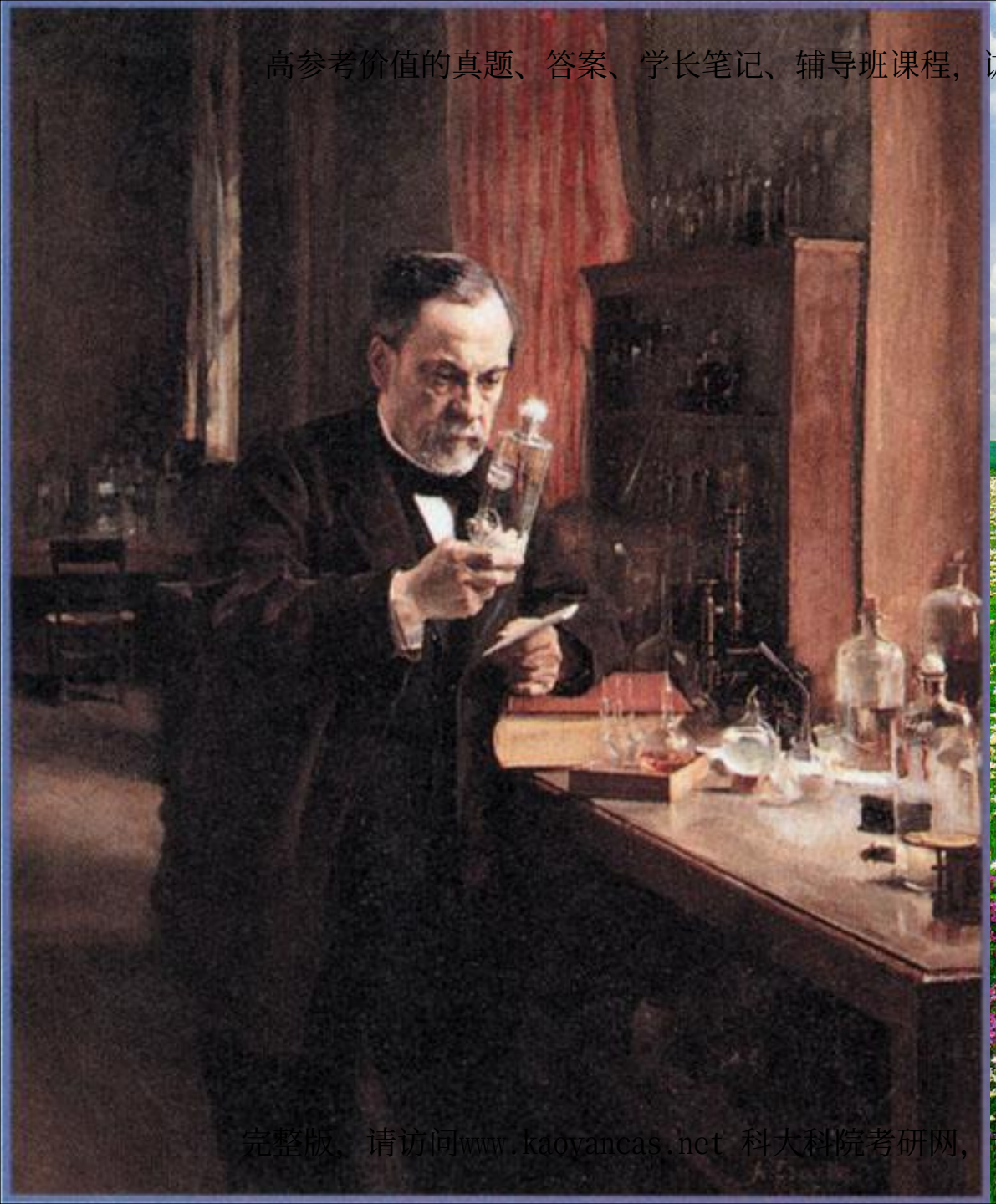


第22章

糖酵解作用

一、糖酵解作用的研究历史

The Nobel Prize in Chemistry 1907

"for his biochemical researches and his discovery of cell-free fermentation"

Eduard Buchner

Germany

Landwirtschaftliche Hochschule (Agricultural College)
Berlin, Germany

1860 - 1917

1897年Buchner兄弟发现糖转化为乙醇不需要活细胞。

The Nobel Prize in Chemistry 1929

"for their investigations on the fermentation of sugar and fermentative enzymes"

Presentation Speech

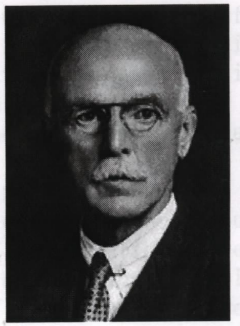
Sir Arthur Harden

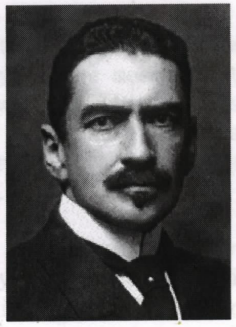
Great Britain

London University
London, Great Britain

1865 - 1940

Biography





Hans von Euler-Chelpin

Sweden

Stockholm University
Stockholm, Sweden

1873 - 1964

Biography
Swedish Nobel Stamps

1905年 Harden A和 Yang W J发现糖分解过程中生成磷酸酯，随后发现这一过程有辅酶参与

30年代 Embden 和 Meyerhof对糖的无氧分解进行深入研究，基本搞清了无氧分解的途径，故这一途径也称作 Embden – Meyerhof 途径。

Hans von Euler-Chelpin,
1873–1964

1874–1933

1884–1951

The Nobel Prize in Physiology or Medicine 1922

Presentation Speech

"for his discovery relating to the production of heat in the muscle"

Sir Archibald Vivian Hill

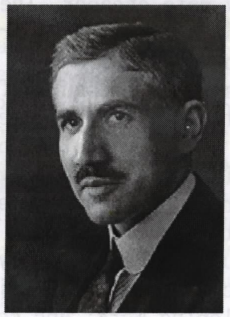
Great Britain

London University
London, Great Britain

1886 – 1977

Biography

"for his discovery of the fixed relationship between the consumption of oxygen and the metabolism of lactic acid in the muscle"



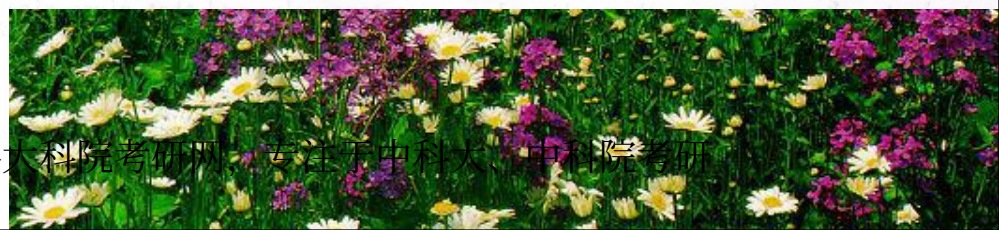
Otto Fritz Meyerhof

Germany

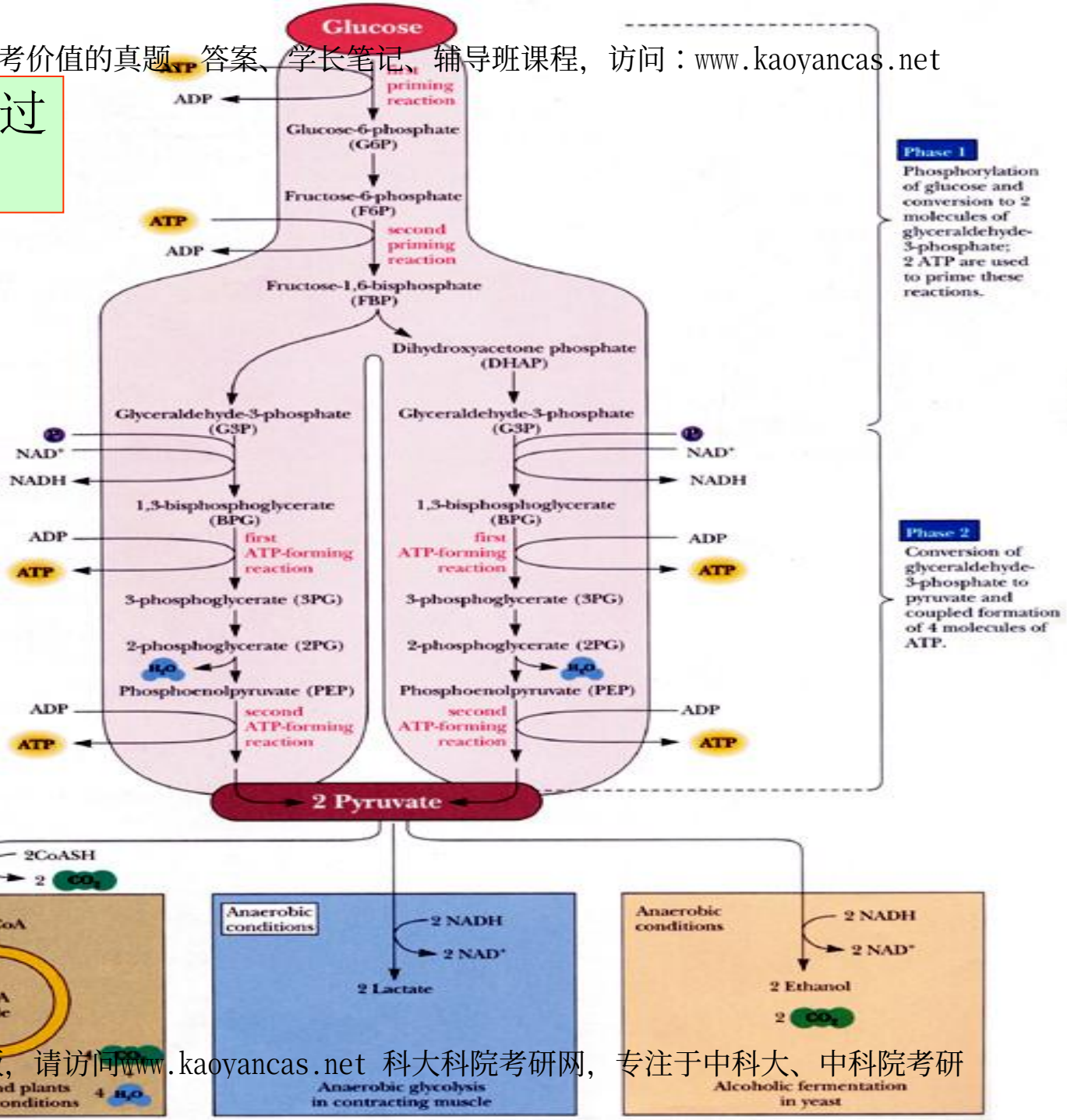
Kiel University
Kiel, Germany

1884 – 1951

Biography



二、糖酵解过程概述



In the first five steps of glycolysis, [illegible] is split into two 3-carbon compounds.

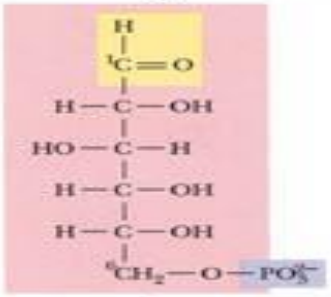
2 molecules of ATP are required to prime these reactions.

ATP

Mg^{2+}

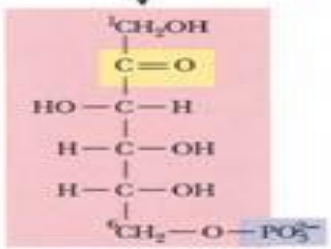
ADP

Hexokinase
glucokinase



D-Glucose-6-phosphate (G6P)

Phosphoglucoisomerase



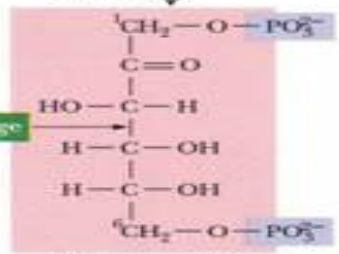
D-Fructose-6-phosphate (F6P)

ATP

Mg^{2+}

ADP

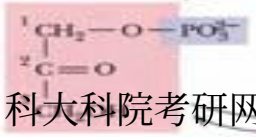
Phosphofructokinase



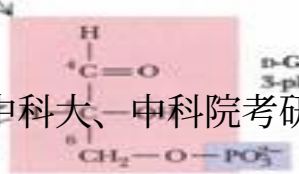
Aldol cleavage

D-Fructose-1,6-bisphosphate (FBP)

Fructose bisphosphate aldolase



Dihydroxyacetone phosphate (DHAP)



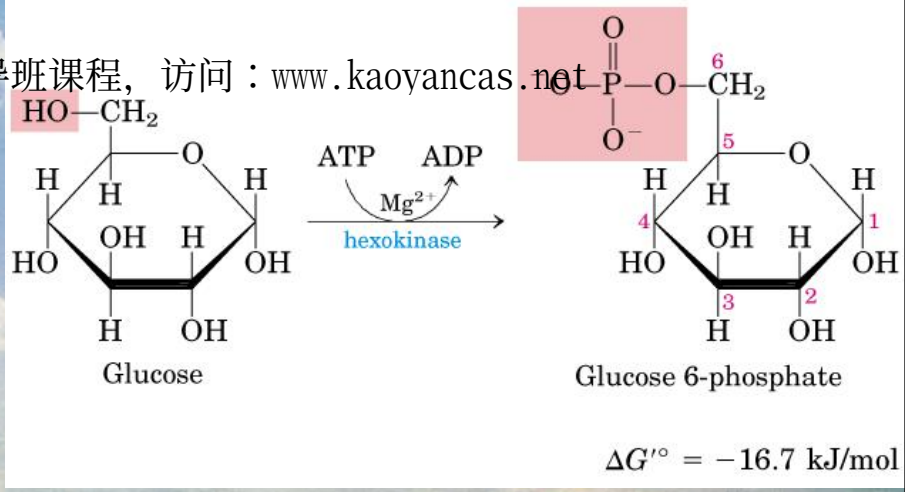
D-Glyceraldehyde-3-phosphate (G3P)

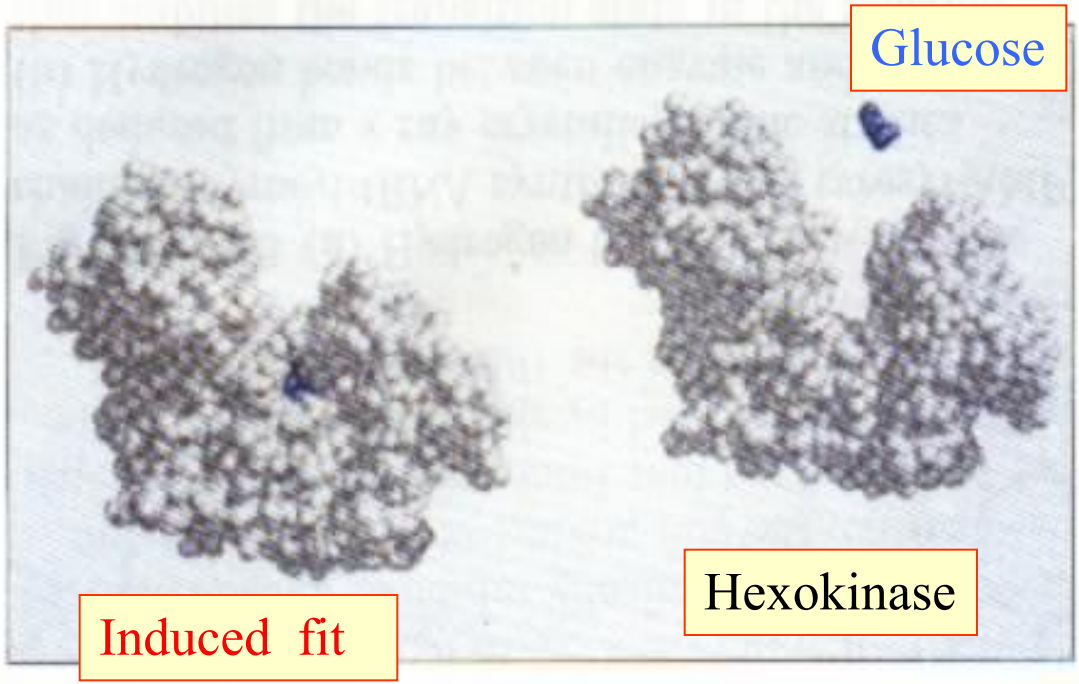
Triose phosphate isomerase

四、糖酵解第一阶段的反应机制

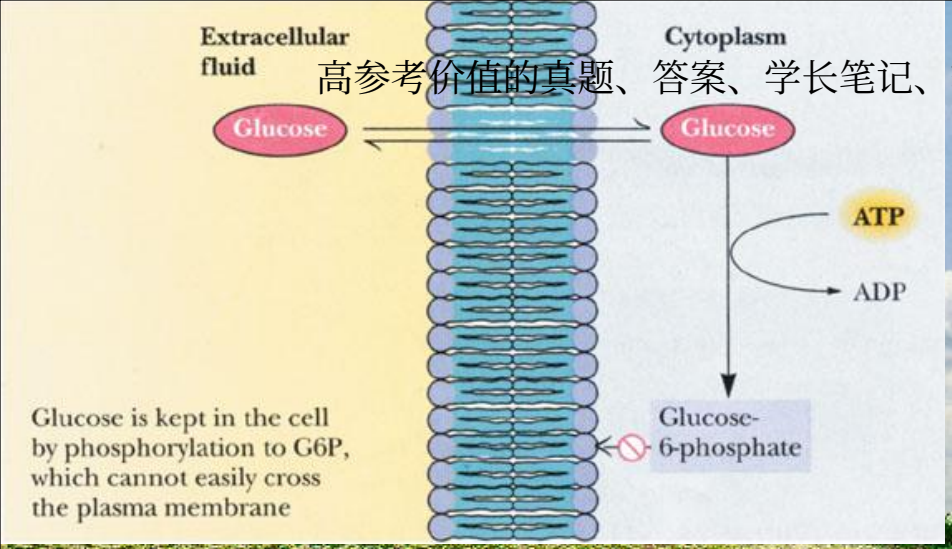
(一) 葡萄糖的磷酸化

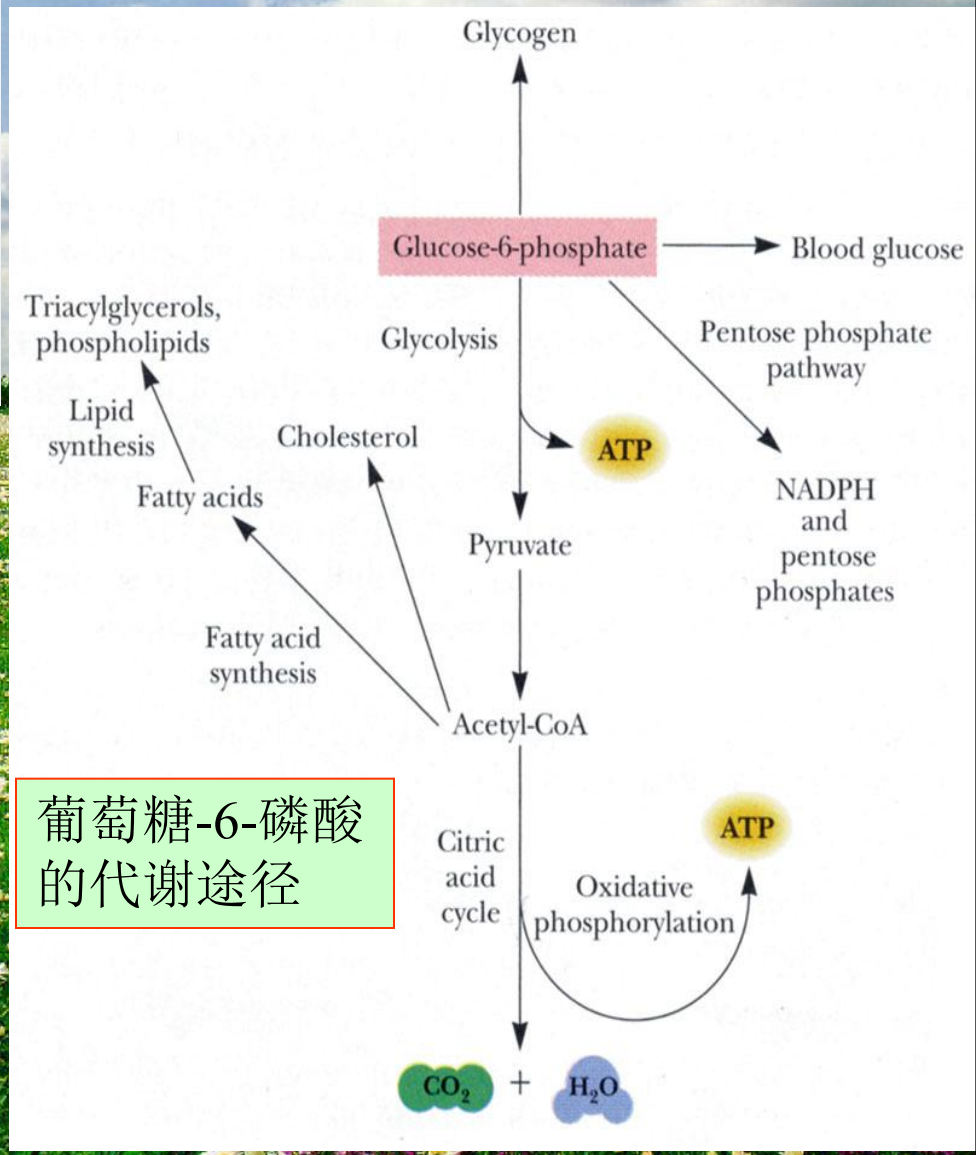
己糖激酶是调控酶，受葡萄糖-6-磷酸的抑制。该酶催化的反应释放大量能量，为不可逆反应。





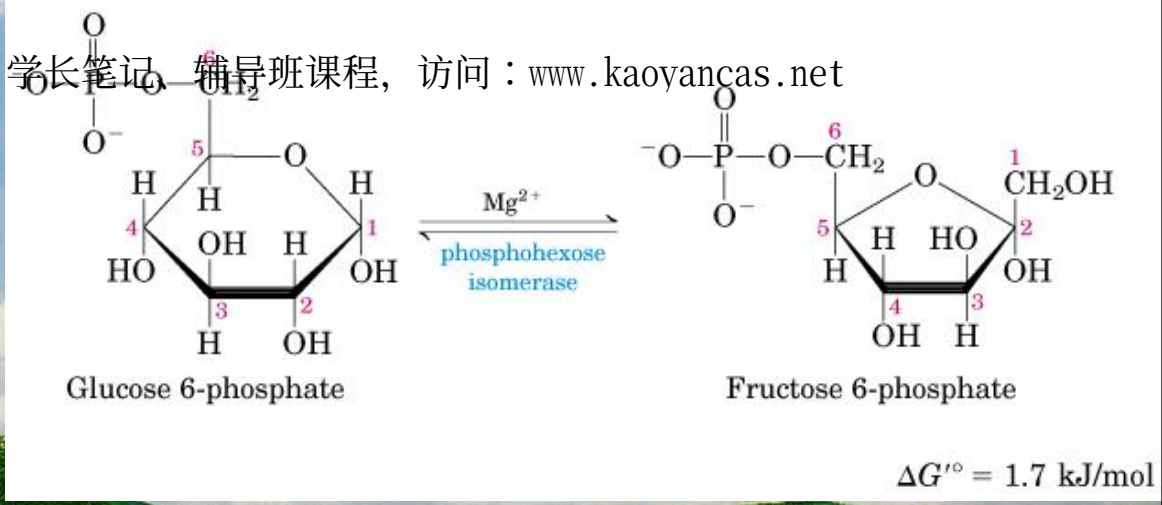
己糖激酶（哺乳动物为单体酶，酵母为二聚体）有4种同工酶，同工酶主要存在于脑和肾，葡萄糖-6-磷酸对该酶有抑制作用，少量的无机磷可解除葡萄糖-6-磷酸的抑制作用，同工酶　主要存在于骨骼肌和心肌，同工酶主要存在于肝脏和肾脏，同工酶　（葡萄糖激酶）只存在于肝脏，其合成受胰岛素的诱导。

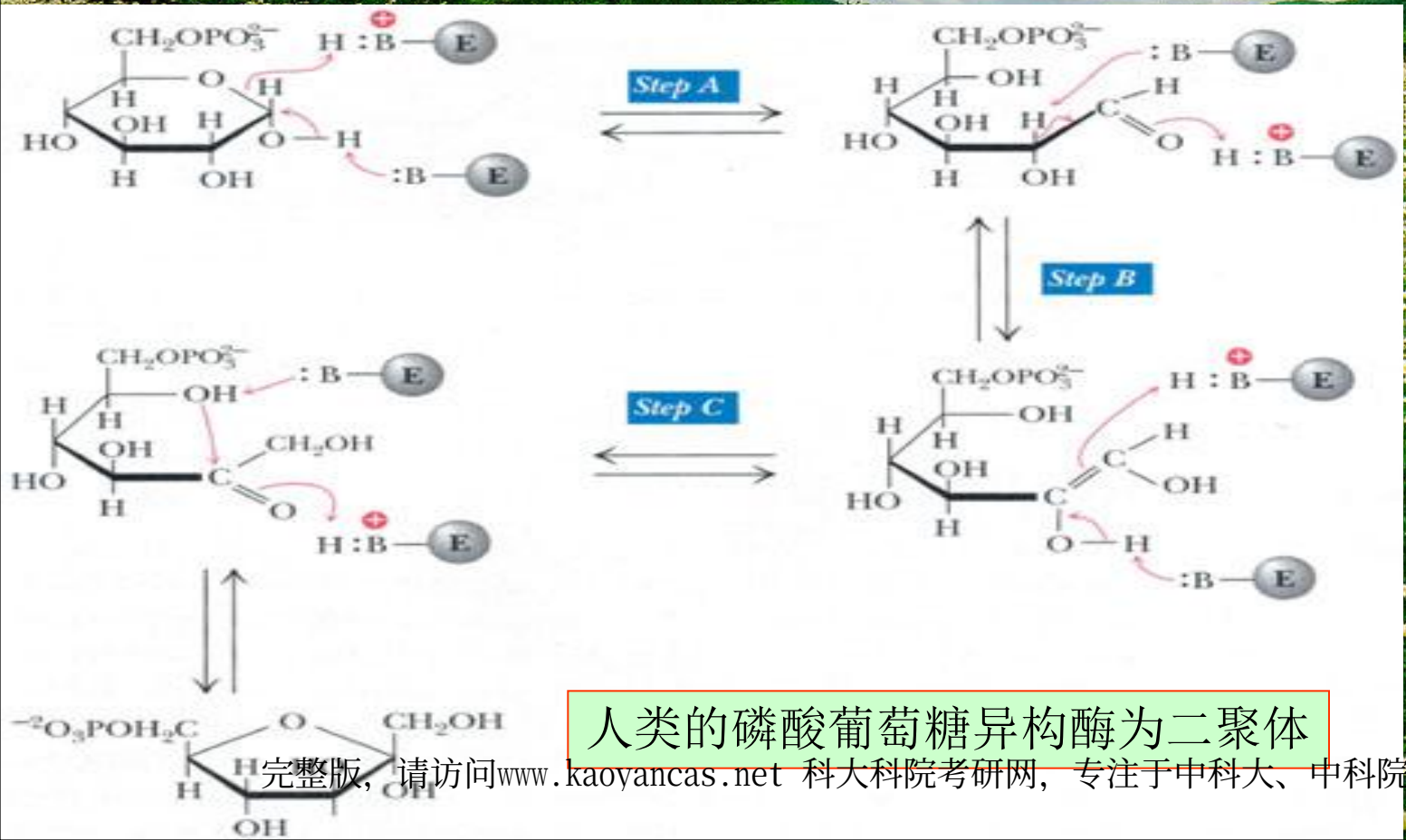




葡萄糖-6-磷酸的代谢途径

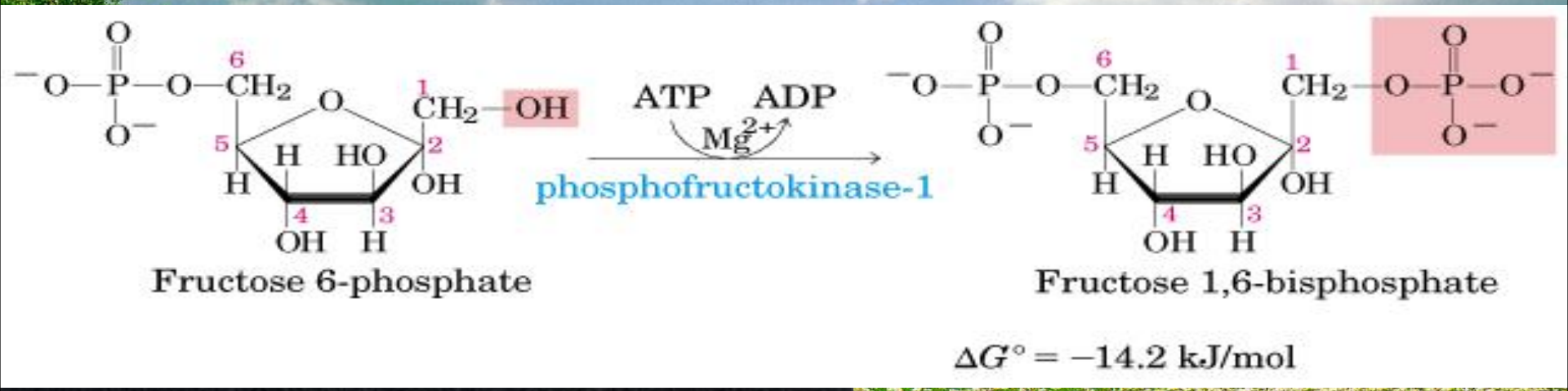
(二) 葡萄糖-6-磷酸异构化形成果糖-6-磷酸



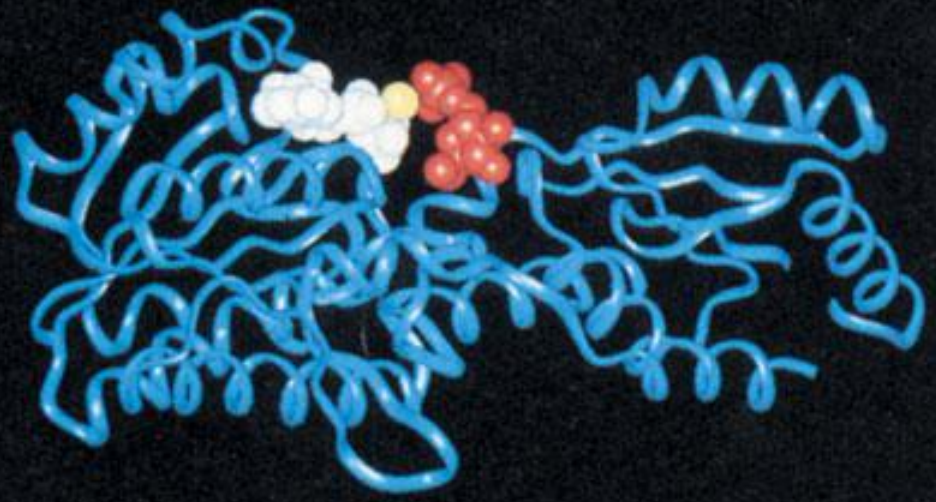


人类的磷酸葡萄糖异构酶为二聚体

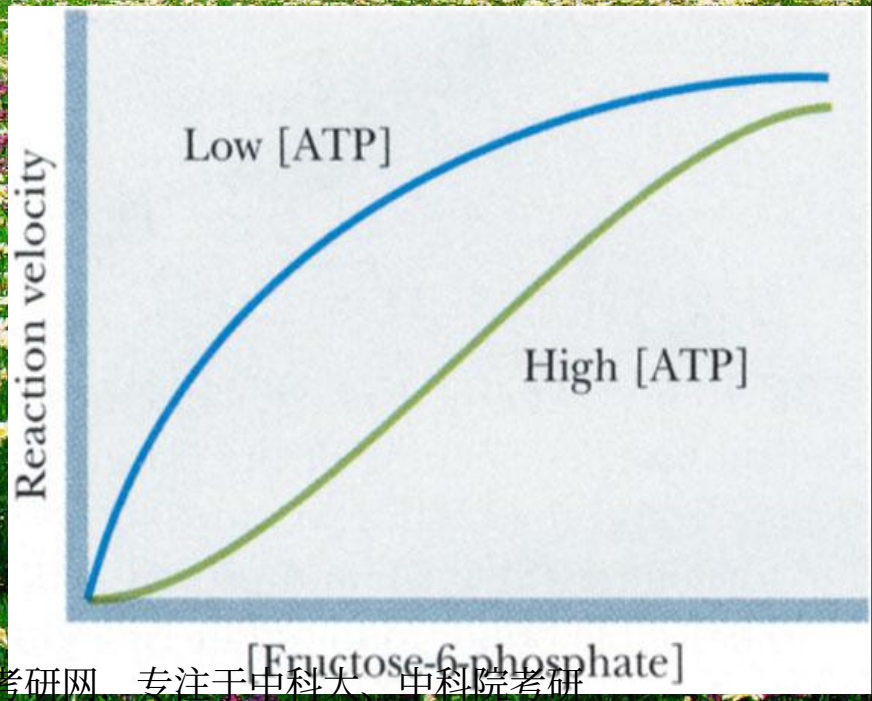
(三) 果糖-6-磷酸形成果糖-1，6-二磷酸

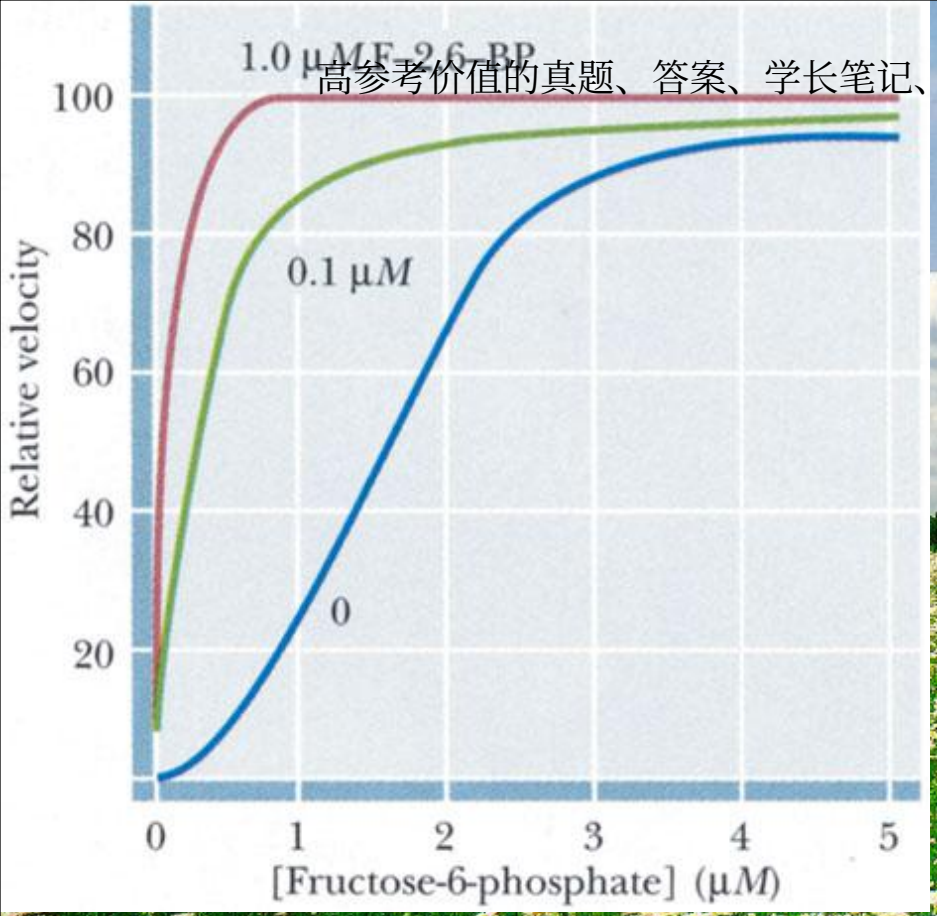


磷酸果糖激酶亚基的结构（四个亚基）

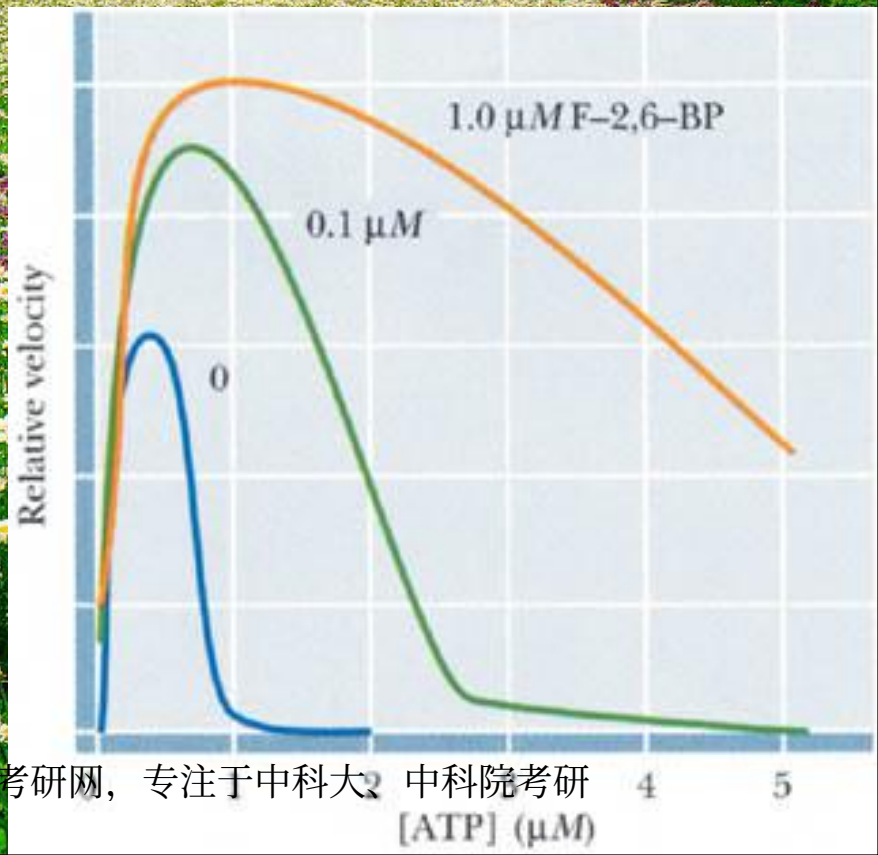


白色为ATP，红色为果糖-6-磷酸



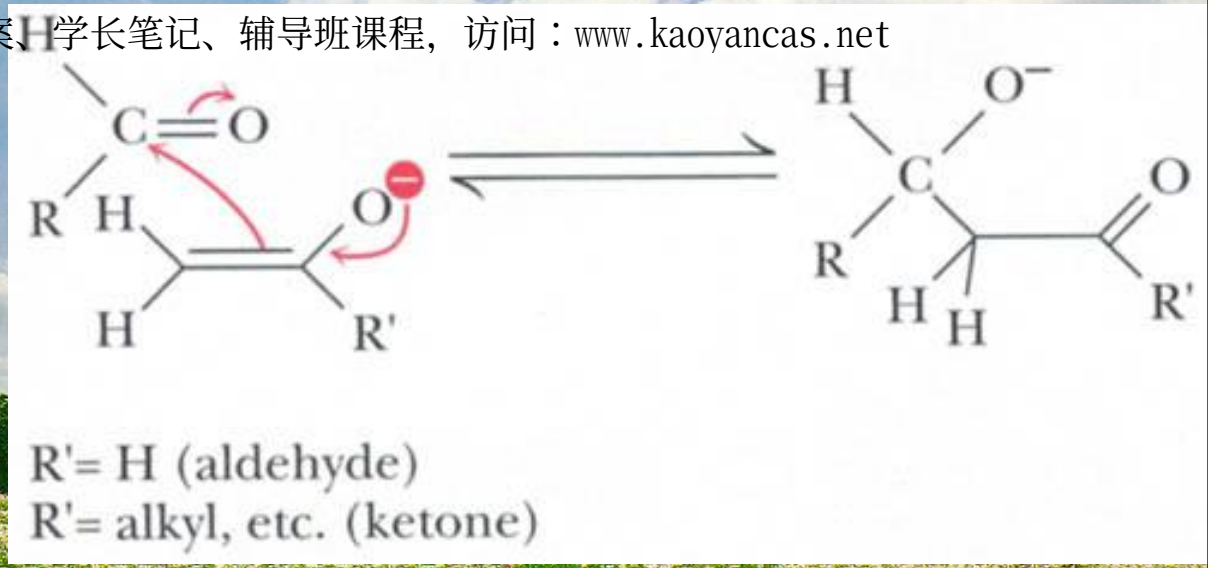


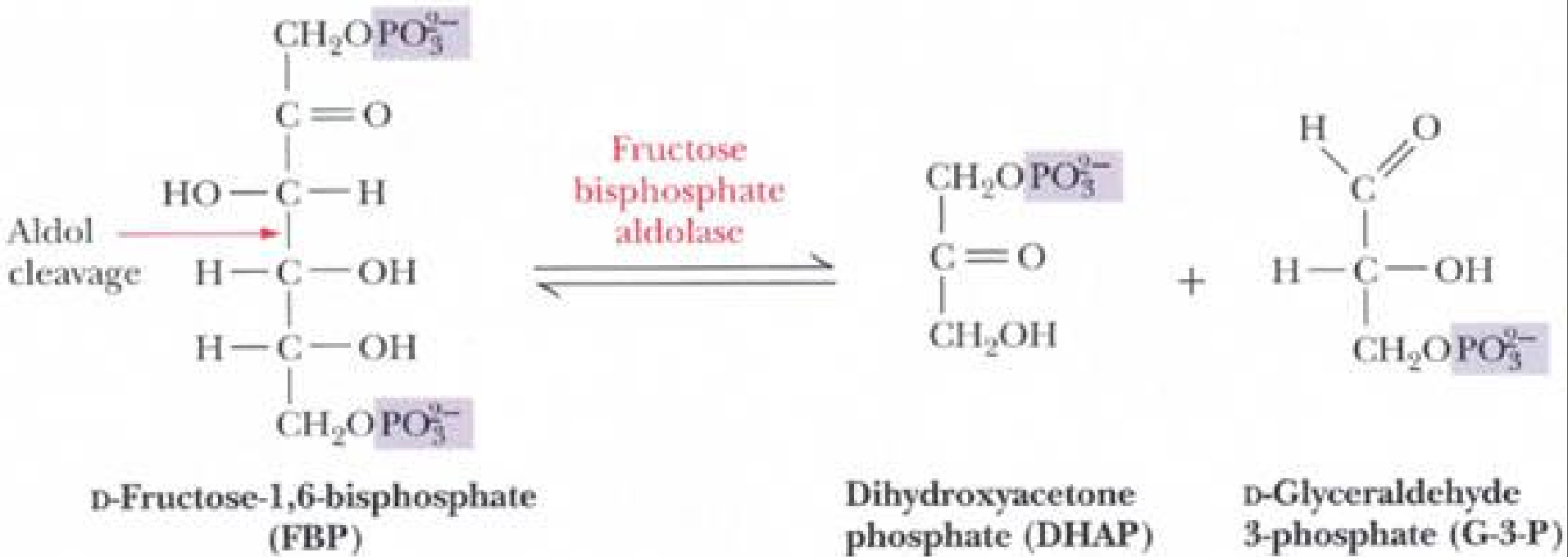
磷酸果糖激酶是关键的调控酶，有4个亚基，3种同工酶，同工酶A存在于骨骼肌和心肌，对磷酸肌酸、柠檬酸、无机磷酸的抑制作用最敏感；同工酶B存在于肝脏和红细胞，对2,3-二磷酸甘油酸（BPG）的抑制作用最敏感；同工酶C存在于脑中，对腺嘌呤核苷酸的作用最敏感。

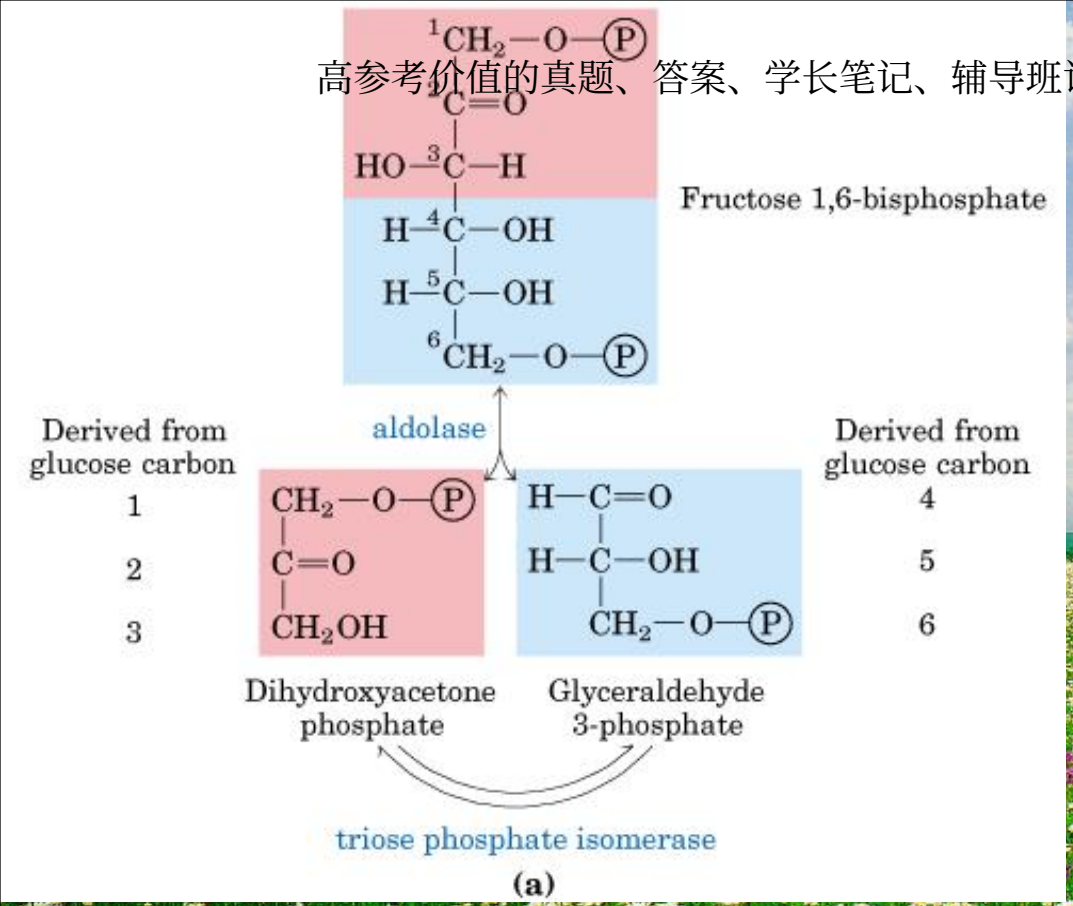


磷酸果糖激酶催化的反应不可逆，ATP是别构抑制剂，F-2,6-BP是别构激活剂。两次磷酸化使葡萄糖转化为反应活性很高的F-1,6-BP，有利于随后的分解反应。

(四) 果糖-1，6-二磷酸转变为甘油醛-3-磷酸和二羟丙酮磷酸







醛缩酶有多种同工酶，型醛缩酶存在于高等动植物，为四聚体，有3种同工酶，A主要存在于肌肉中，B主要存在于肝脏，C主要存在于脑组织， 3种同工酶均由4中不同的亚基组成。

型醛缩酶存在于微生物，相对分子质量只有 型醛缩酶的一半，含有二价金属离子。

果糖-1，6-二磷酸浓度较低时，容易转变为甘油醛-3-磷酸和二羟丙酮磷酸。

$23970 = -8.314 \quad 310 \ln K$

$K=10^{-4}$

FBP↔DHAP+G3P

1-X X X

若FBP为: 1mol/L

$10^{-4}= X^2 / (1 \quad X)$

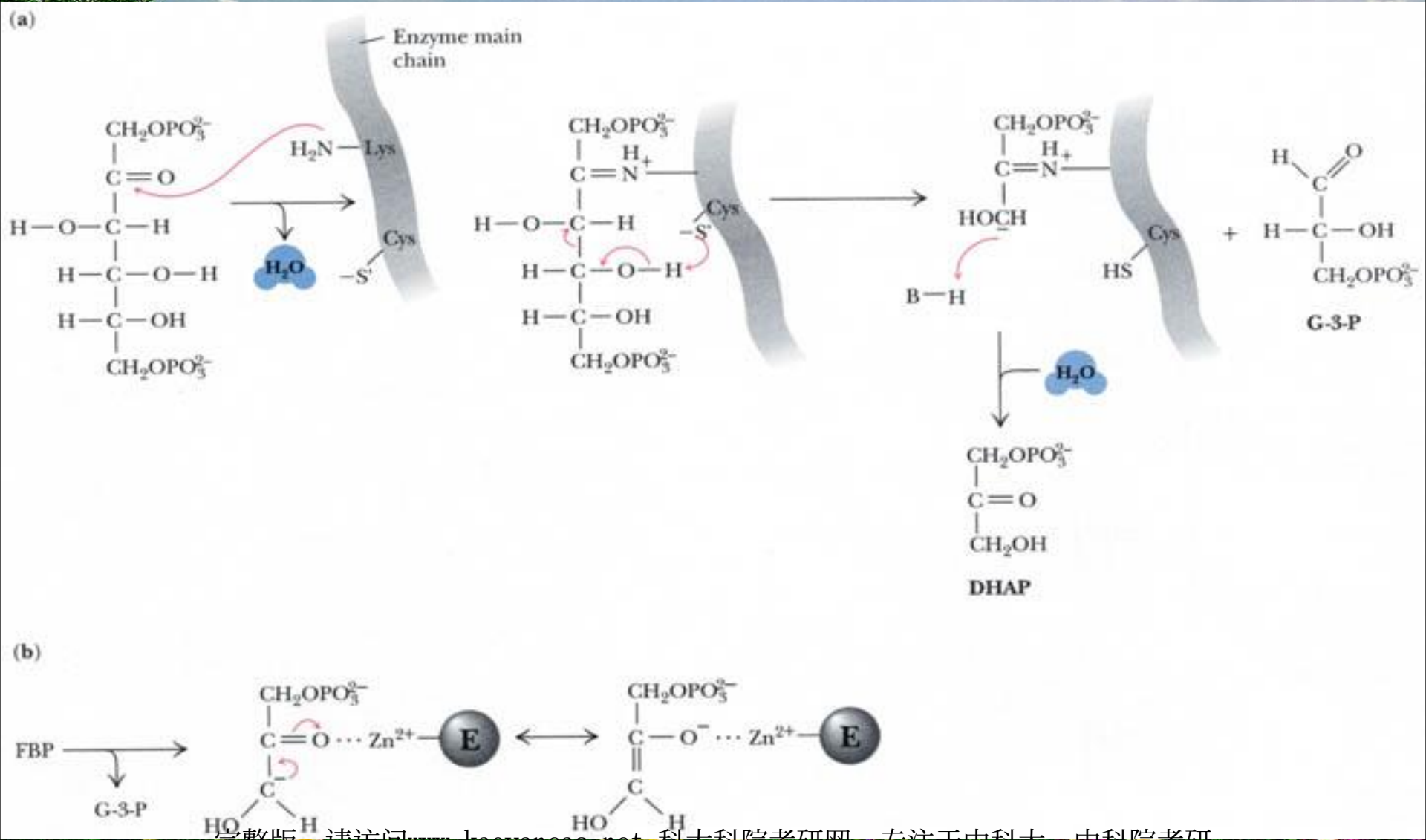
$X=10^{-2}$

若FBP为: 10^{-5}mol/L

$10^{-4}= X^2 / (10^{-5} \quad X)$

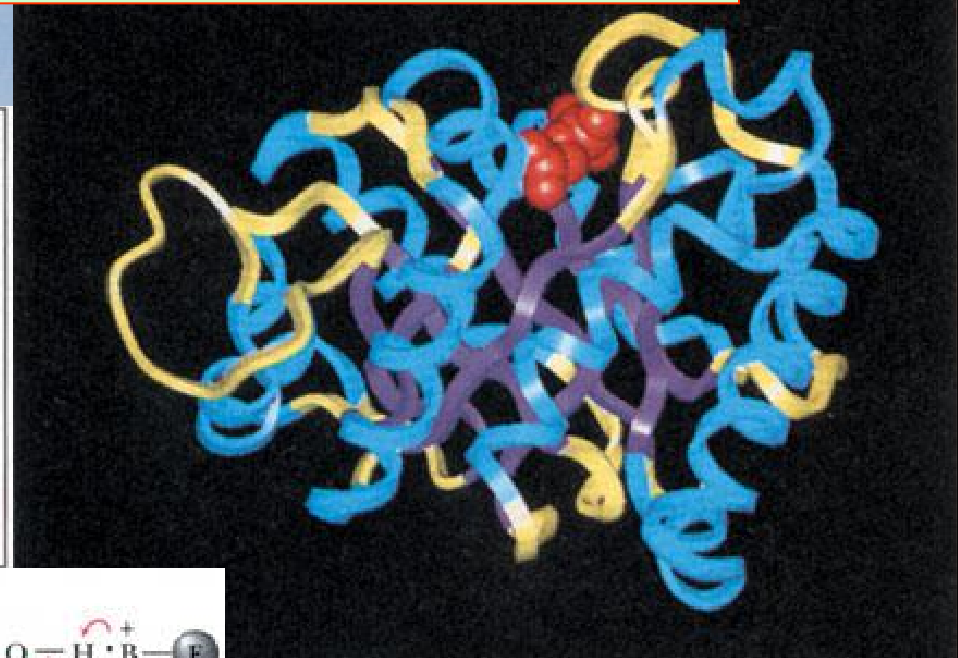
$X=0.92 \quad 10^{-5}$

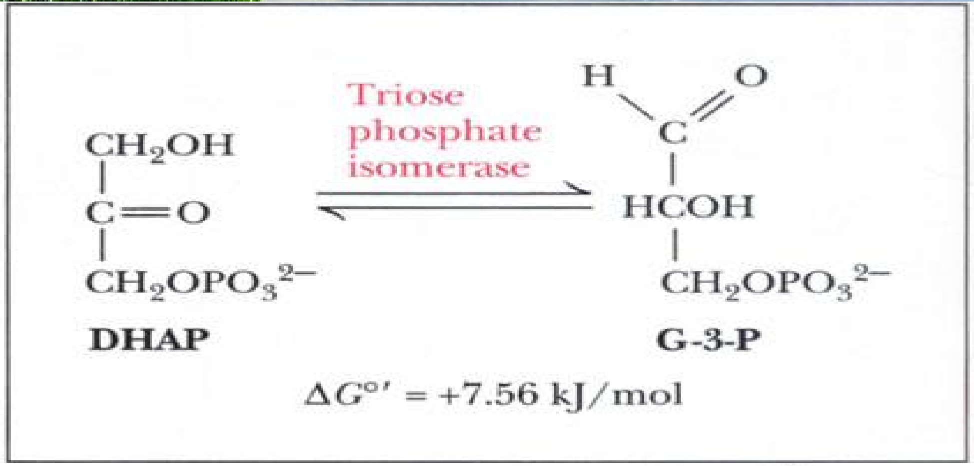
果糖-1，6-二磷酸转变为甘油醛-3-磷酸和二羟丙酮磷酸的反应机制

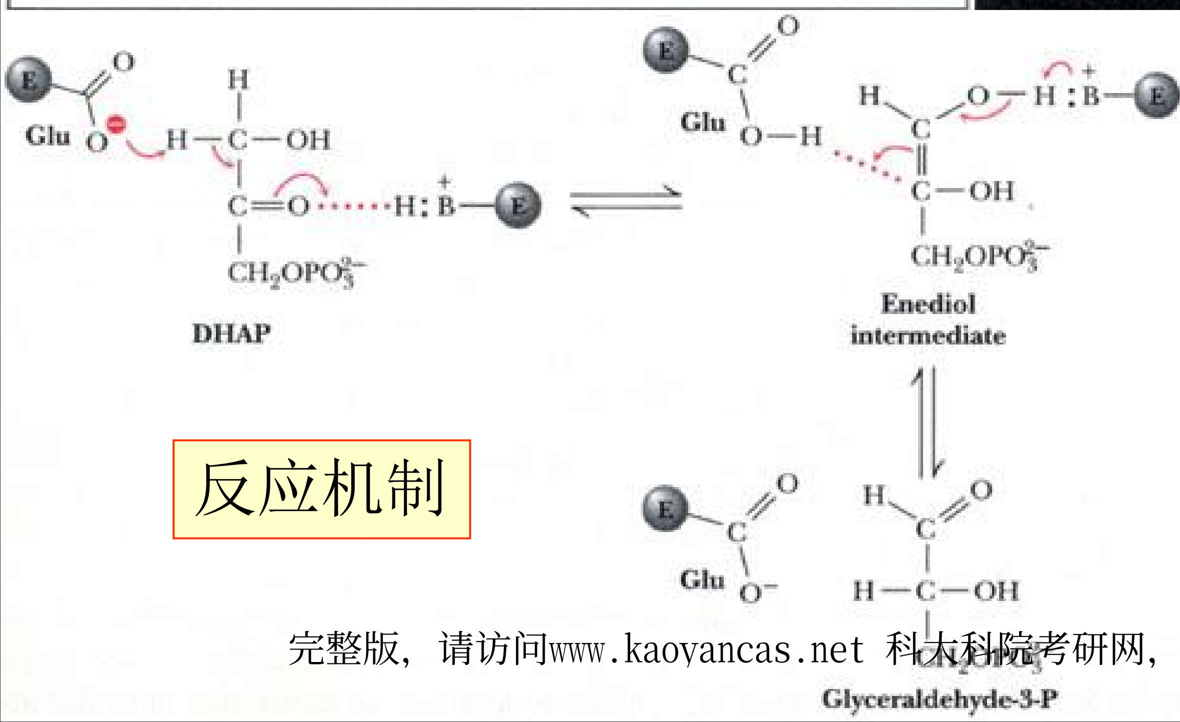


(五) 二羟丙酮磷酸转变为甘油醛-3-磷酸

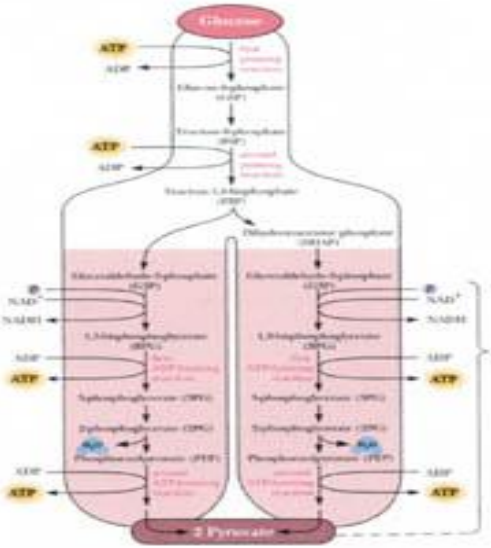
丙糖磷酸异构酶的四聚体，图中所示为单体的结构，红色为二羟丙酮磷酸。





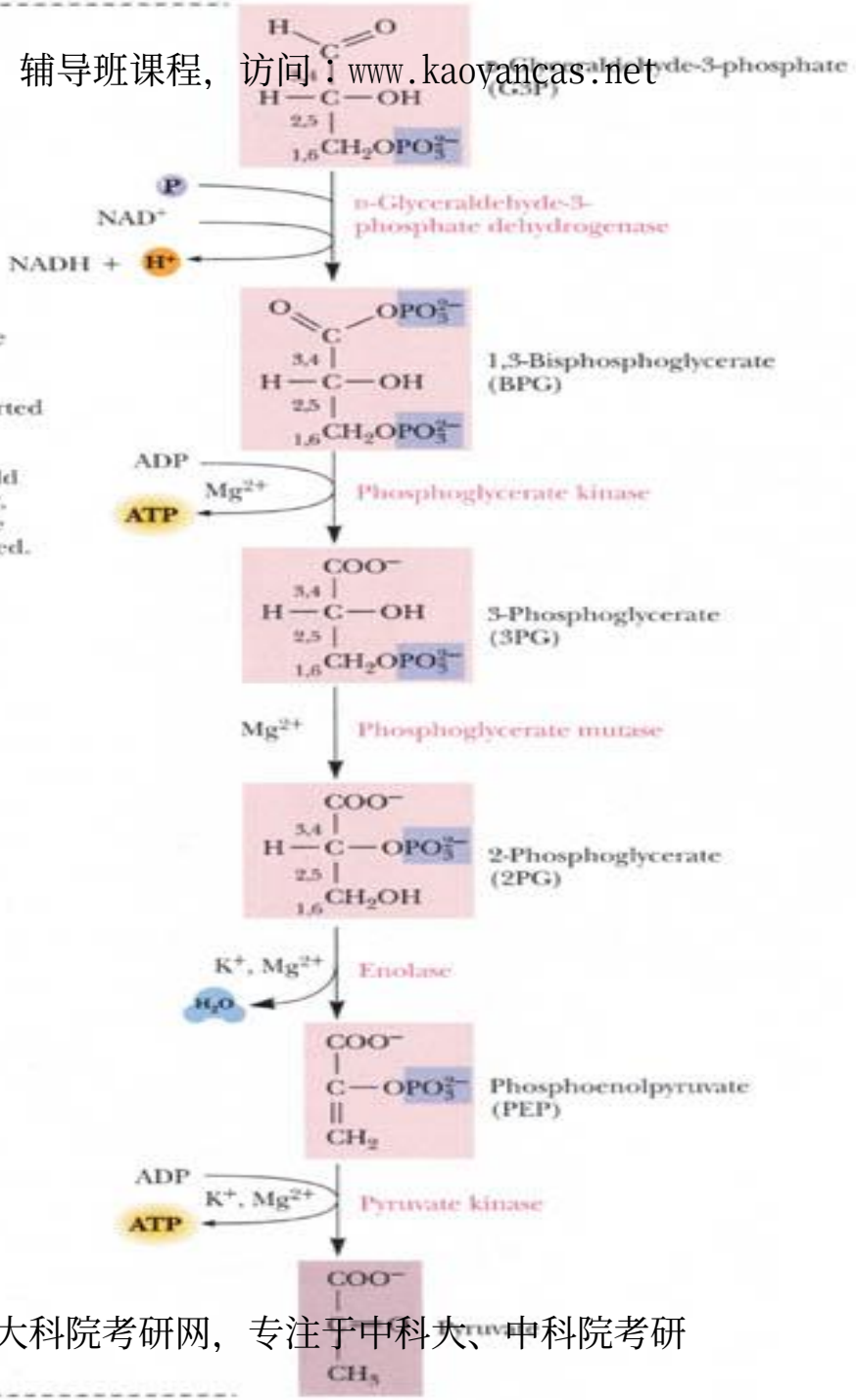


反应机制



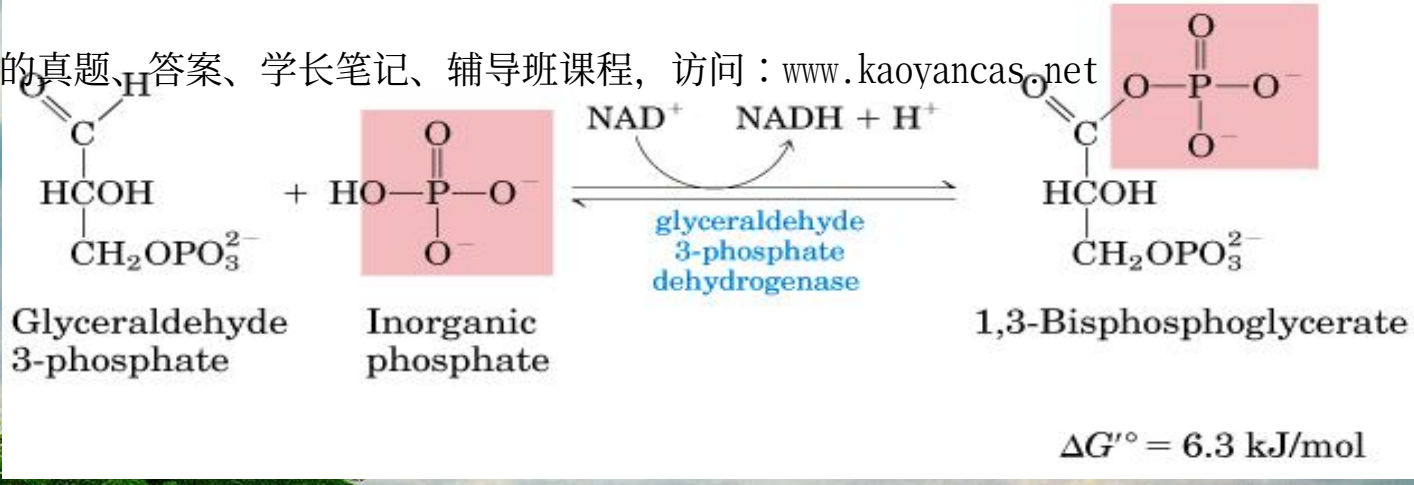
In the second phase of glycolysis, glyceraldehyde-3-phosphate is converted to pyruvate.

These reactions yield 4 molecules of ATP, 2 for each molecule of pyruvate produced.

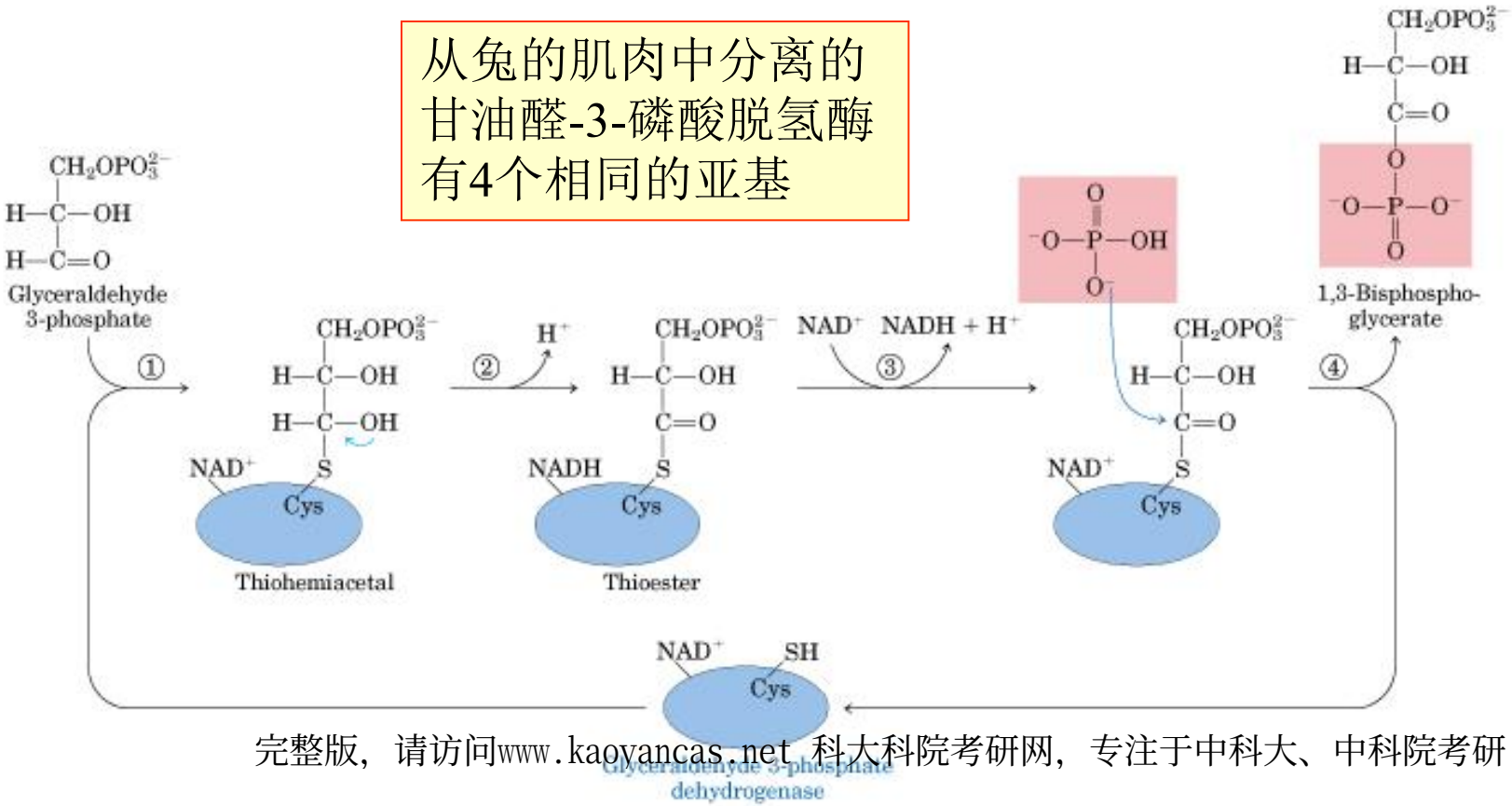


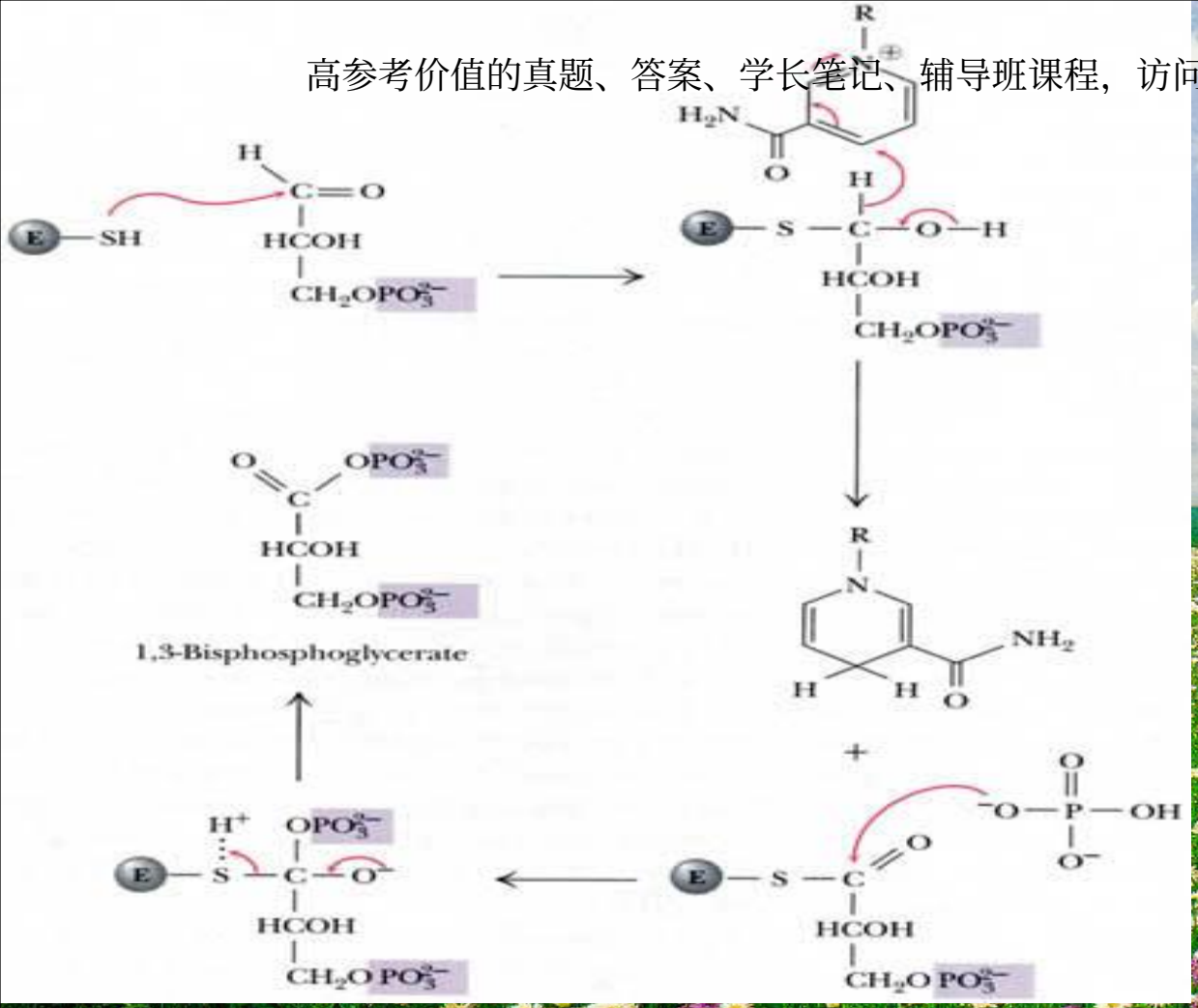
五、酵解第二阶段放能阶段的反应机制

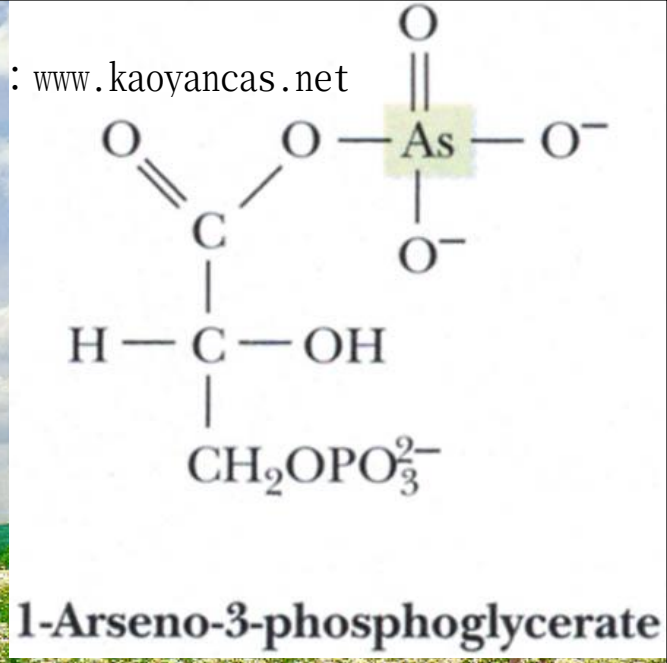
(一) 甘油醛-3-磷酸氧化成1，3-二磷酸甘油酸



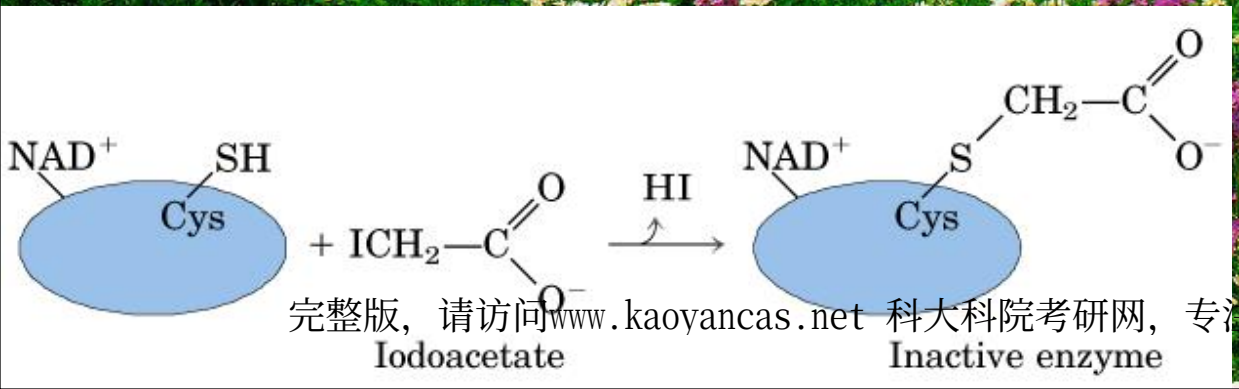
从兔的肌肉中分离的甘油醛-3-磷酸脱氢酶有4个相同的亚基



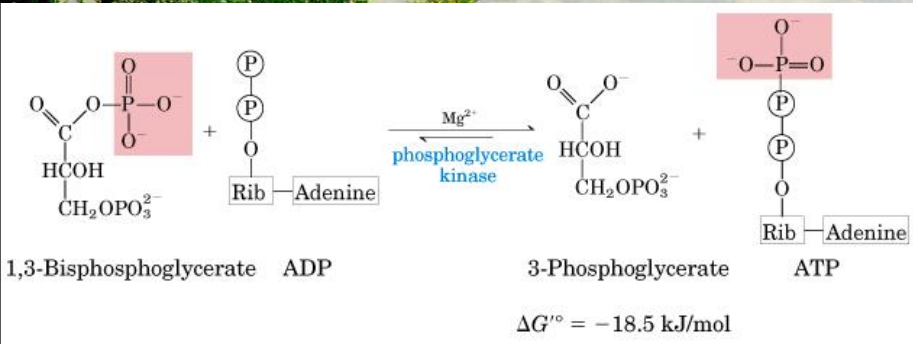


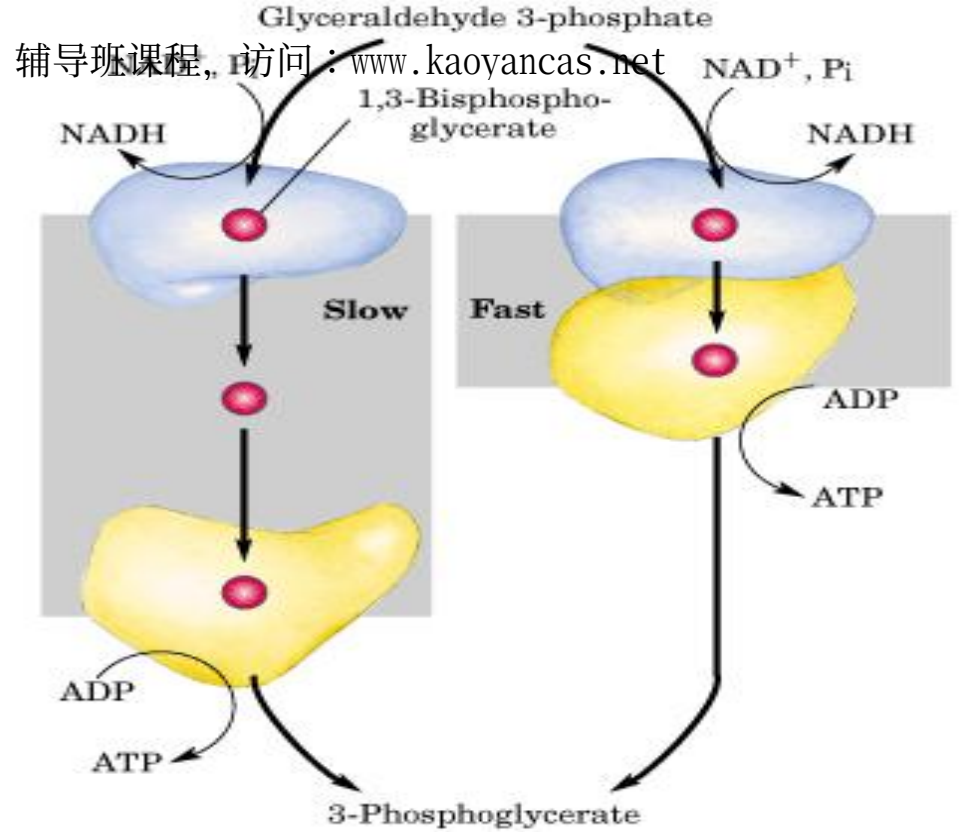


砷酸化合物迅速分解，不能生成ATP



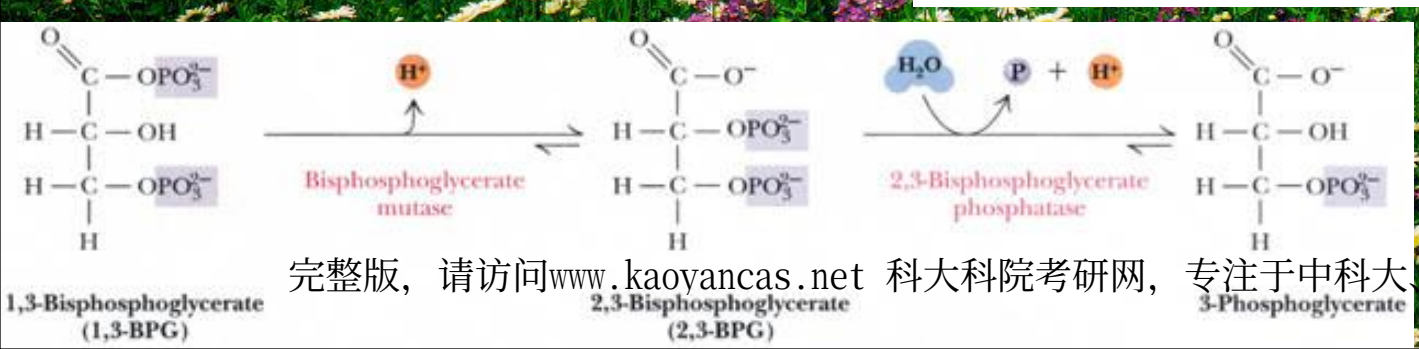
(二) 1，3-二磷酸甘油酸转移高能磷酸基团形成ATP



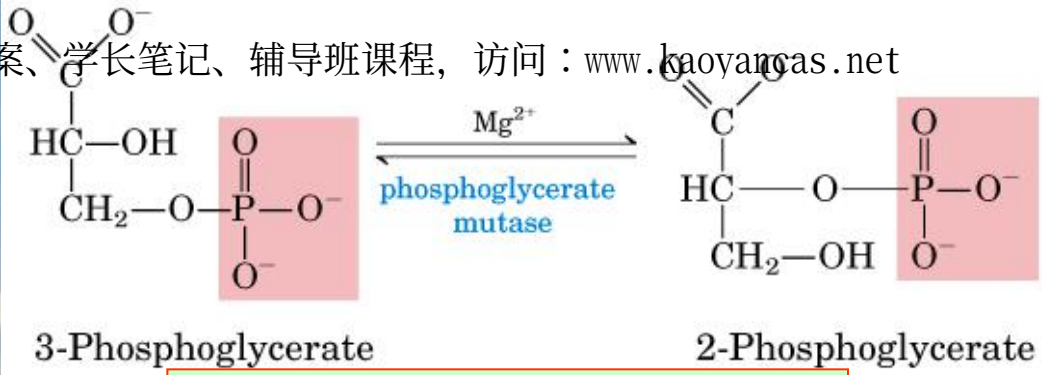


Sequential action of two separate enzymes: the product of the first enzyme (1,3-bisphosphoglycerate) diffuses to the second enzyme.

Substrate channeling through a functional complex of two enzymes: the intermediate (1,3-bisphosphoglycerate) is never released to the solvent.



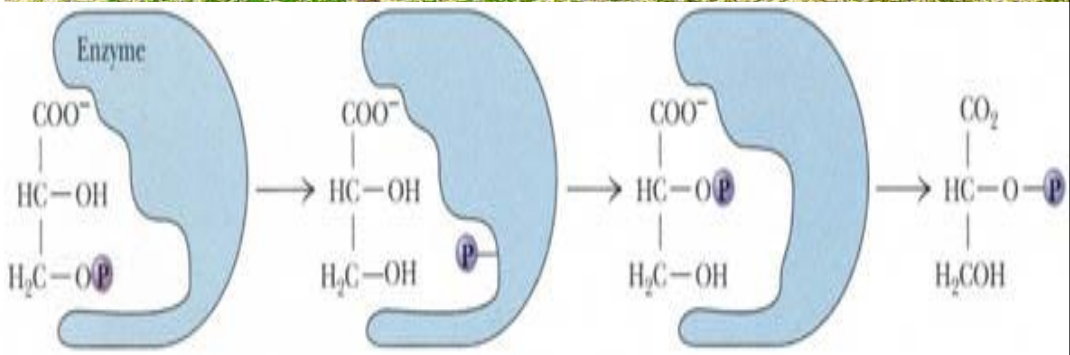
(三) 3-磷酸甘油酸转变为2-磷酸甘油酸

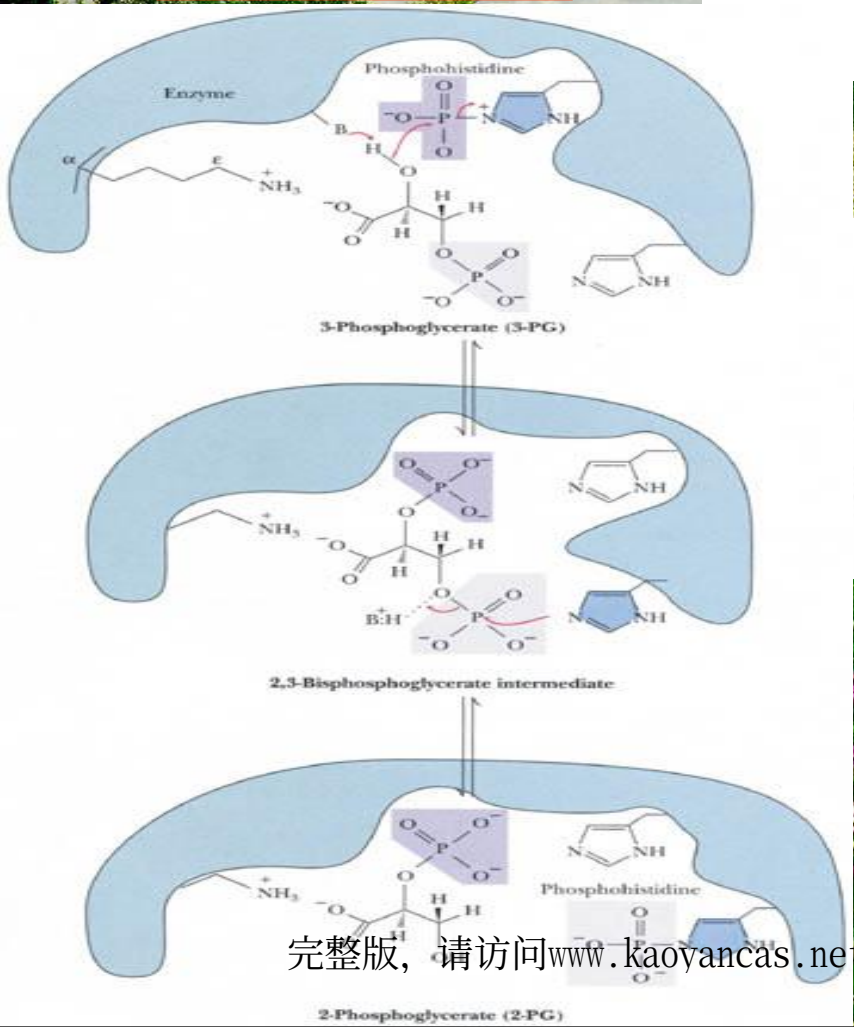


酵母的反应机制

磷酸甘油酸变位酶为二聚体

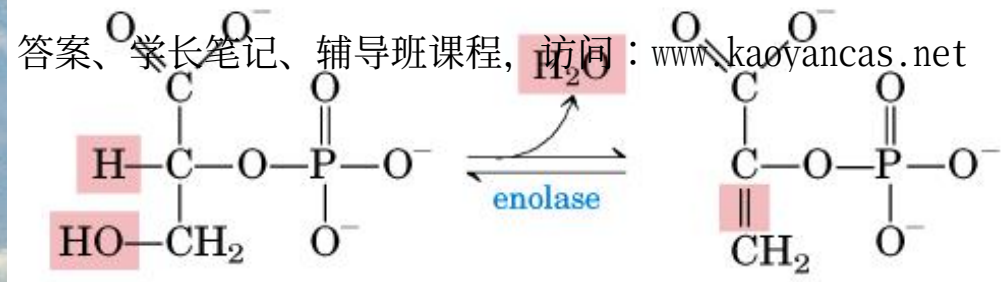
$\Delta G'^{\circ} = 4.4$ kJ/mol





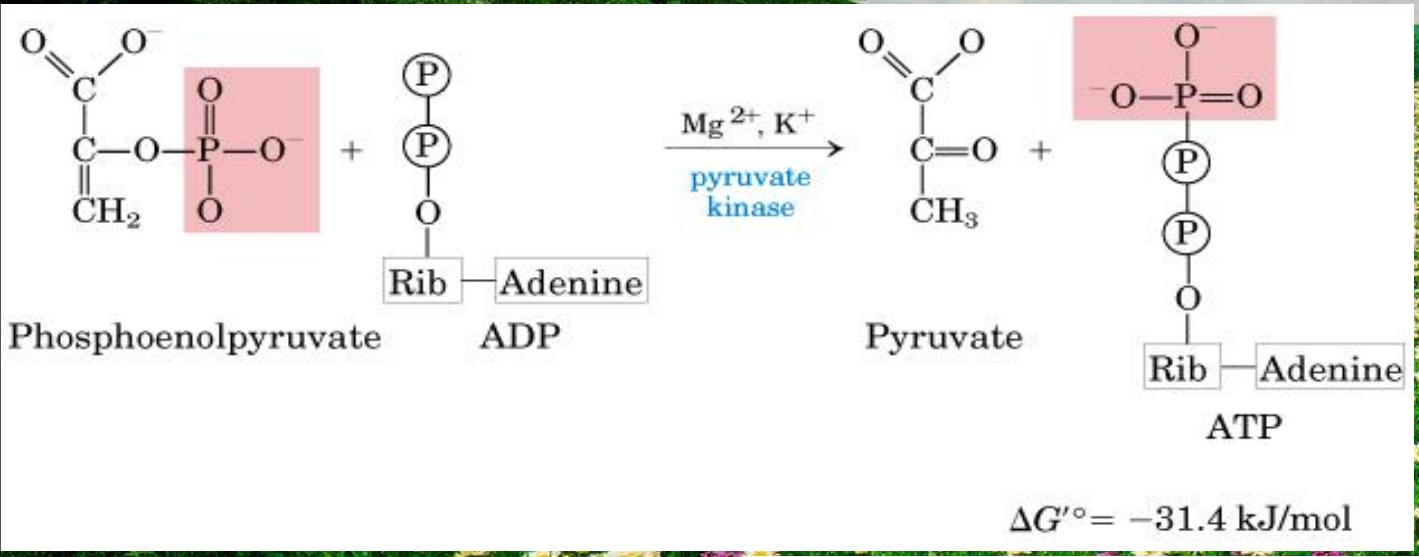
麦芽的反应机制

(四) 2-磷酸甘油酸脱水生成磷酸烯醇式丙酮酸



烯醇化酶为二聚体

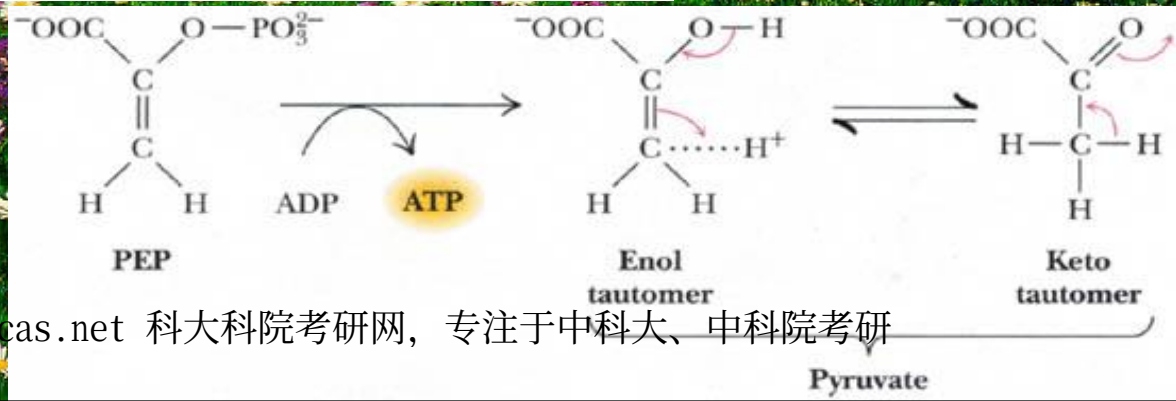
$\Delta G'^{\circ} = 7.5$ kJ/mol



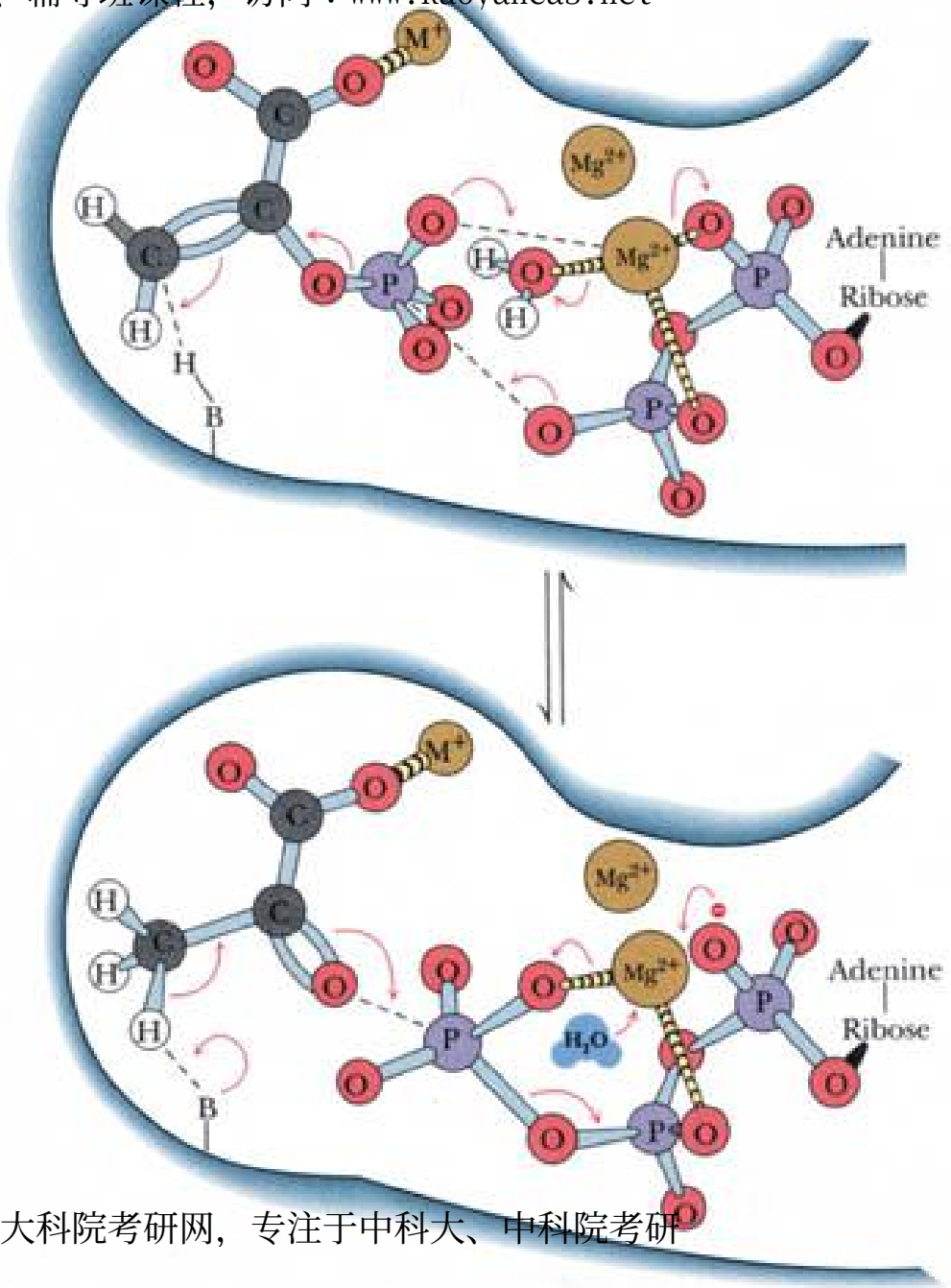
$\Delta G'^{\circ} = -31.4$ kJ/mol

(五) 磷酸烯醇式丙酮酸转变为丙酮酸并产生一个ATP分子

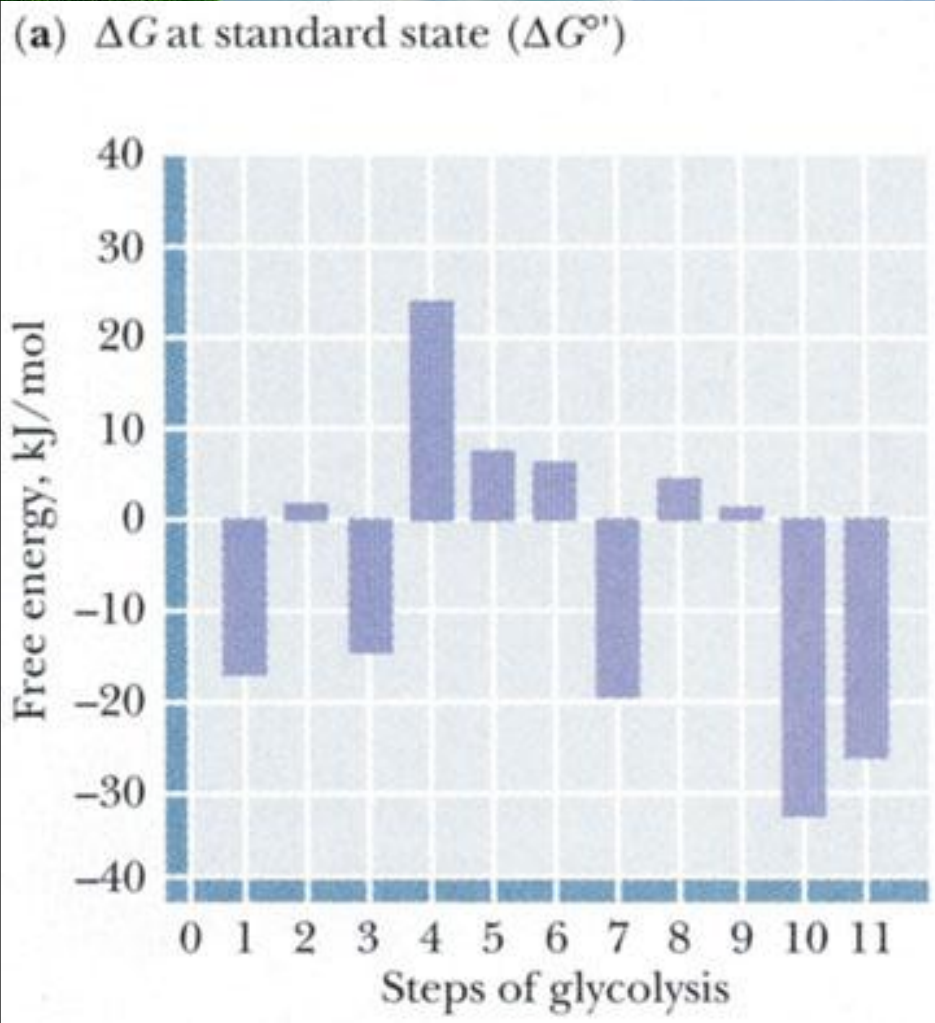
丙酮酸激酶为四聚体，反应可以看作2步。

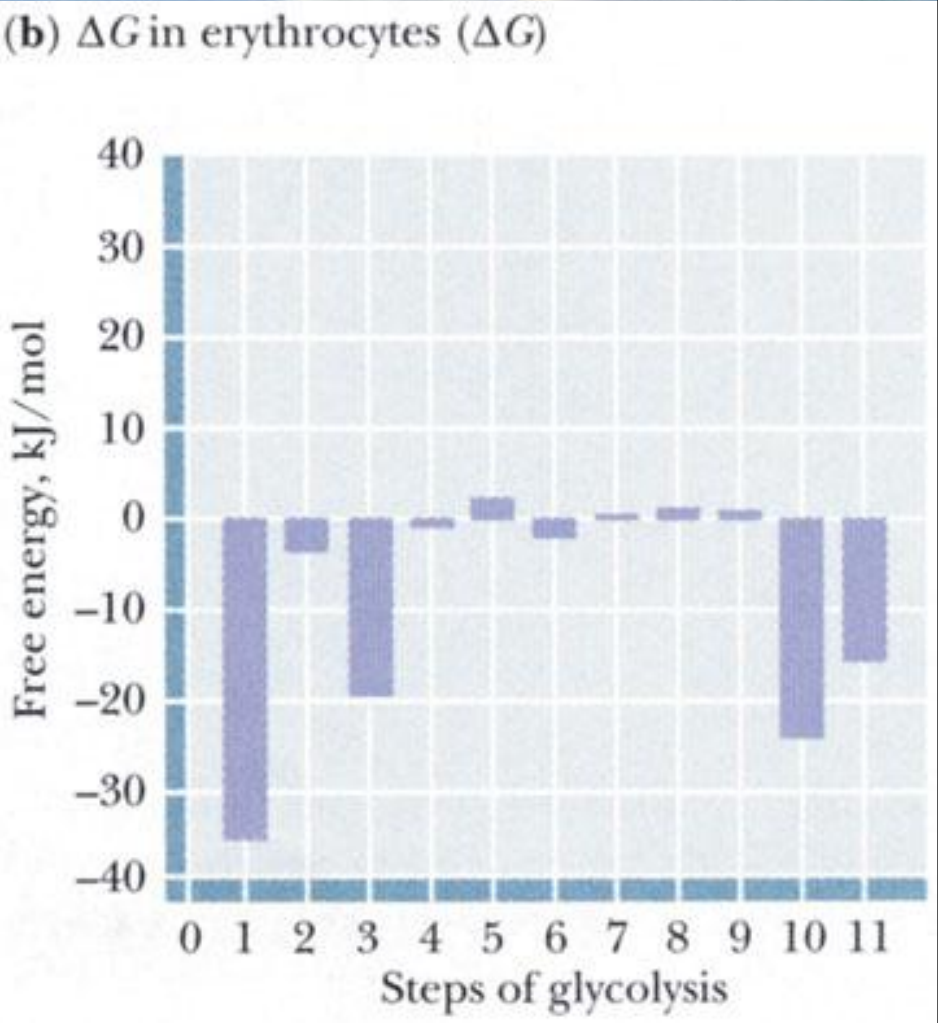


A mechanism for the pyruvate kinase reaction: (a) a water on the Mg^{2+} ion coordinated to ADP is replaced by the phosphoryl group of PEP; (b) Mg^{2+} dissociates from the α-P of ADP; (c) the phosphoryl group is transferred;and (d) the enolate of pyruvate is protonated.



六、由葡萄糖转变为两分子丙酮酸能量转变的估算





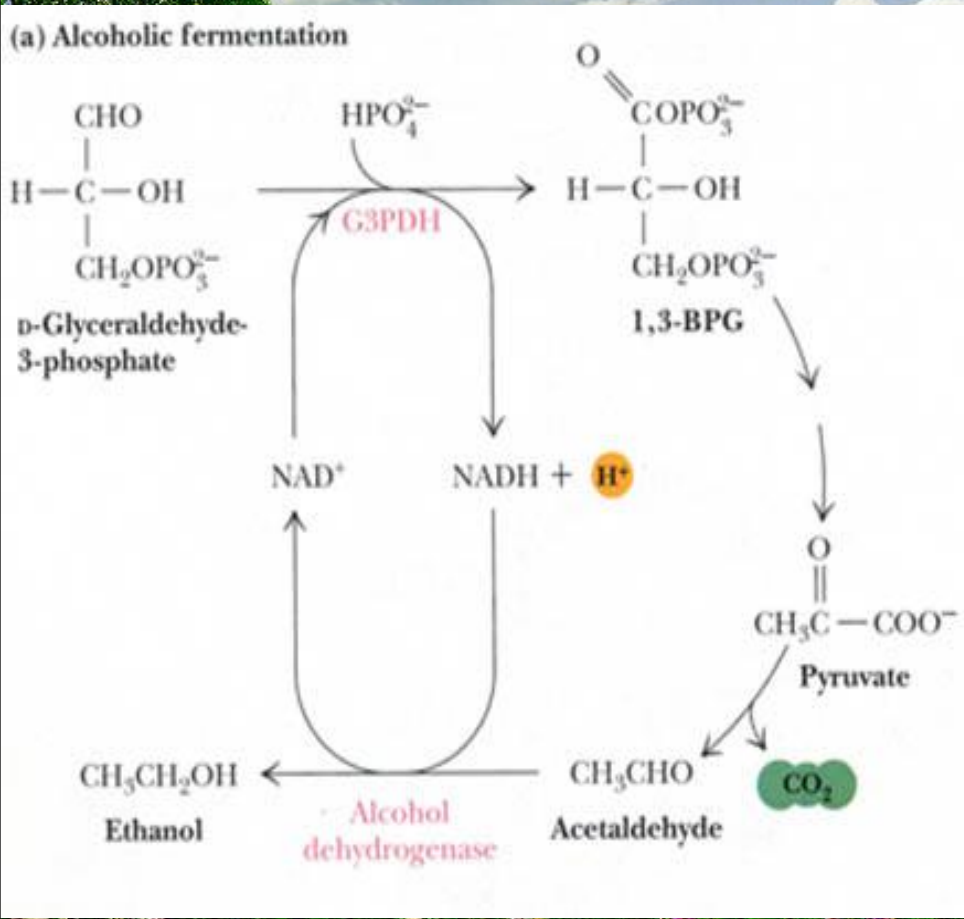
ATP的生成数

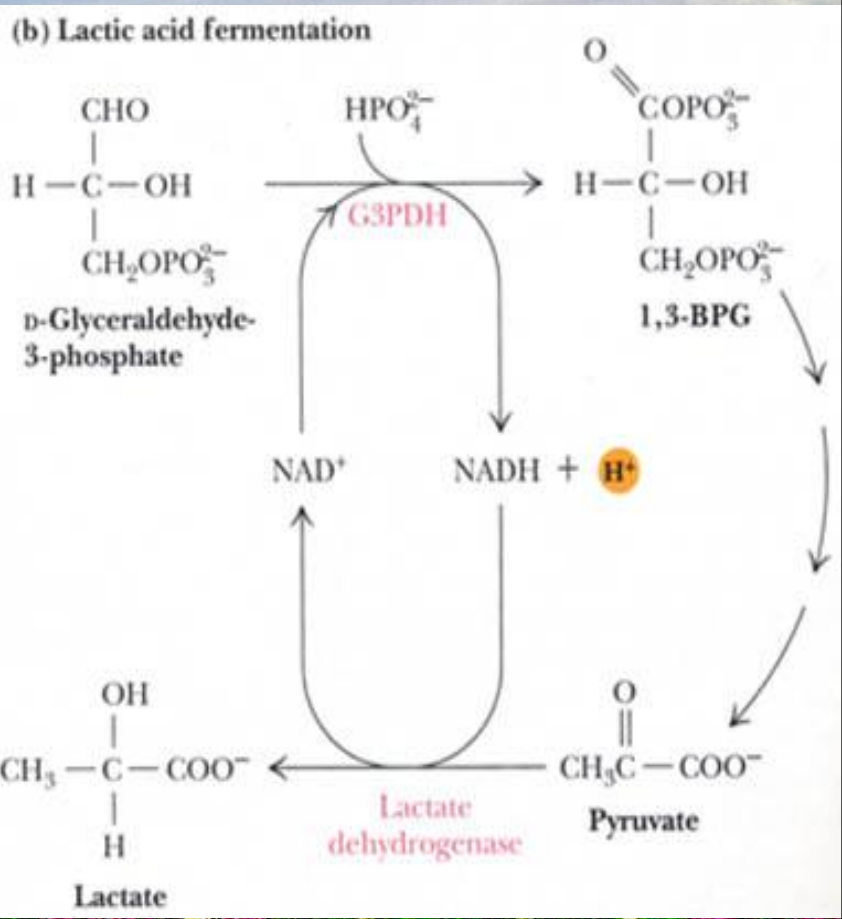
表 22－1　酵解过程中 ATP 的消耗和产生

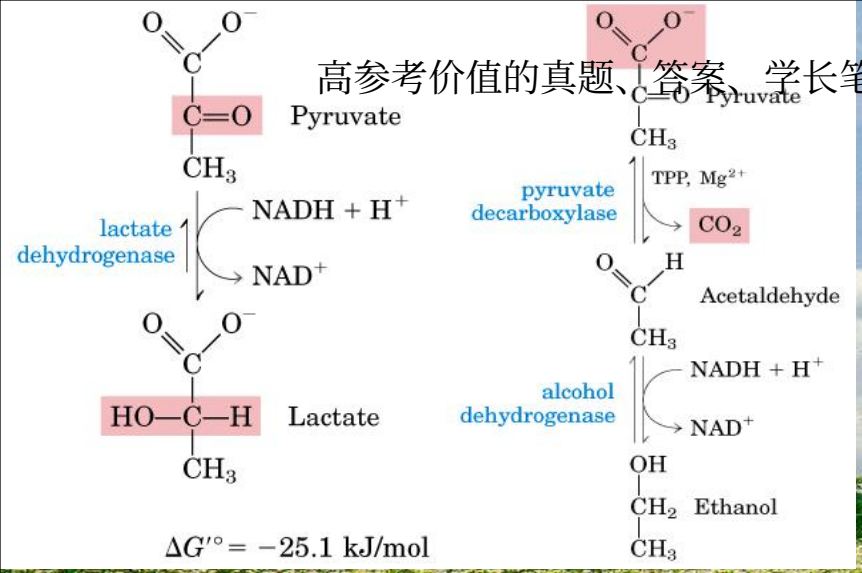
消耗或产生 ATP 的反应	每分子 ATP 葡萄糖 ATP 变化的分子数
葡萄糖→葡萄糖 6－磷酸	－1
果糖－6－磷酸→果糖－1,6－二磷酸	－1
2×1,3－二磷酸甘油酸→2×3－磷酸甘油酸	+2
2×磷酸烯醇式丙酮酸→2×丙酮酸	+2
总　　计	+2

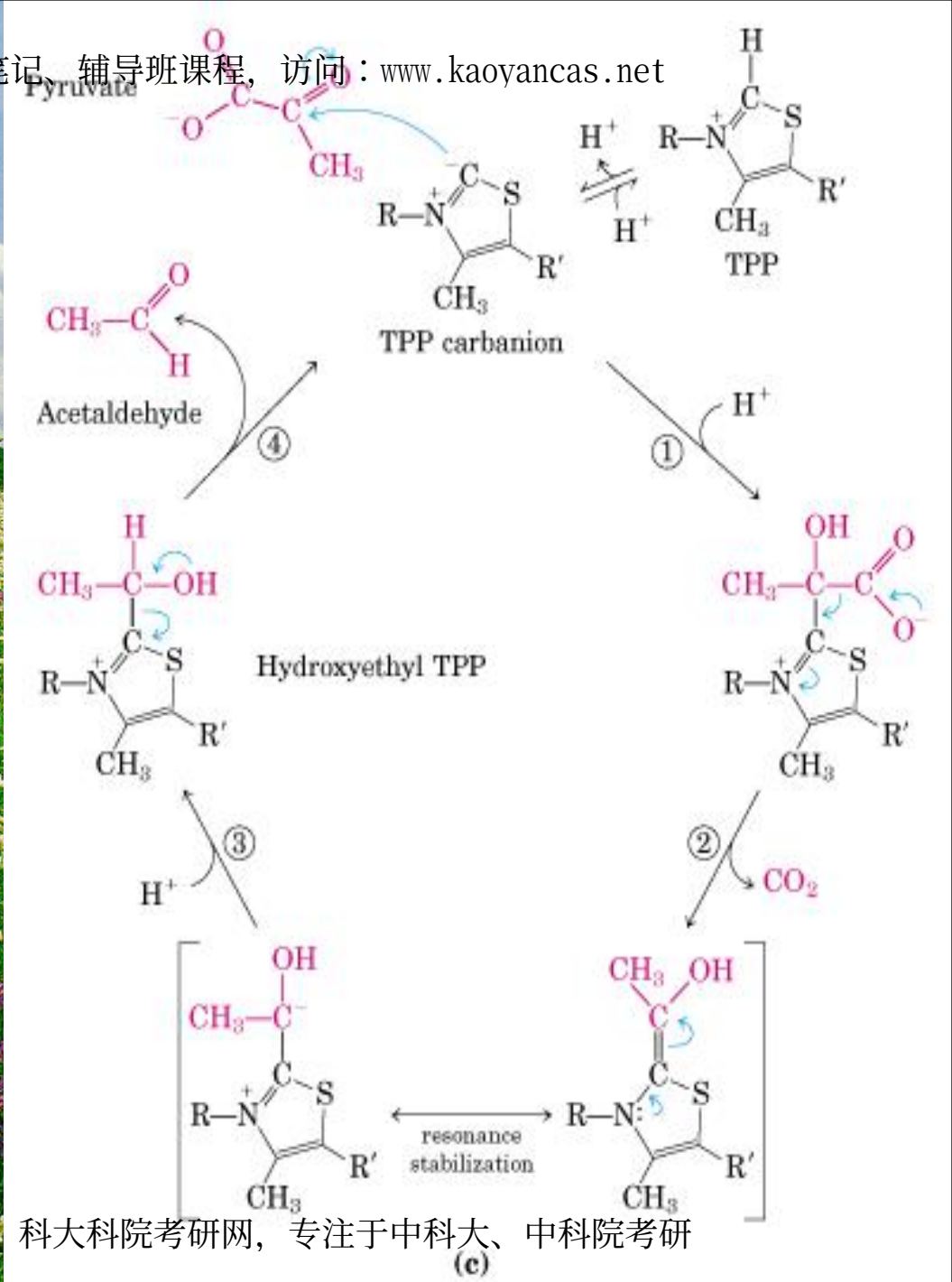
注：负号(－)代表消耗，正号代表产生。

七、丙酮酸的去路和NAD+ 的再生









九、其他六碳糖进入糖酵解途径

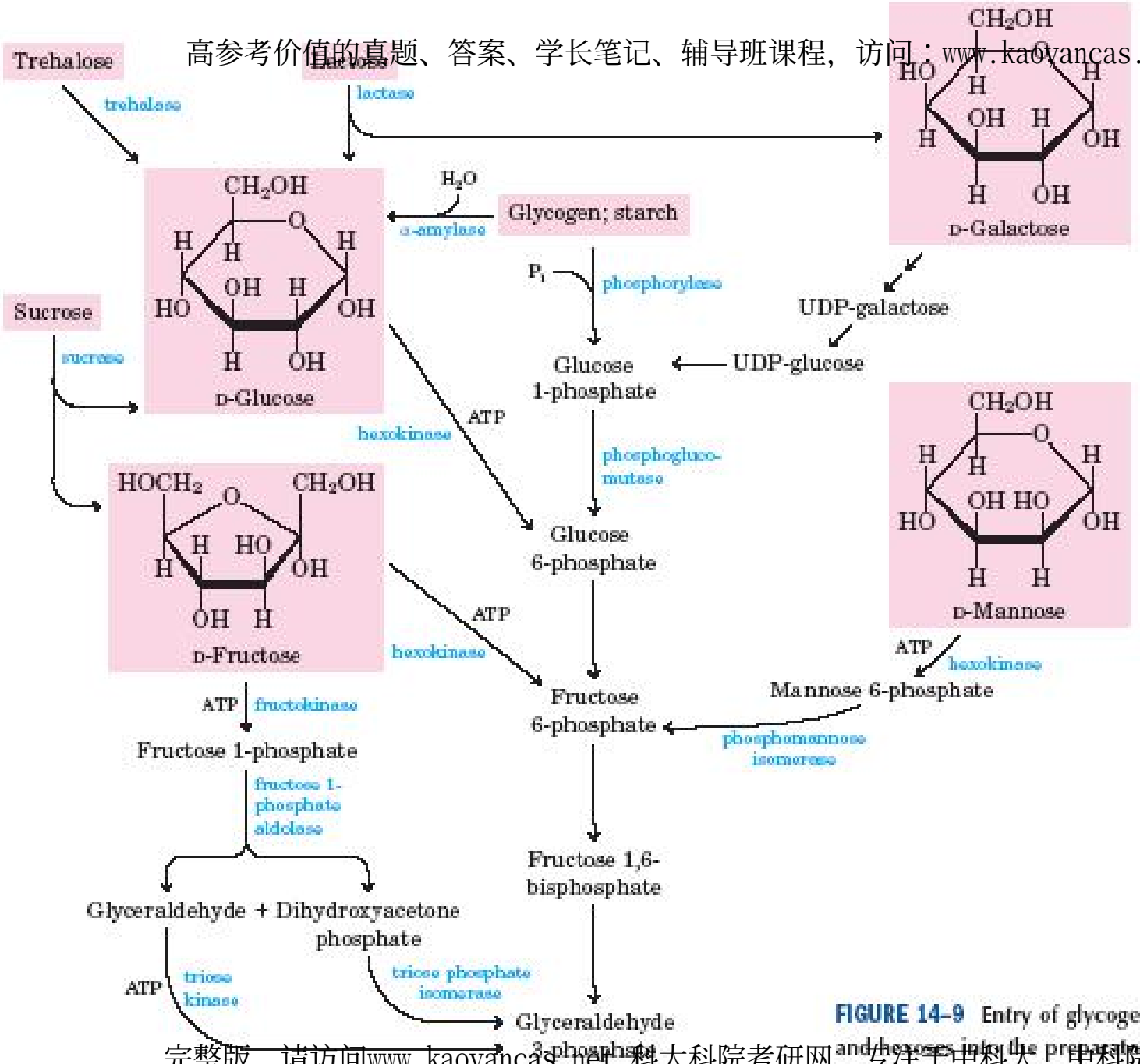
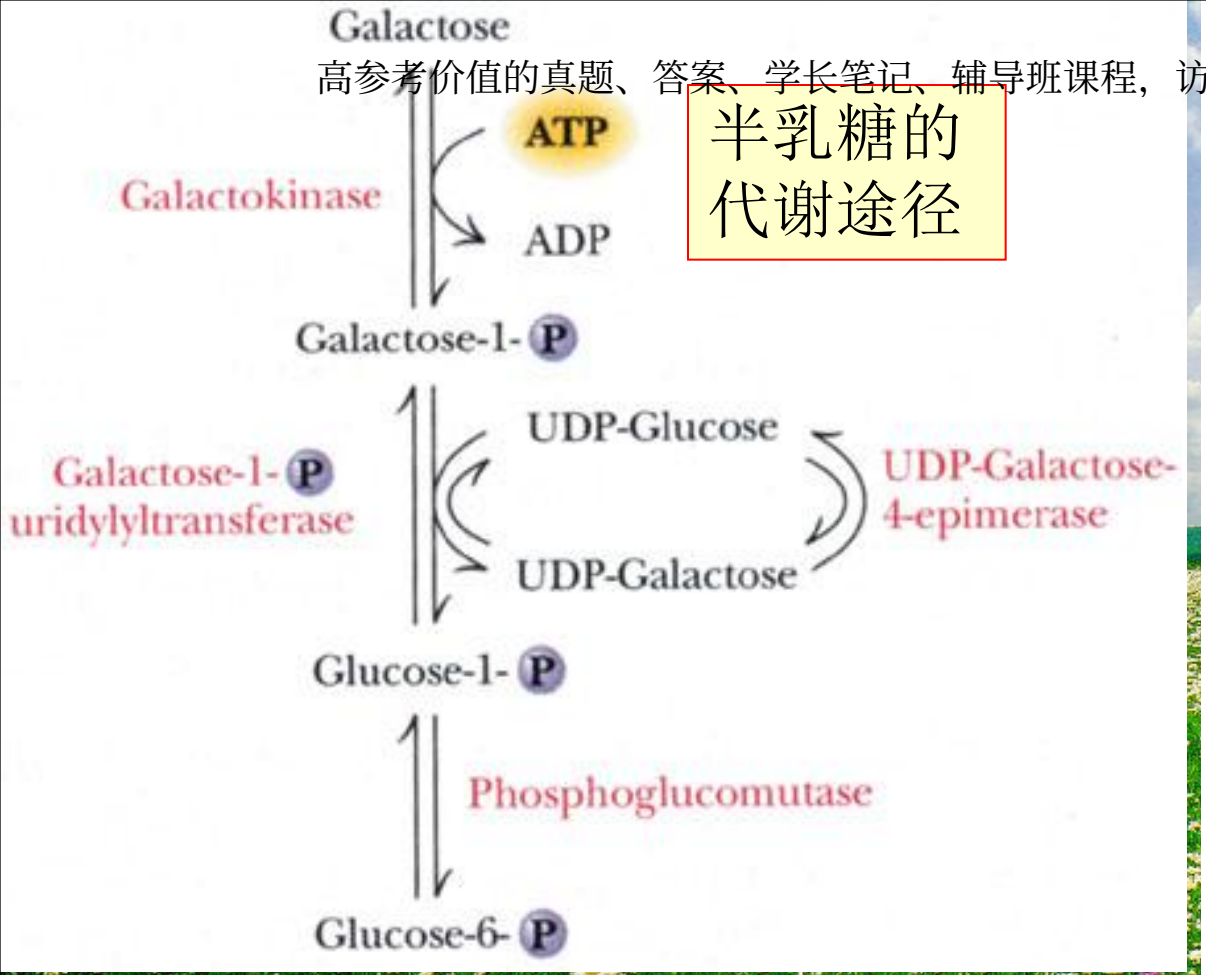
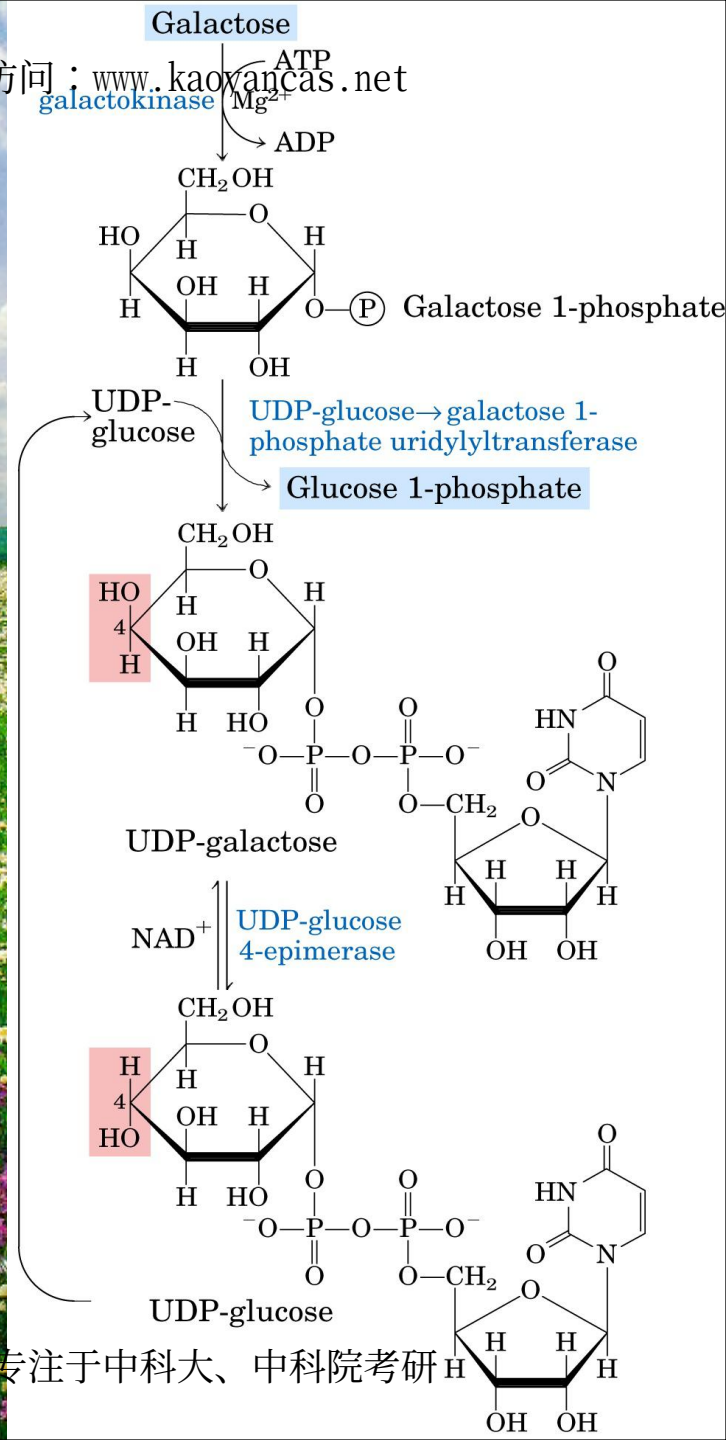


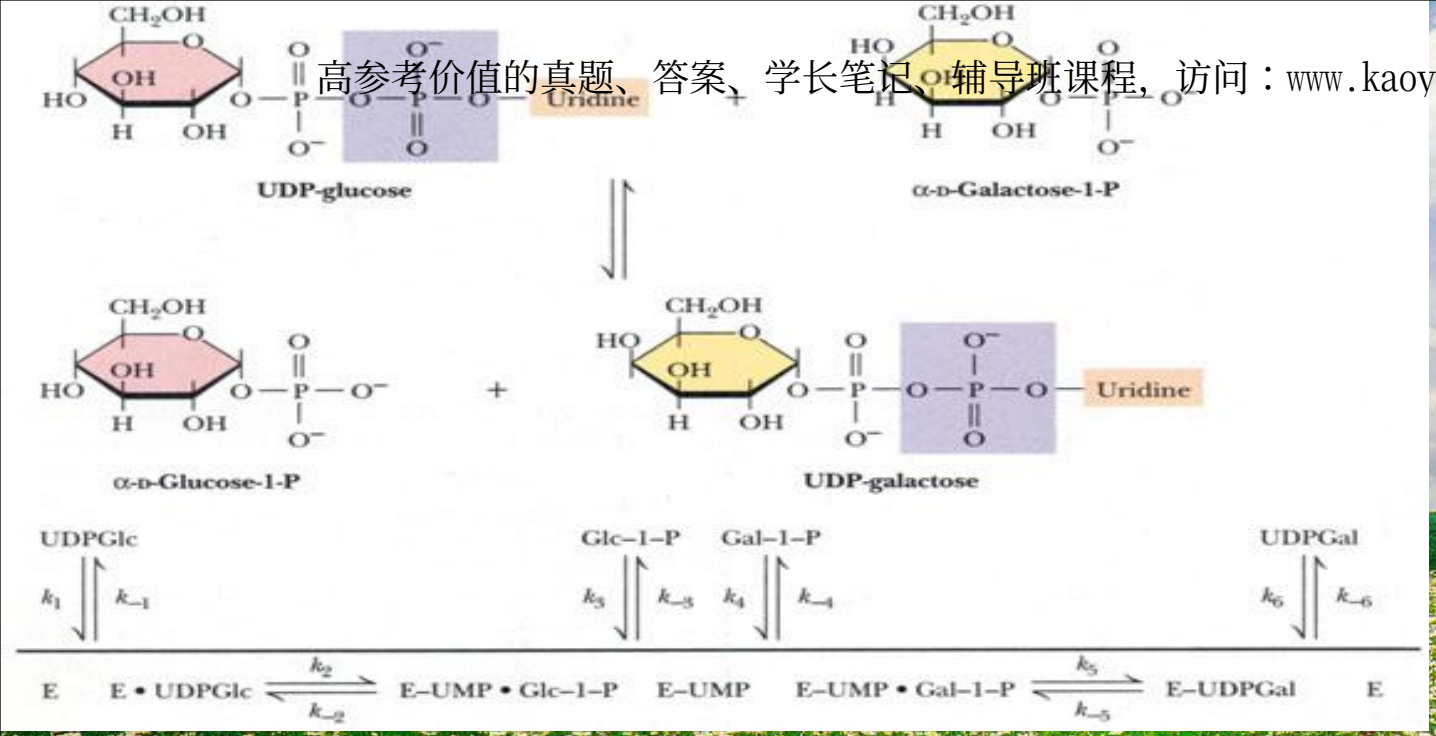
FIGURE 14–9 Entry of glycogen, starch, disaccharides, and hexoses into the preparatory stage of glycolysis.

半乳糖的代谢途径

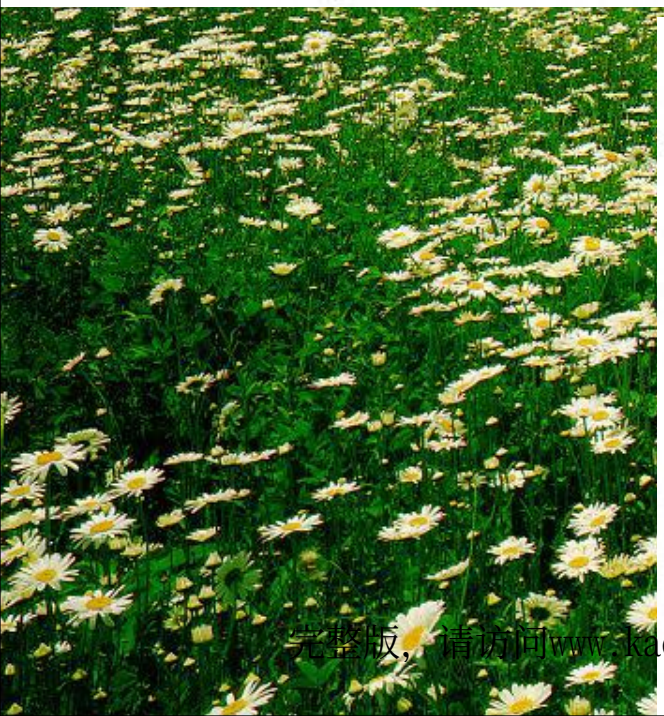


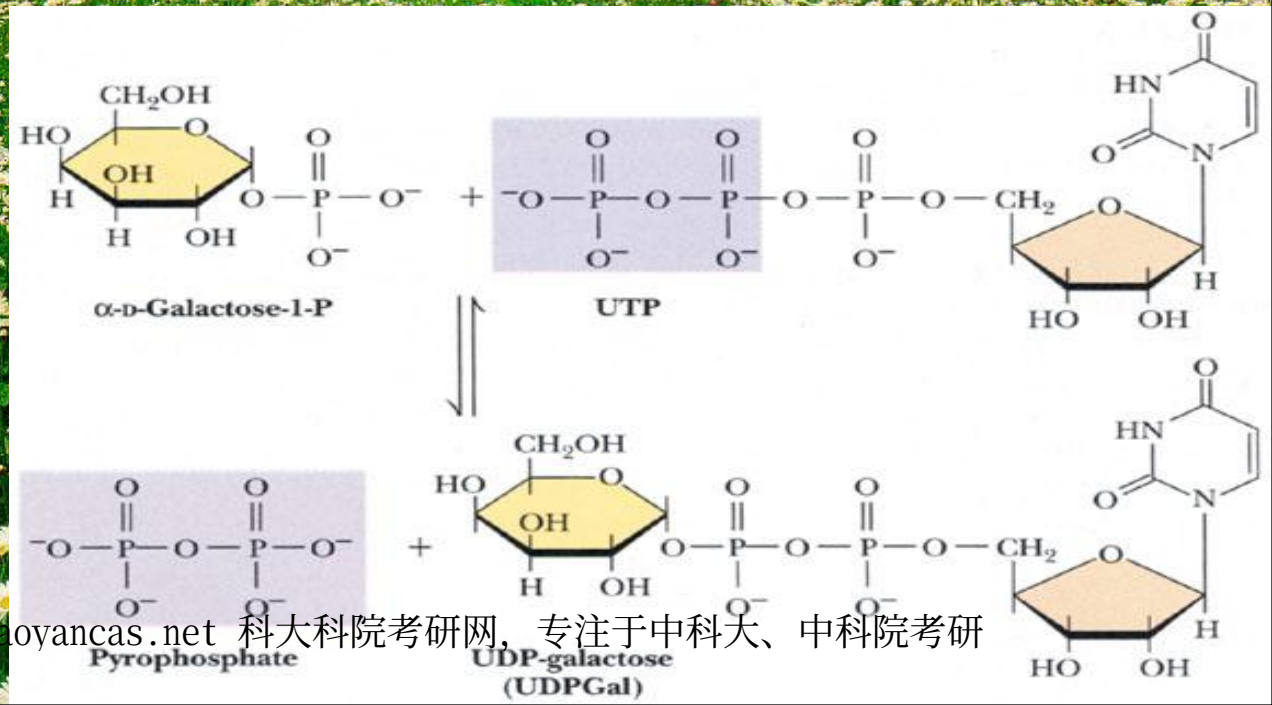
缺乏半乳糖-1-磷酸尿苷酰转移酶使晶状体半乳糖增高，引起白内障，严重时引起生长停滞，智力迟钝，甚至引起肝损伤导致死亡。





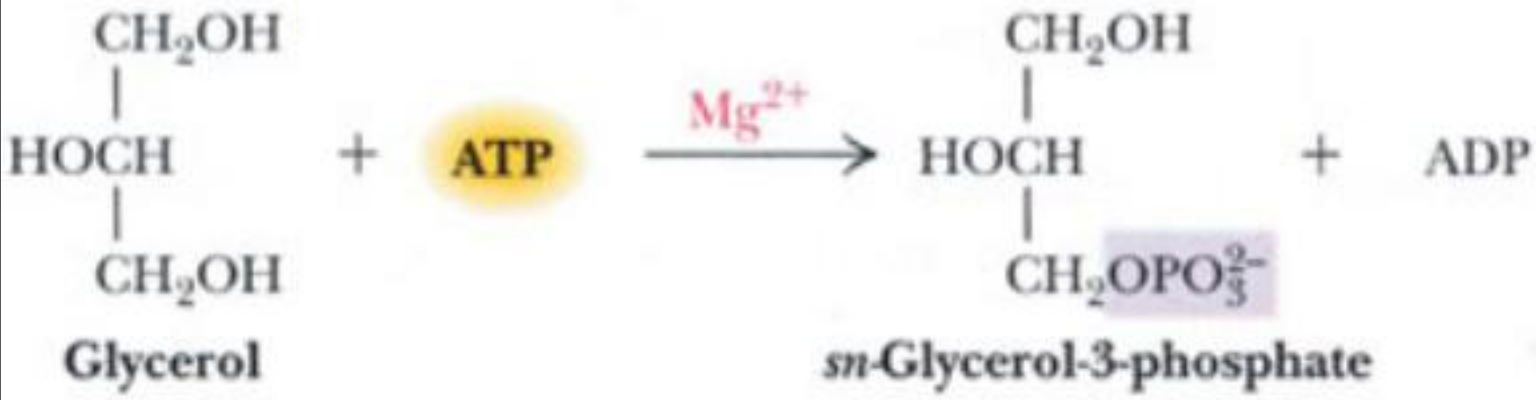
反应的乒乓动力学机制





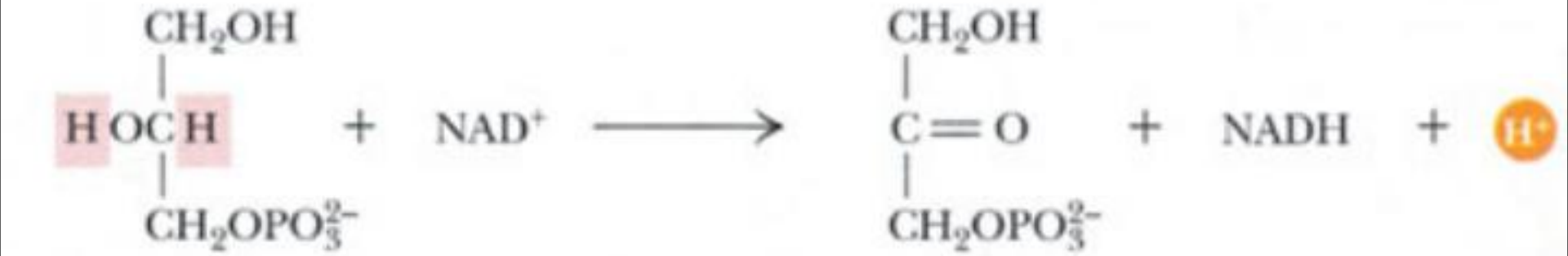
甘油分解的途径

The glycerol kinase reaction



Glycerol → ***sn*-Glycerol-3-phosphate**

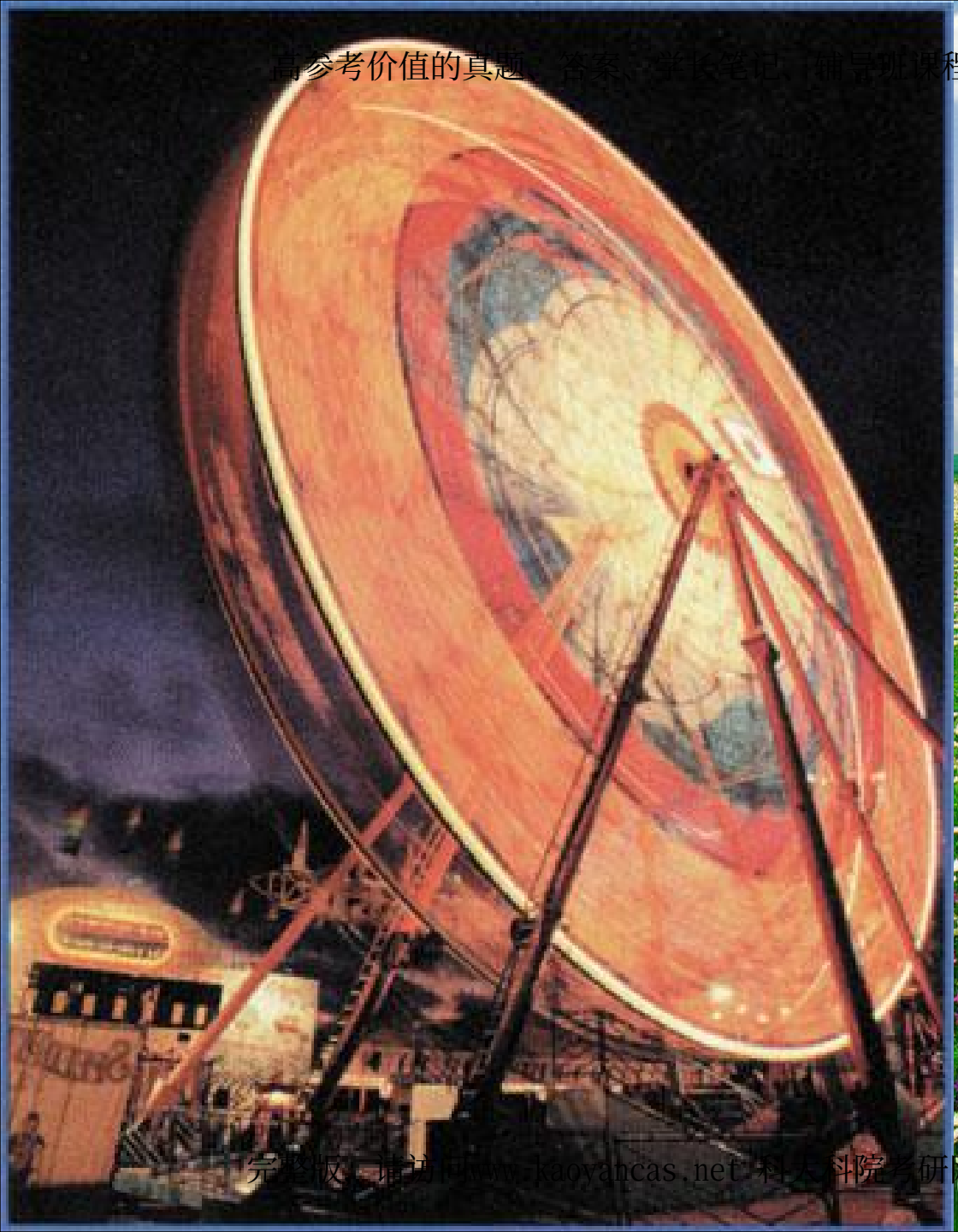
The glycerol phosphate dehydrogenase reaction



***sn*-Glycerol-3-phosphate** → **Dihydroxyacetone phosphate**

基本要求

1. 熟悉糖酵解作用的研究历史。
2. 掌握糖酵解过程的概况。（重点）
3. 熟悉糖酵解作用的反应过程。（难点）
4. 掌握糖酵解过程的能量计算。（重点）
5. 掌握丙酮酸的去路。（重点）
6. 掌握糖酵解作用的调节。（重点）
7. 熟悉其他六碳糖进入糖酵解的途径。



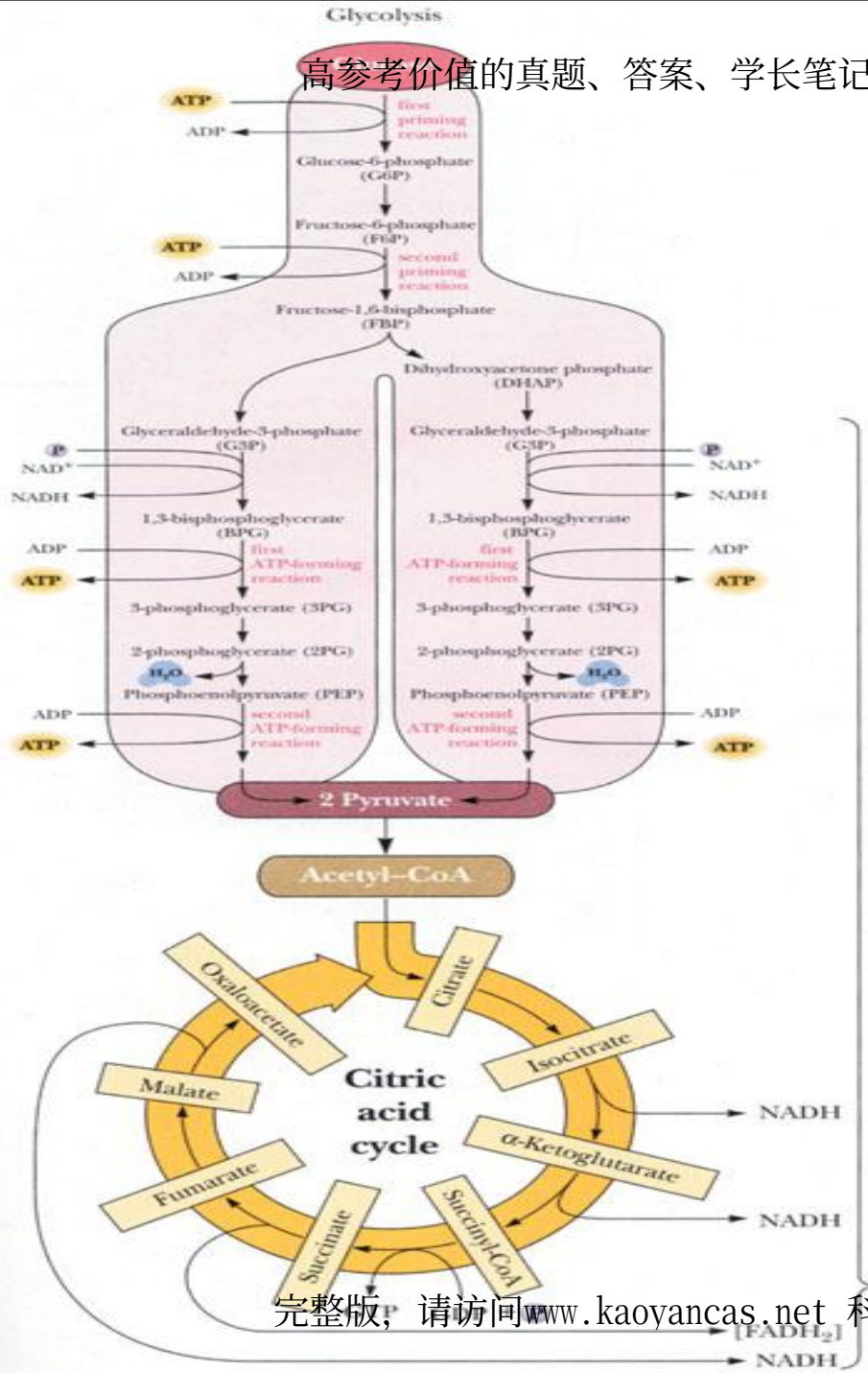
第23章

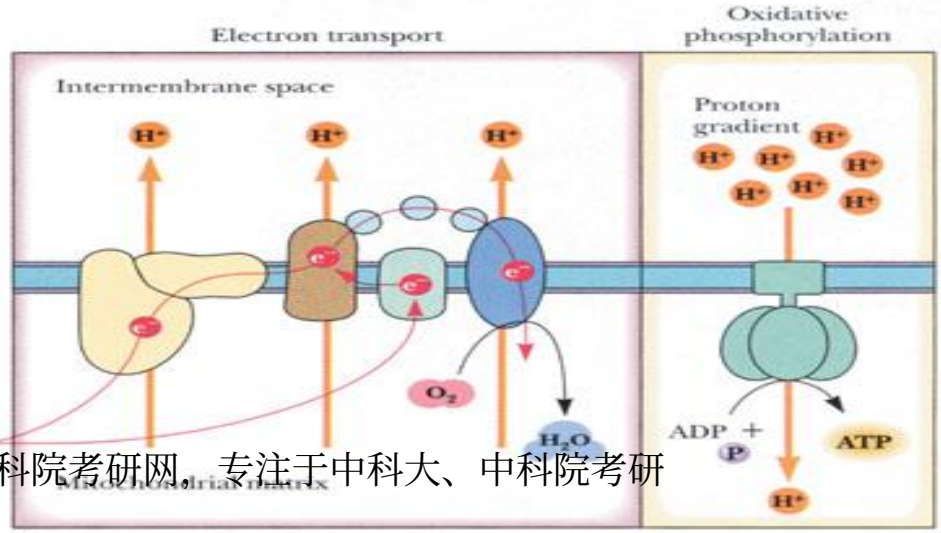
柠檬酸循环

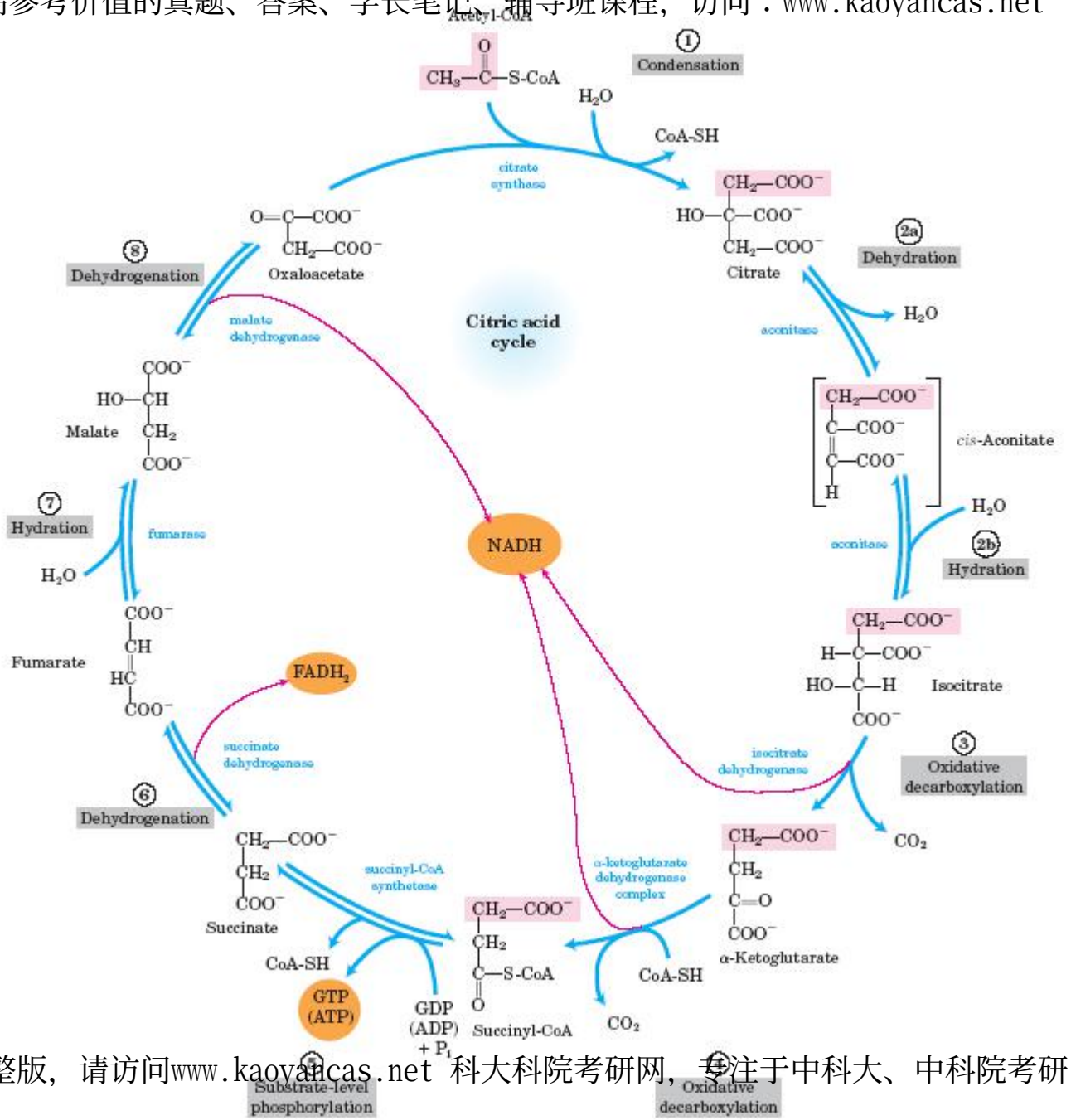
一、柠檬酸循环概貌

又称三羧酸循环

（Tricarboxylic acid cycle,TCA），**或Krebs循环，是物质代谢的枢纽。**

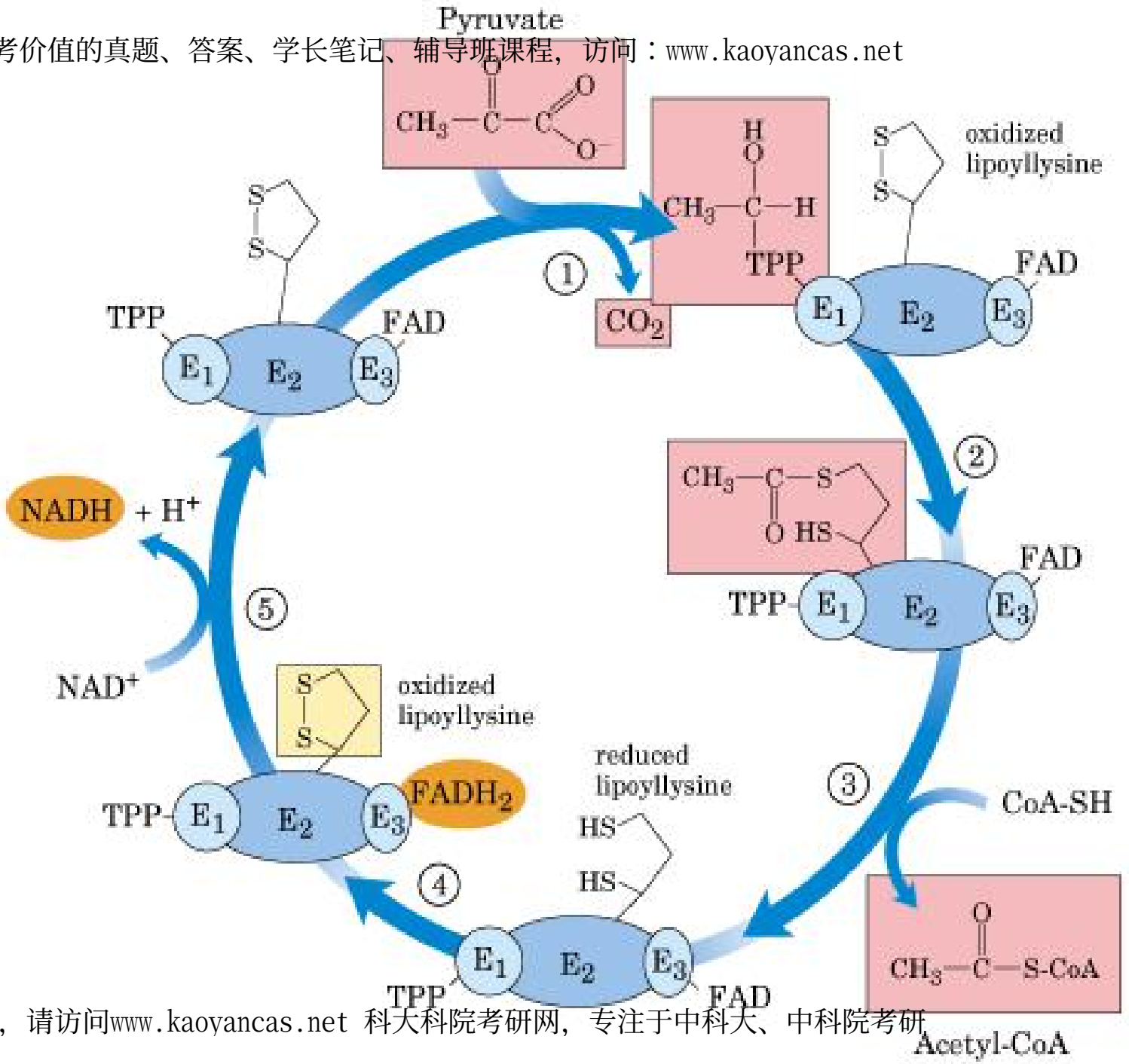




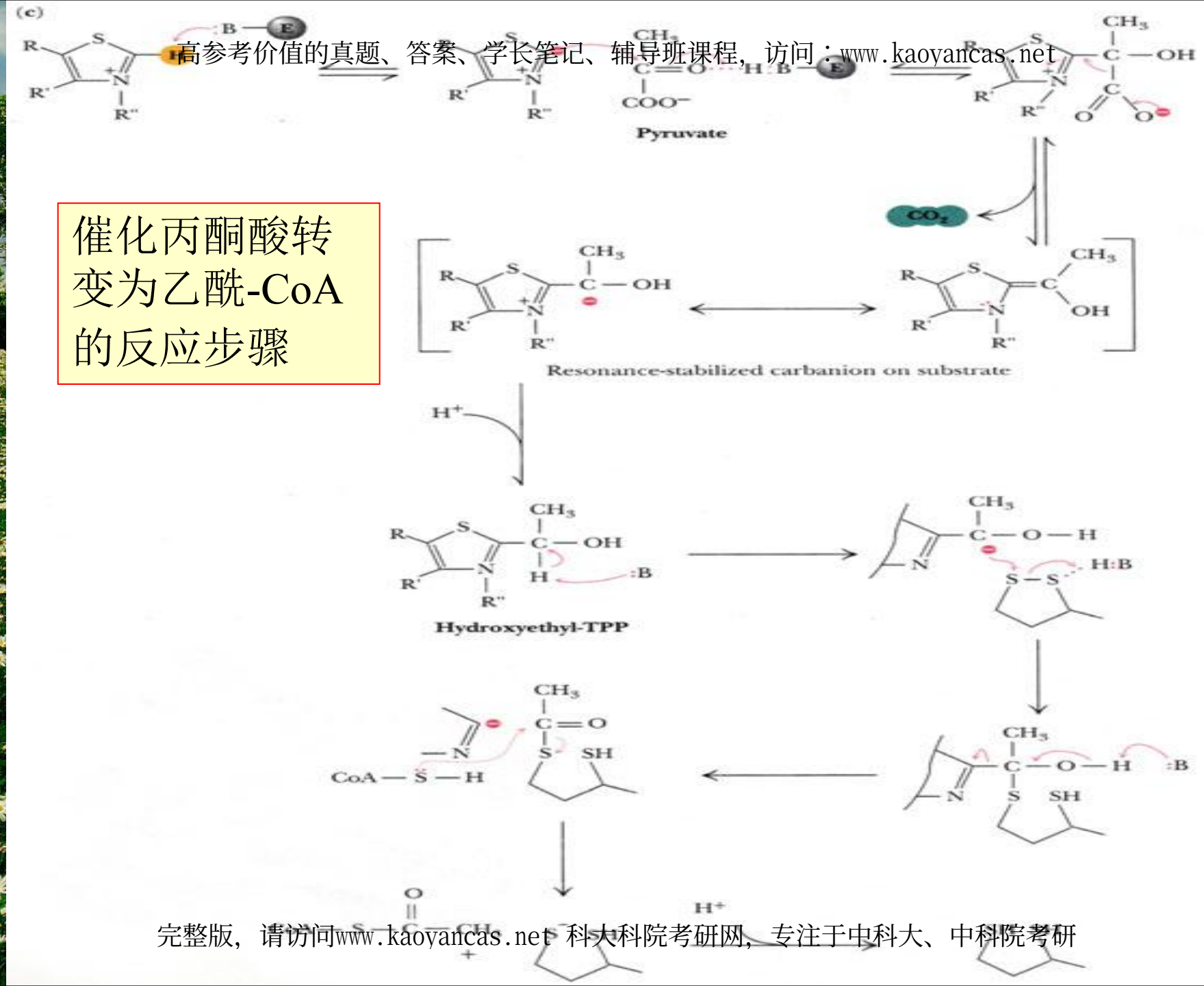


二、丙酮酸进入柠檬酸循环的准备阶段:形成乙酰-CoA

E_1：丙酮酸脱氢酶，E_2：二氢硫辛酸转乙酰基酶，E_3：二氢硫辛酸脱氢酶。有5种辅酶参与反应。

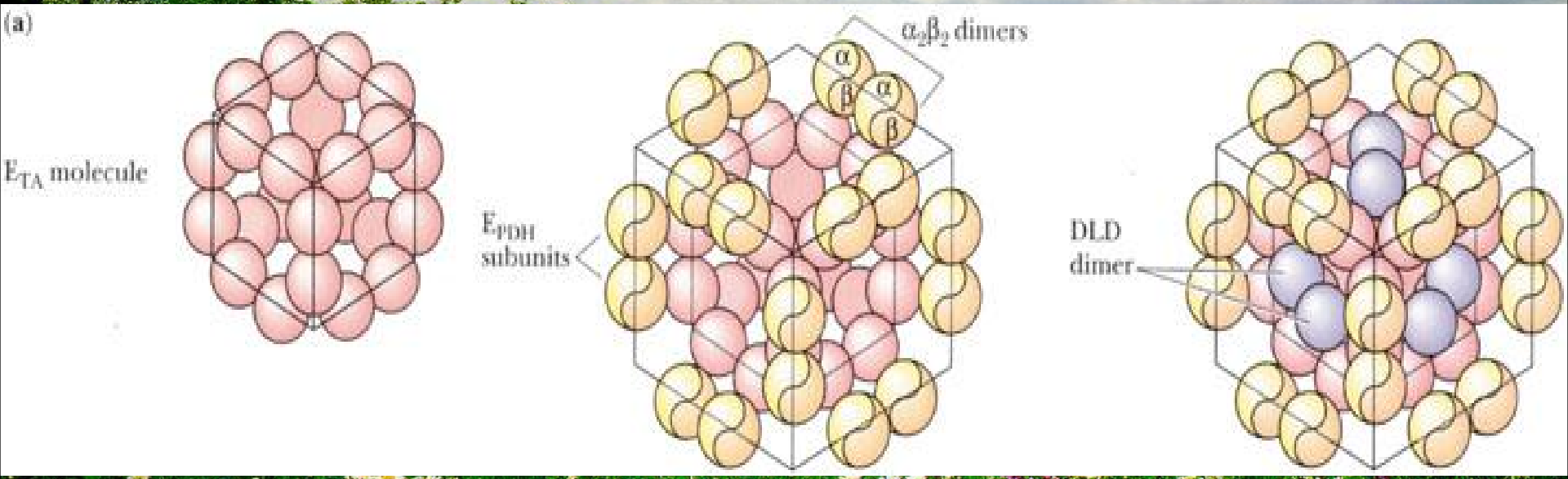


催化丙酮酸转变为乙酰-CoA的反应步骤



丙酮酸脱氢酶复合体的结构

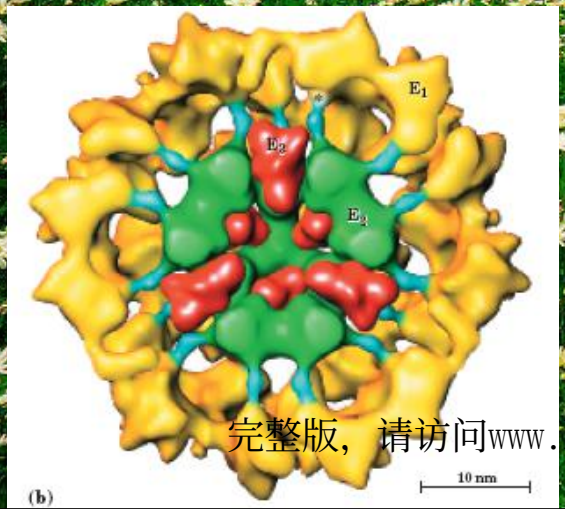
12个丙酮酸脱氢酶二聚体（PDH，E_1，黄色）构成立方体，8个二氢硫辛酸转乙酰基酶三聚体（TA，E_2，粉色）构成另一个立方体，6个二氢硫辛酸脱氢酶二聚体（DLD，E_3，蓝色）与上述两个立方体相互嵌合，构成一个由60个亚基组成的复杂的复合体。

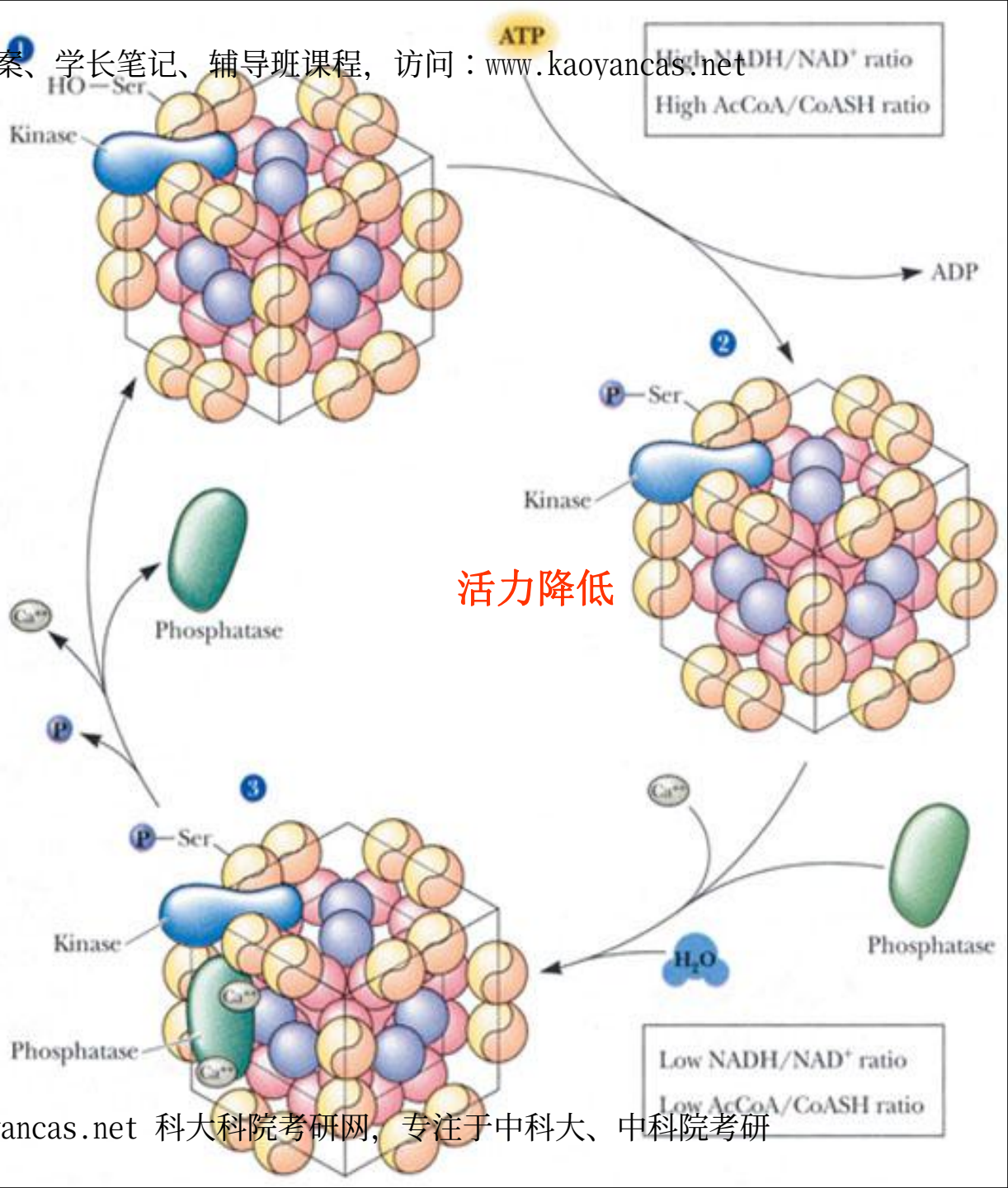


丙酮酸脱氢酶复合体的调控

E_1被磷酸化活力降低，脱磷酸活力增高，乙酰-CoA竞争性抑制E_2，NADH竞争性抑制E_3。

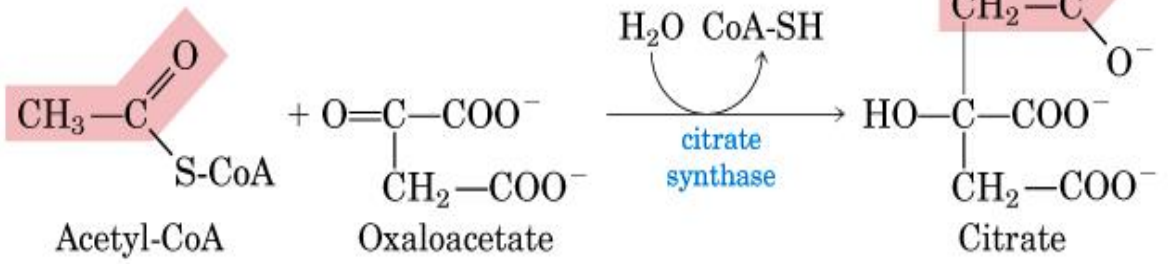
亚砷酸盐及有机砷化合物抑制作用的机制之一是使还原型硫辛酰胺形成无催化能力的砷化物。同样的机制还可抑制α-酮戊二酸脱氢酶复合体。





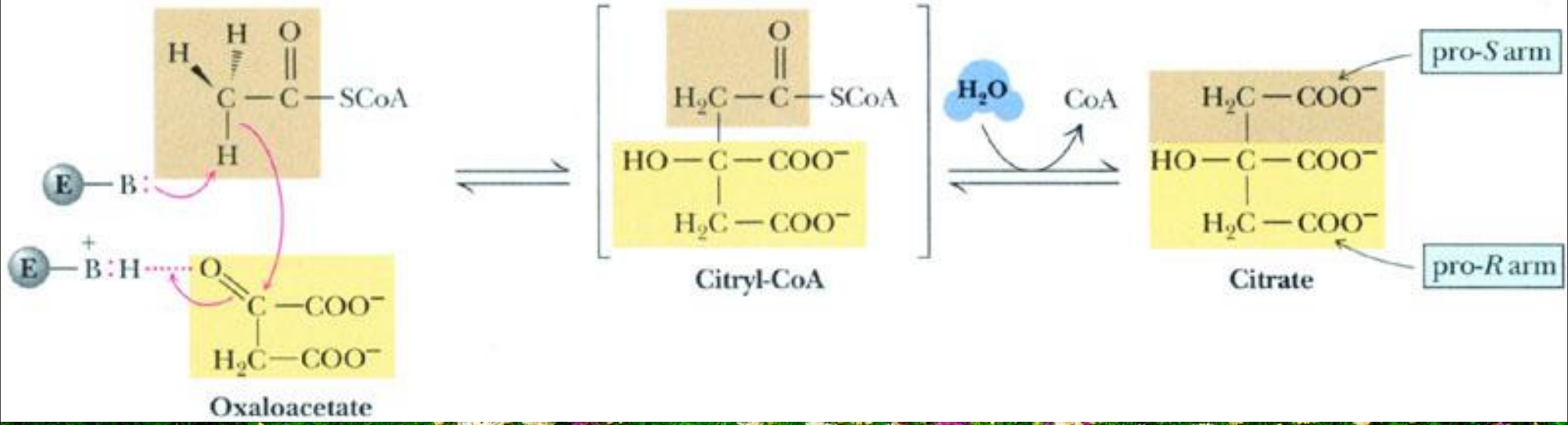
三、柠檬酸循环的反应机制

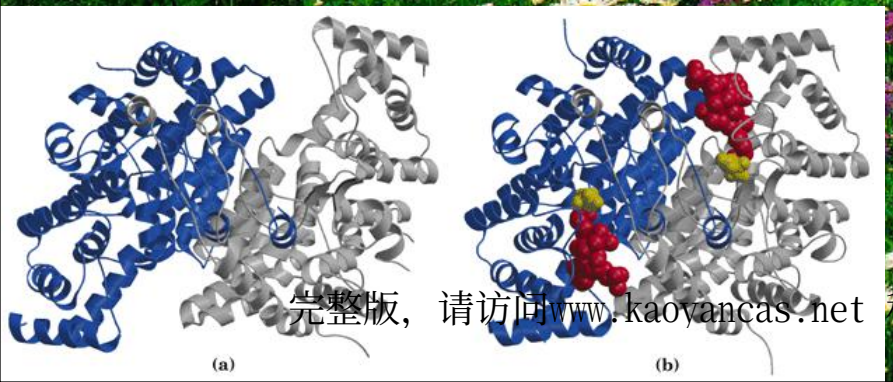
(一) 草酰乙酸(oxaloacetate)与乙酰-CoA缩合(condense)形成柠檬酸



反应不可逆.

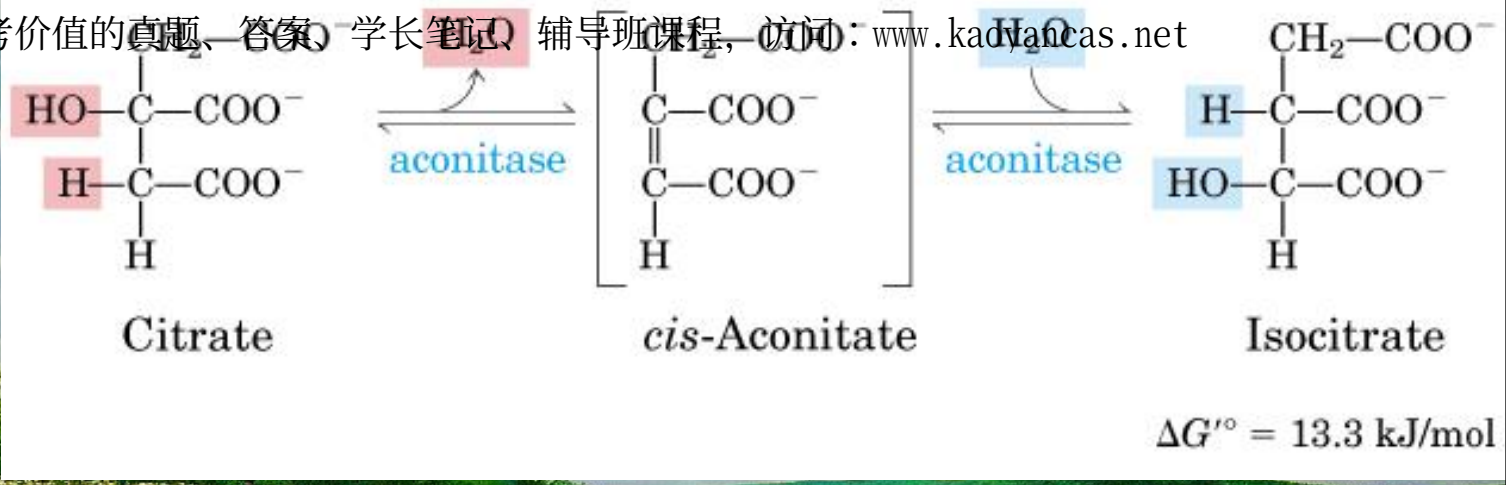
$\Delta G'^{\circ} = -32.2$ kJ/mol

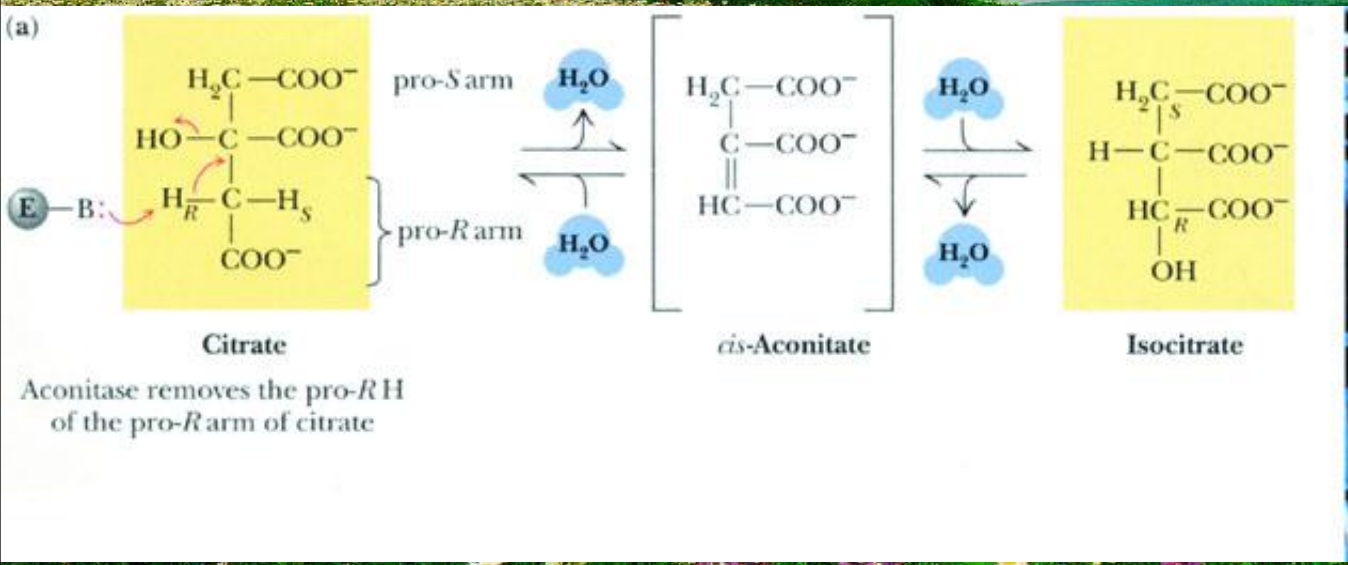




柠檬酸合酶为二聚体，图中所示为亚基的结构。草酰乙酸为黄色，CoA为红色。ATP，NADH，琥珀酰- CoA，酯酰-CoA抑制该酶的活性。

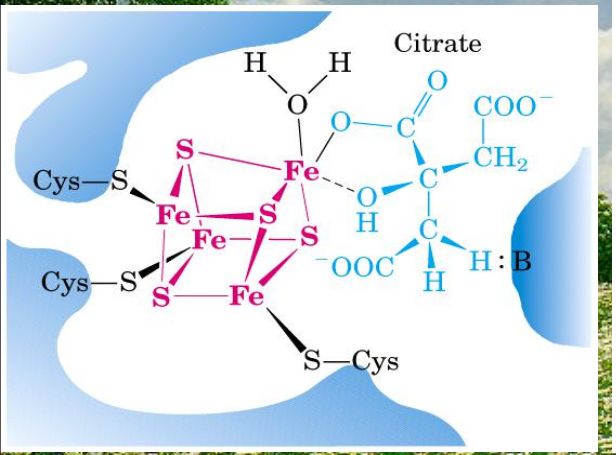
(二) 柠檬酸异构化形成异柠檬酸

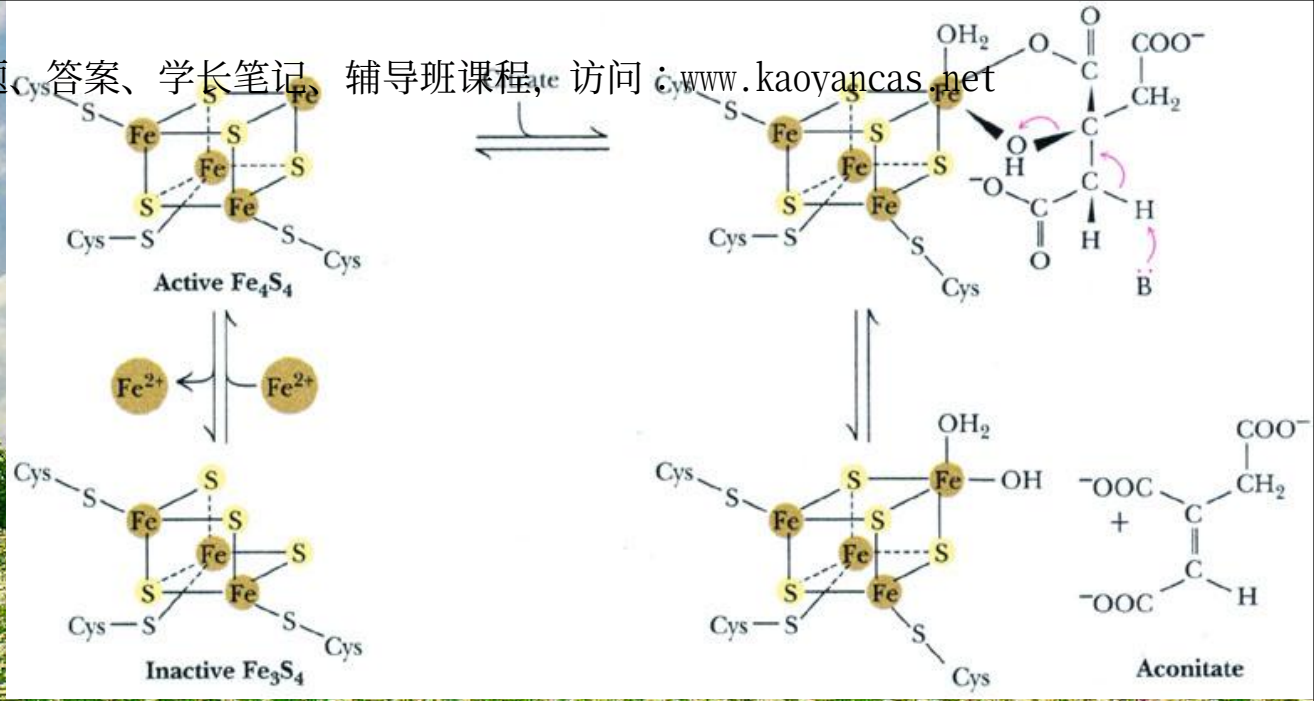




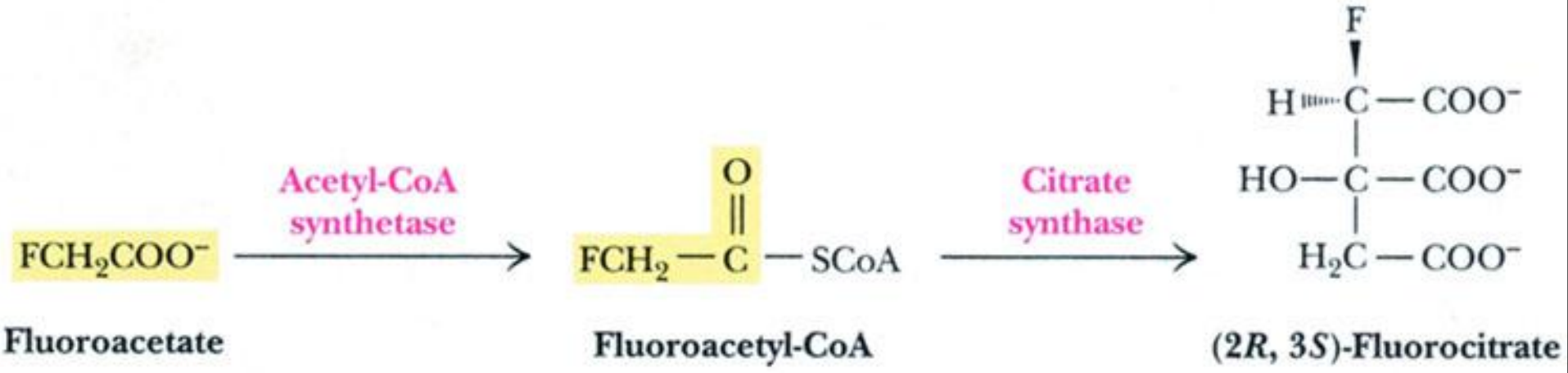
乌头酸酶为二聚体，图中所示为活性部位的结构。铁硫串为红色，半胱氨酸为黄色，异柠檬酸为白色。

乌头酸酶的铁-硫中心在反应中的作用

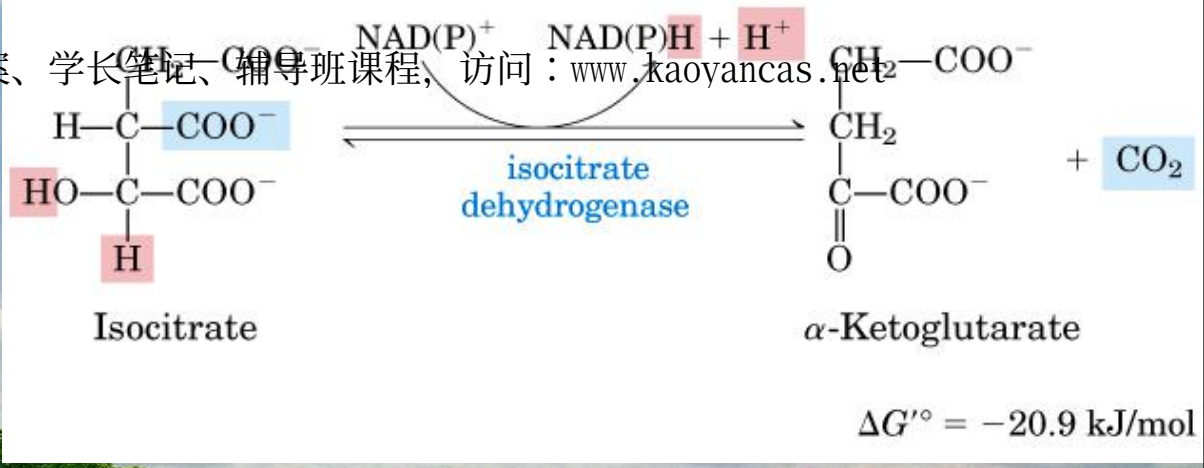




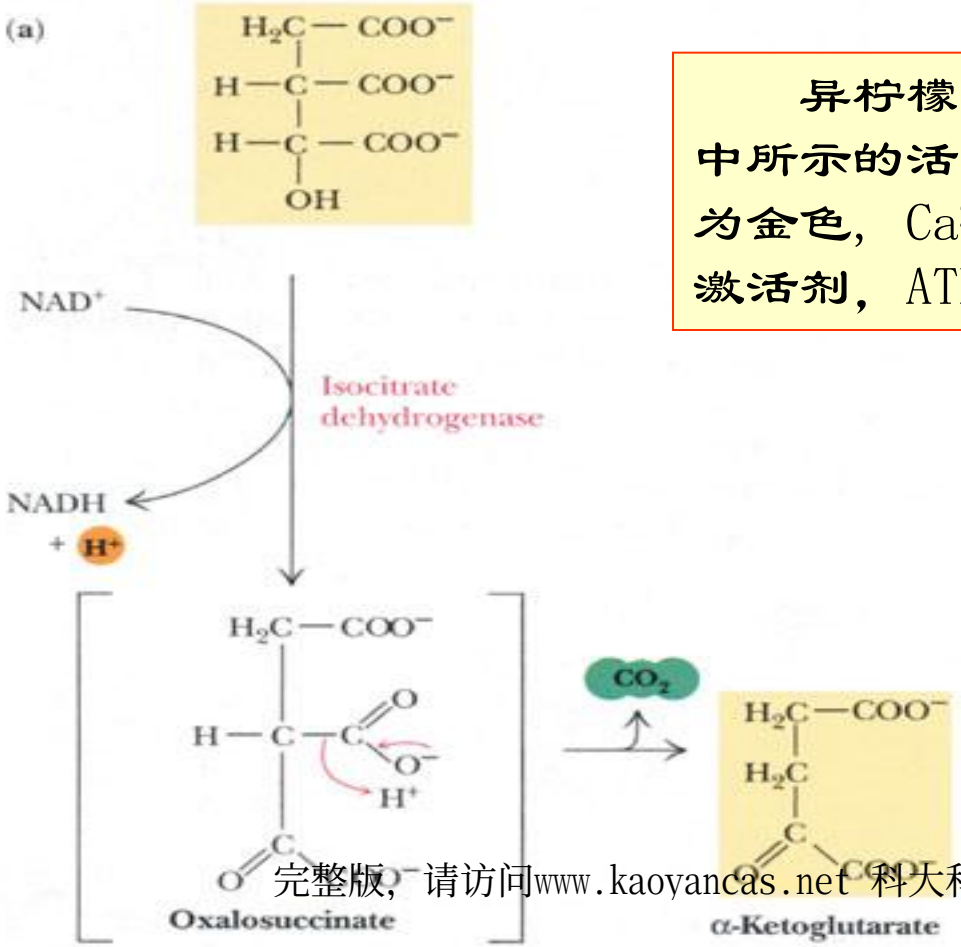
氟乙酸形成的氟柠檬酸对乌头酸酶有抑制作用

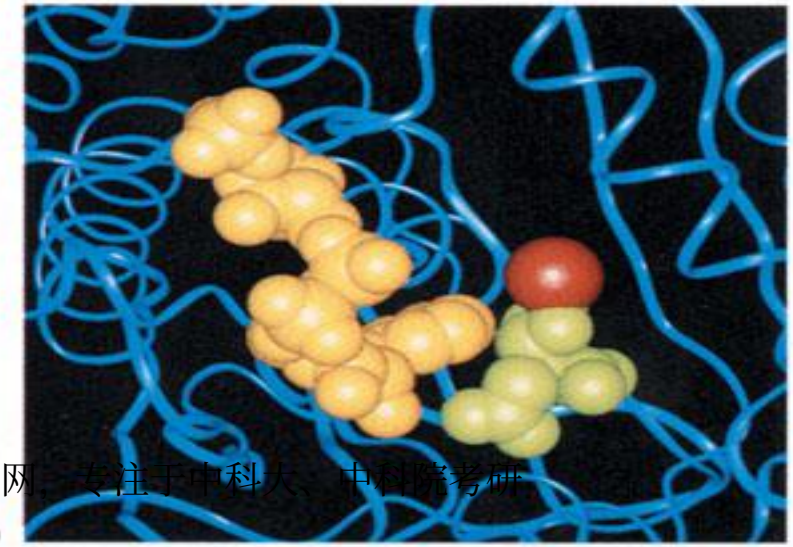


(三) 异柠檬酸氧化形成α-酮戊二酸

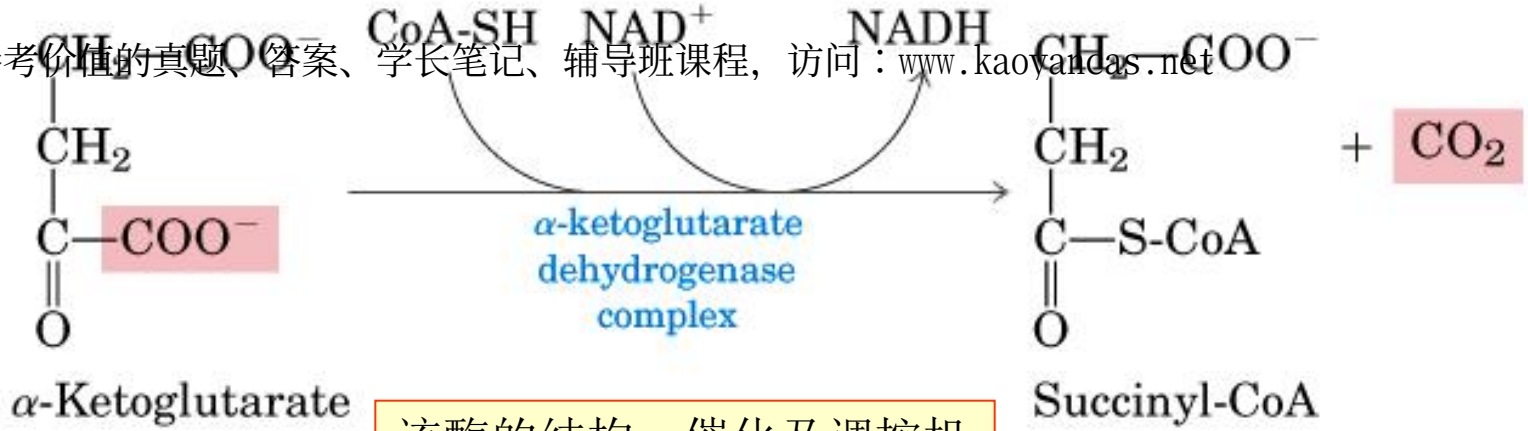


异柠檬酸脱氢酶为四聚体（$\alpha_2\beta\gamma$），图中所示的活性部位。异柠檬酸为绿色，$NADP^+$为金色，Ca^{2+}为红色。ADP，NAD是该酶的别构激活剂，ATP，NADH是该酶的别构抑制剂。





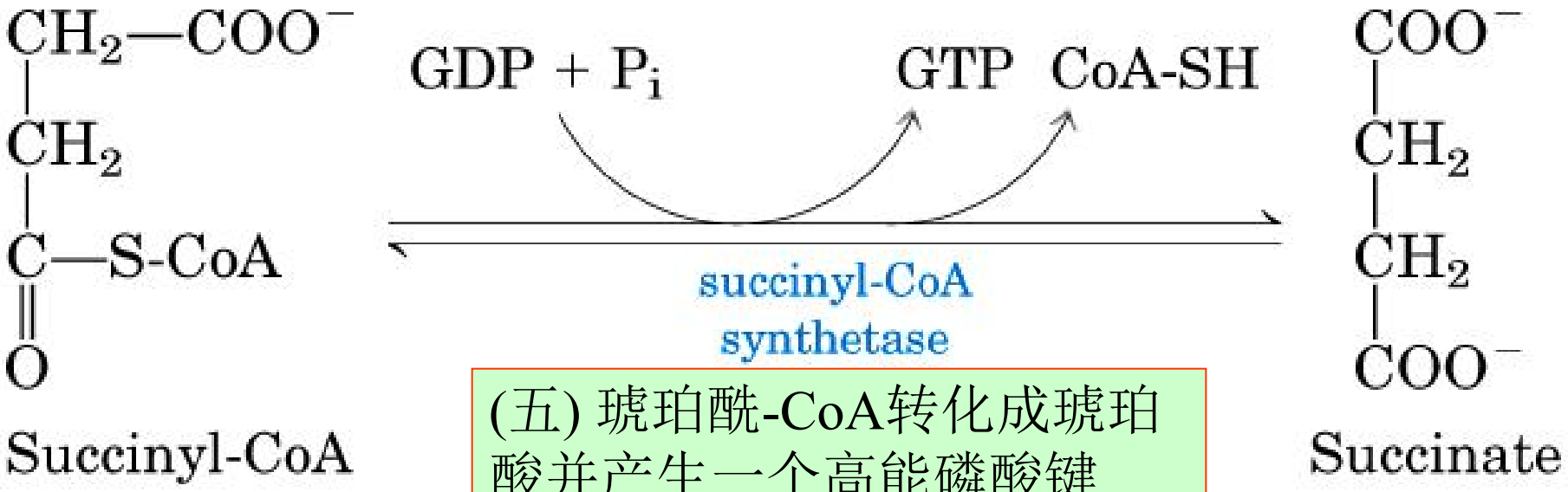
(四) α-酮戊二酸氧化脱羧形成琥珀酰-CoA



该酶的结构，催化及调控机制与丙酮酸脱氢酶相似。

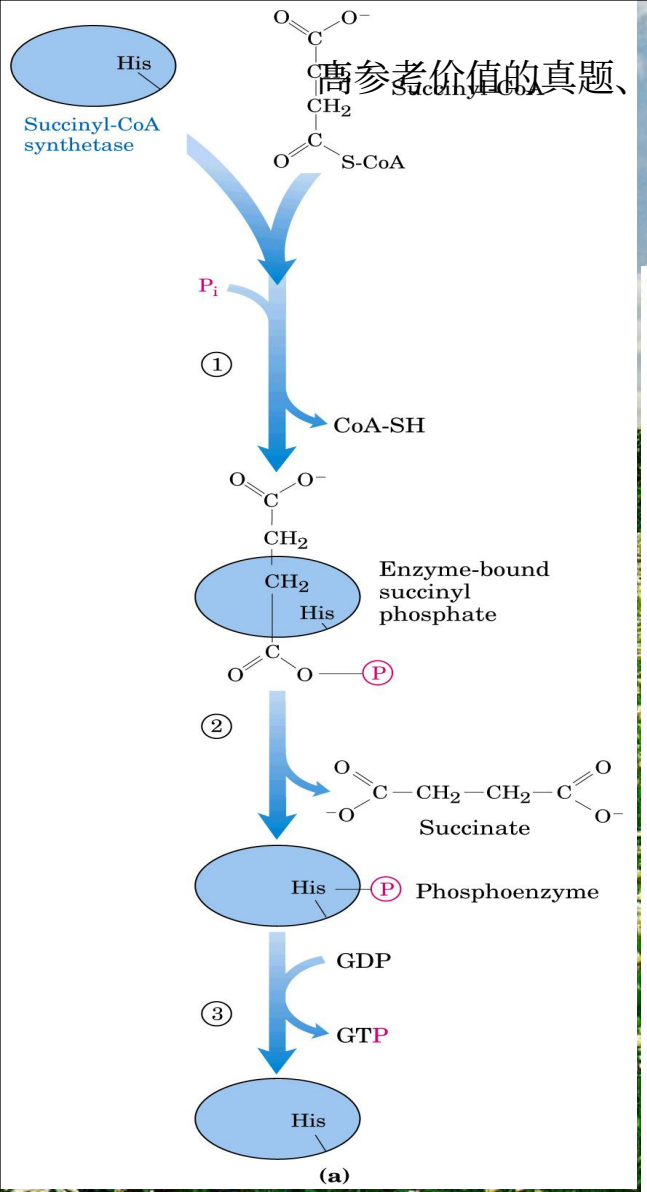
$\Delta G'^{\circ} = -33.5$ kJ/mol

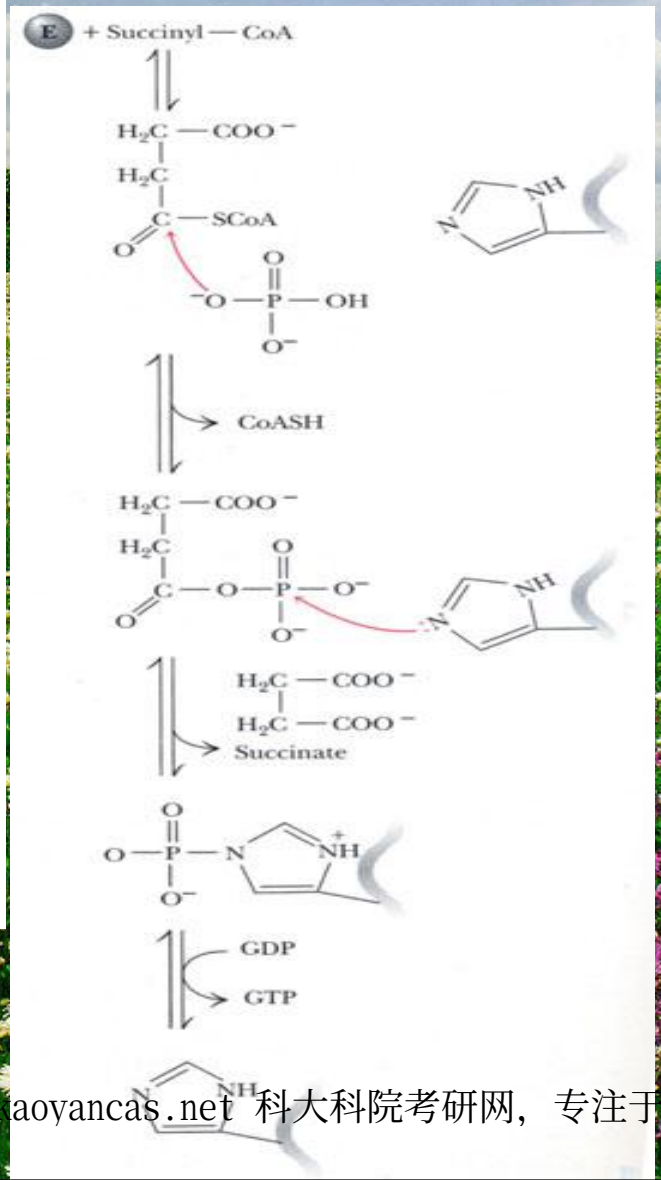
(五) 琥珀酰-CoA转化成琥珀酸并产生一个高能磷酸键

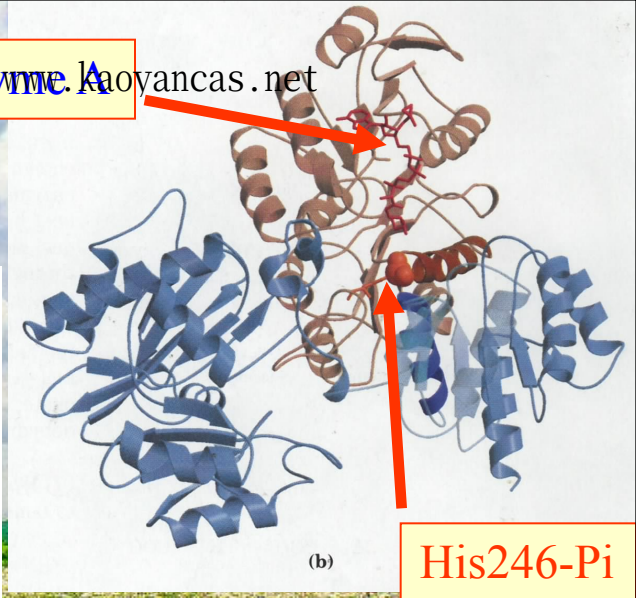


琥珀酰-CoA合成酶为 α β 二聚体，反应的 $\Delta G'^{\circ}$ 为0，反应可逆

$\Delta G' = -2.9$ kJ/mol

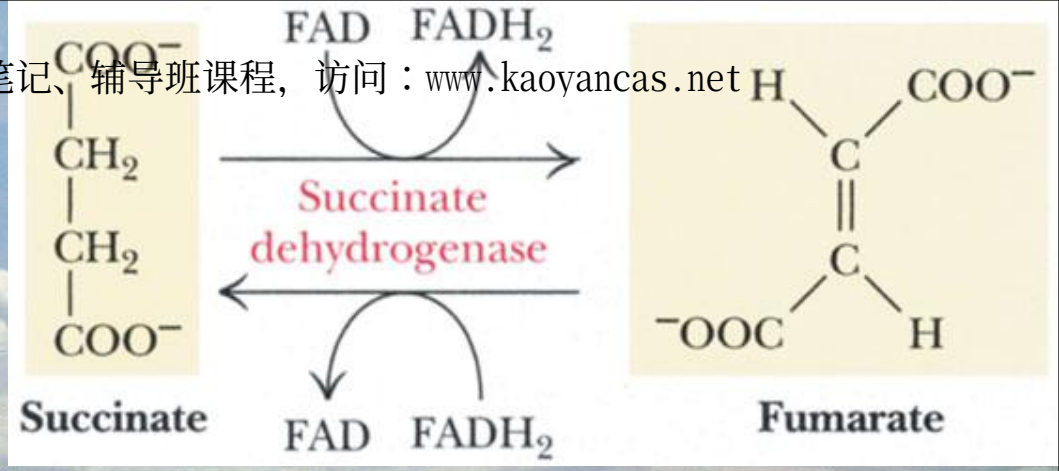


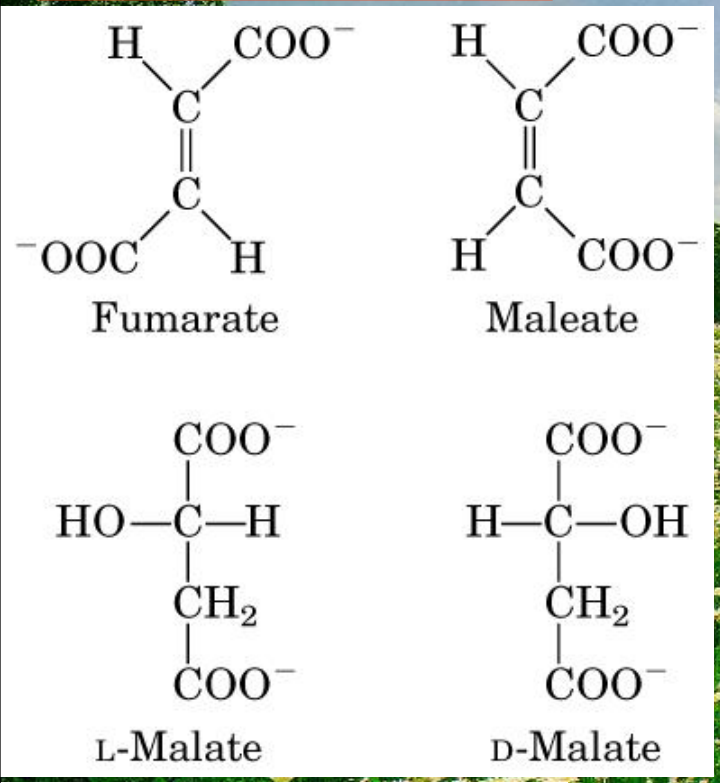


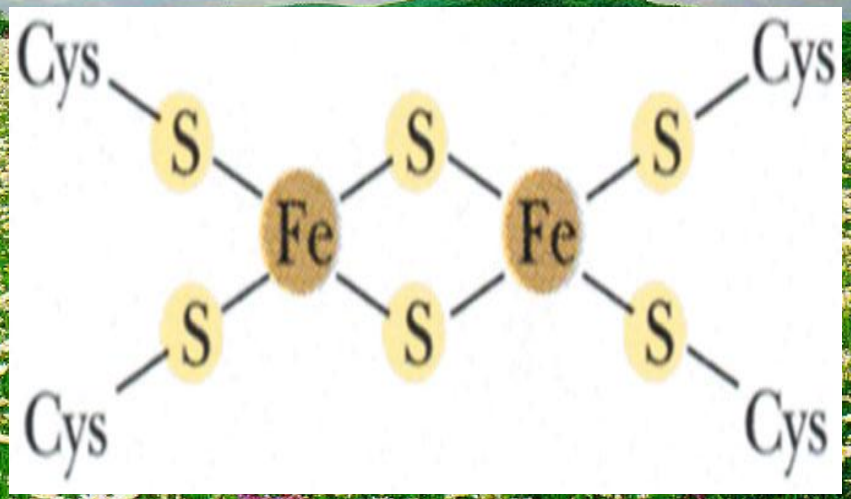


Succinyl-CoA Synthetase from *E. coli*

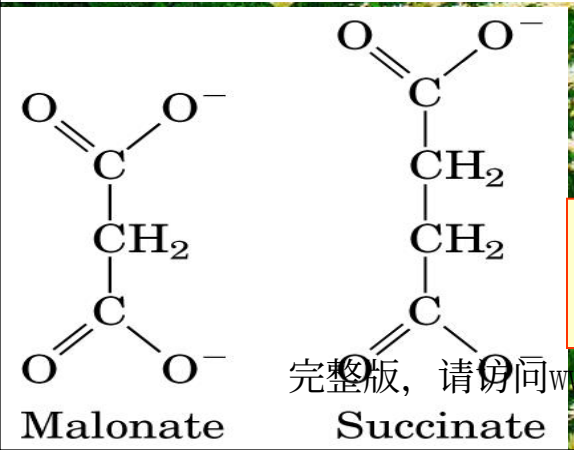
(六) 琥珀酸脱氢形成延胡索酸





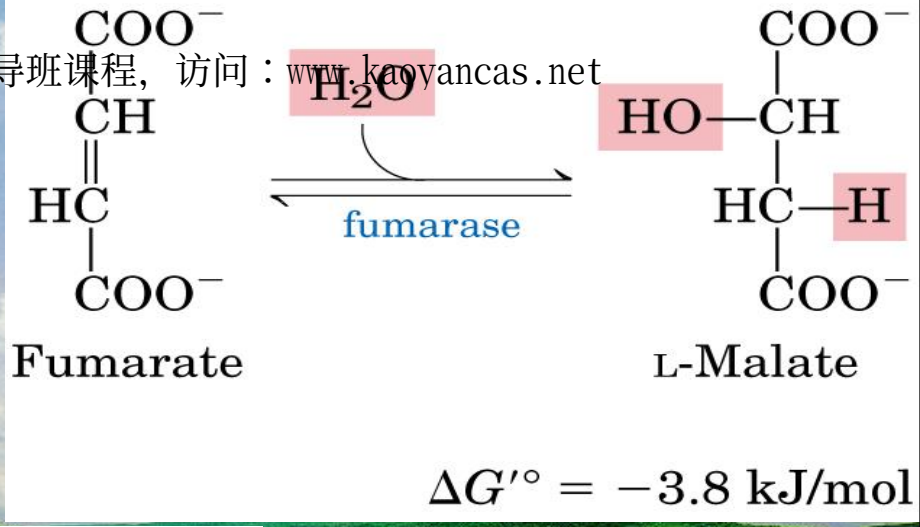


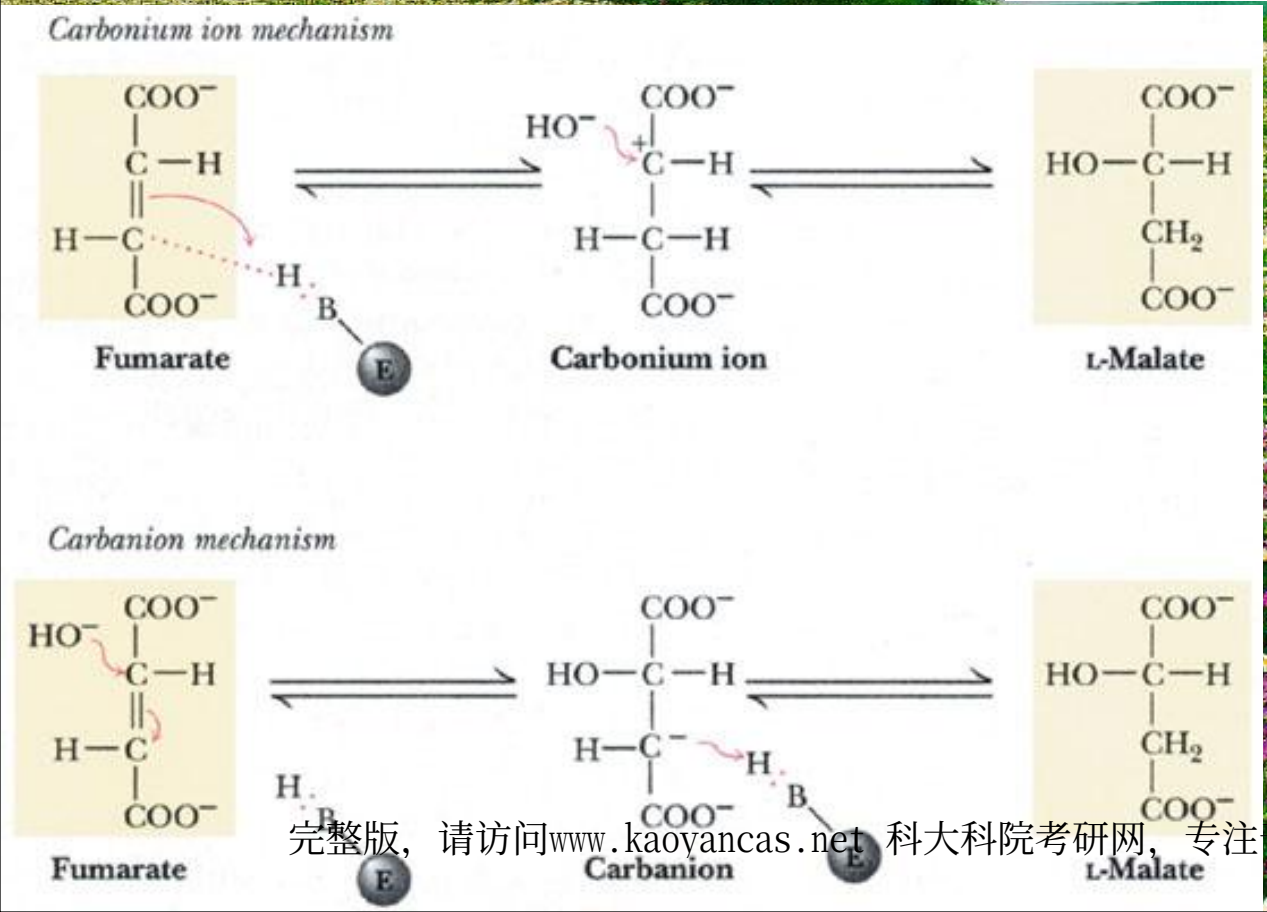
琥珀酸脱氢酶为αβ二聚体，活性部位有铁硫串。

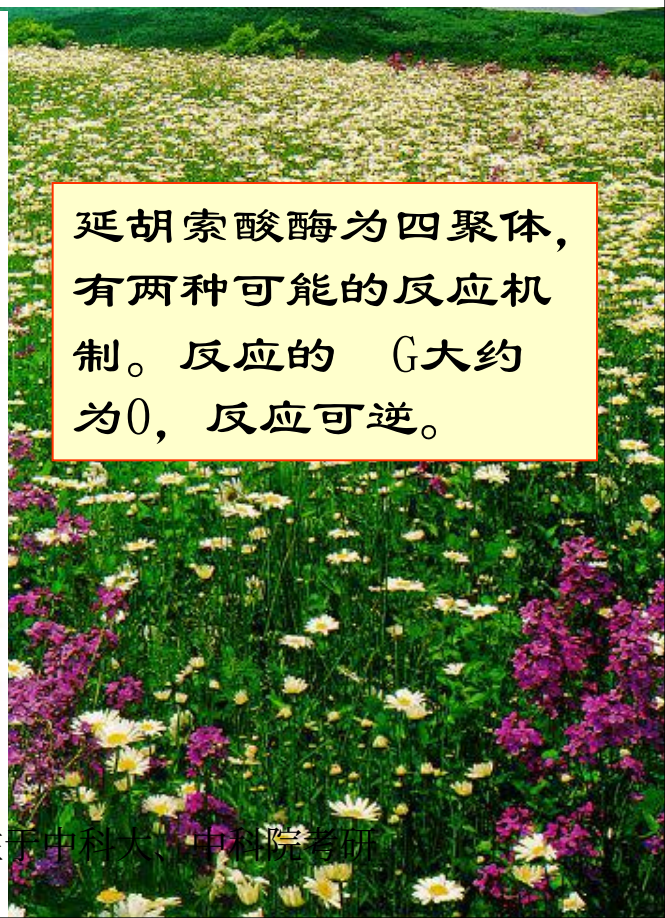


琥珀酸脱氢的抑制剂

(七) 延胡索酸水合形成L-苹果酸

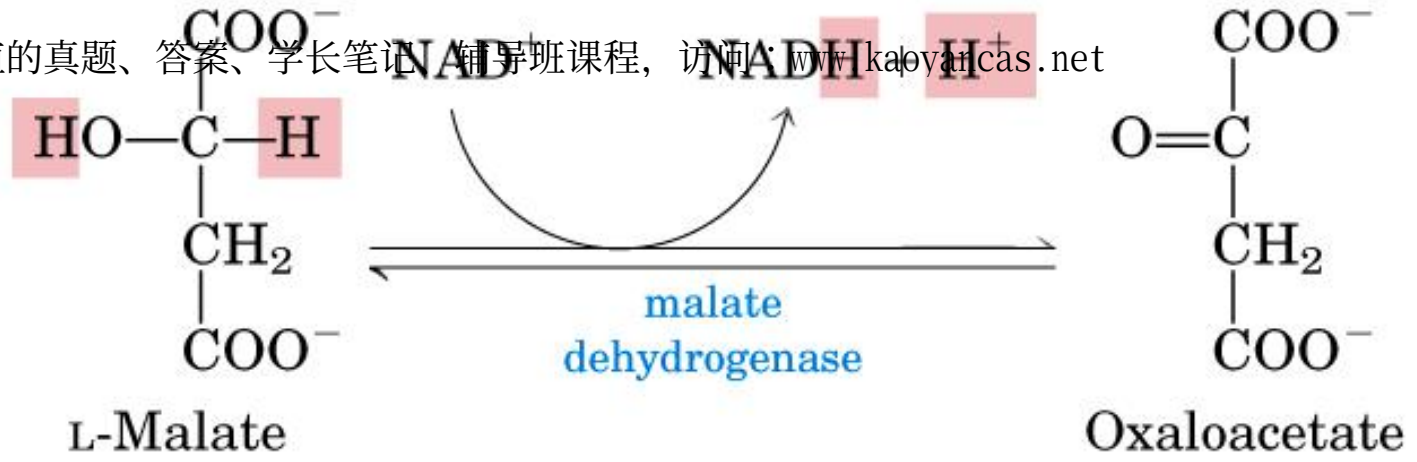






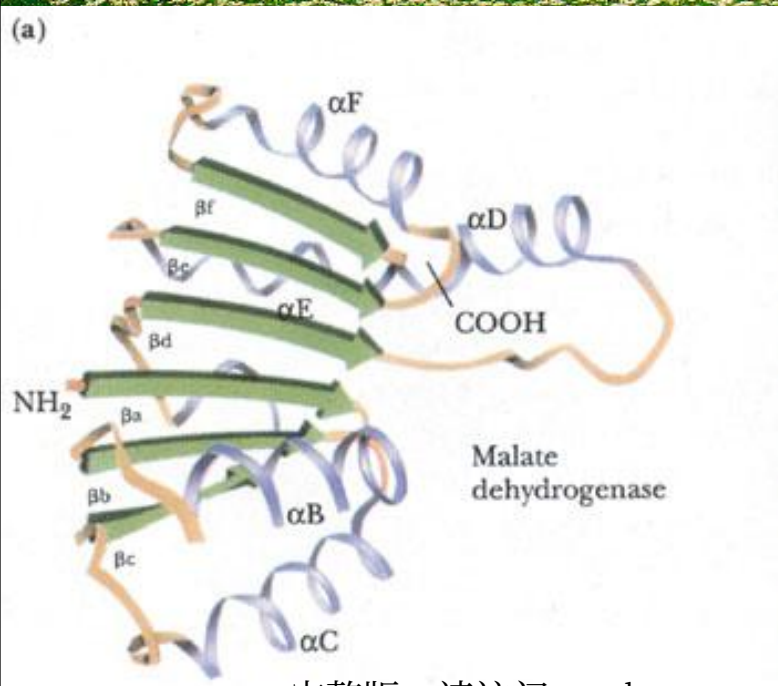
延胡索酸酶为四聚体，有两种可能的反应机制。反应的 G大约为0，反应可逆。

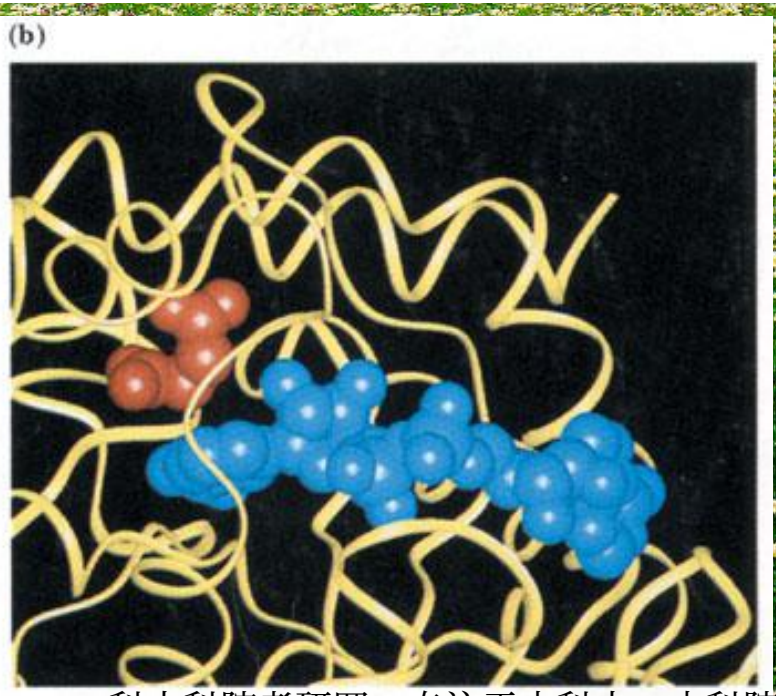
(八) L-苹果酸脱氢形成草酰乙酸



苹果酸脱氢酶为二聚体，反应的 G大约为0，反应可逆。

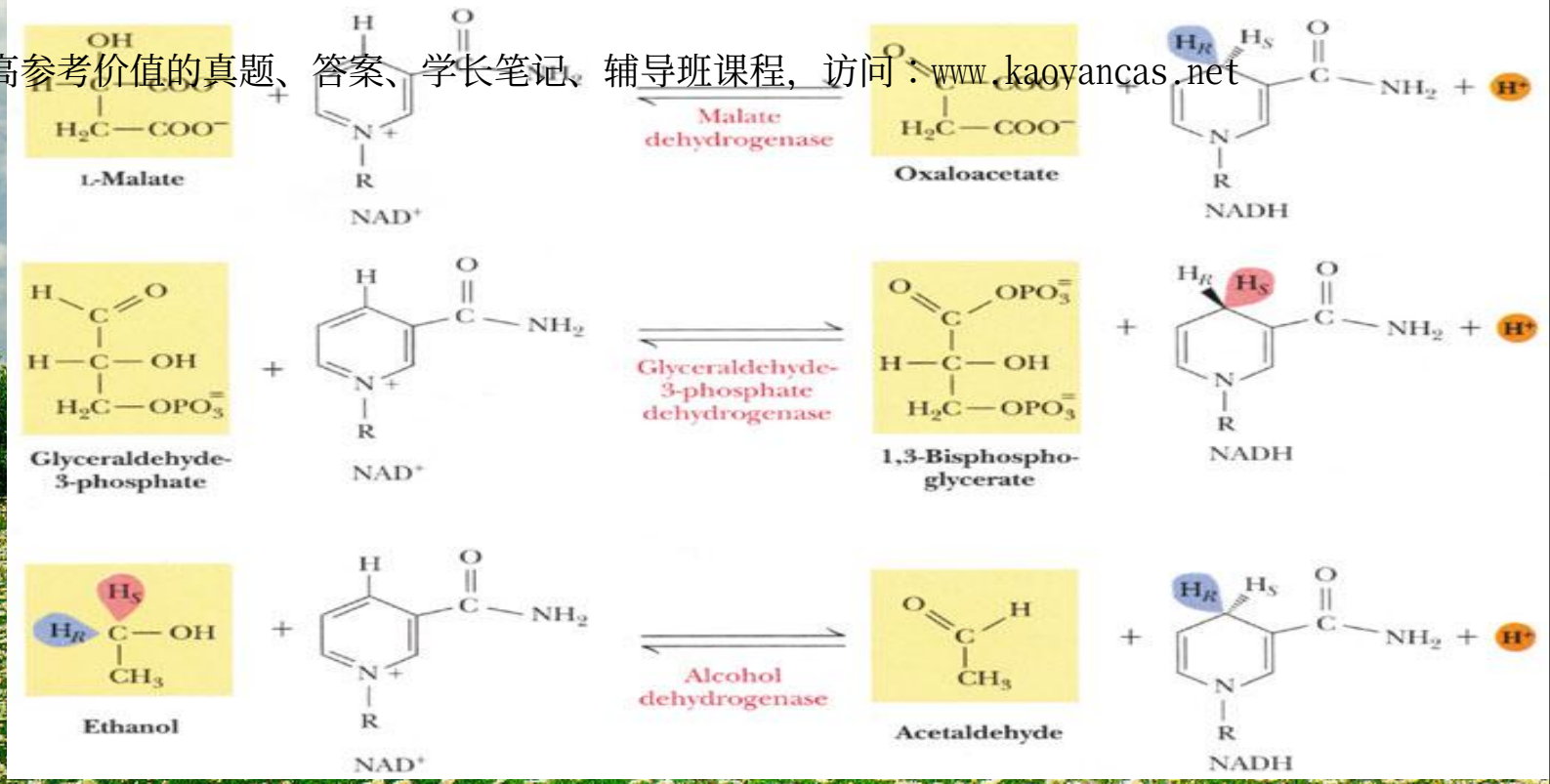
$\Delta G'^{\circ} = 29.7$ kJ/mol

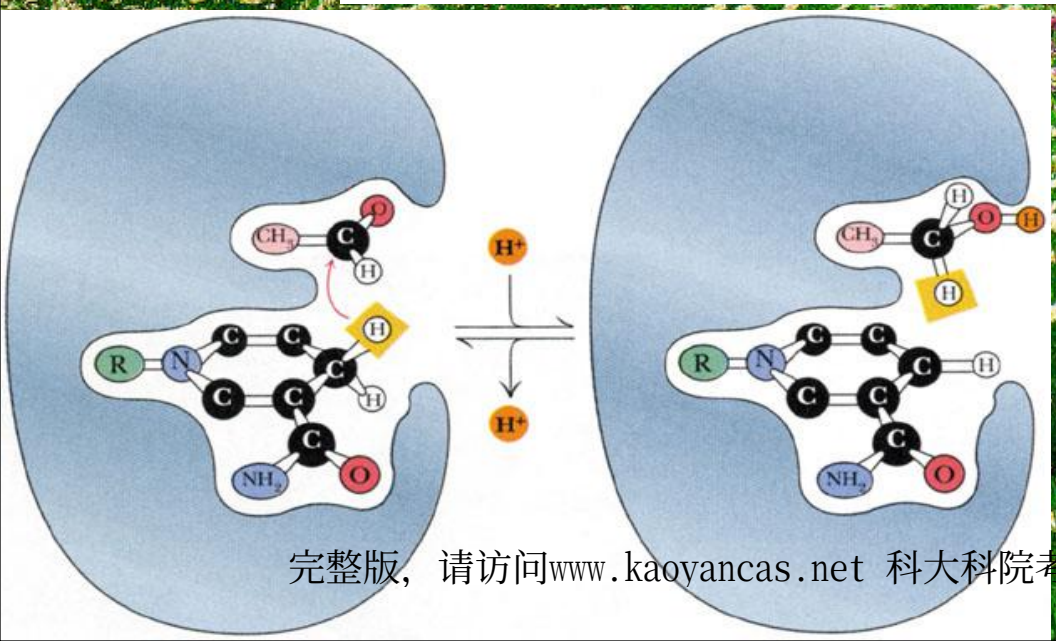




L-苹果酸脱氢酶的结构苹果酸为红色，NAD^+为蓝色。

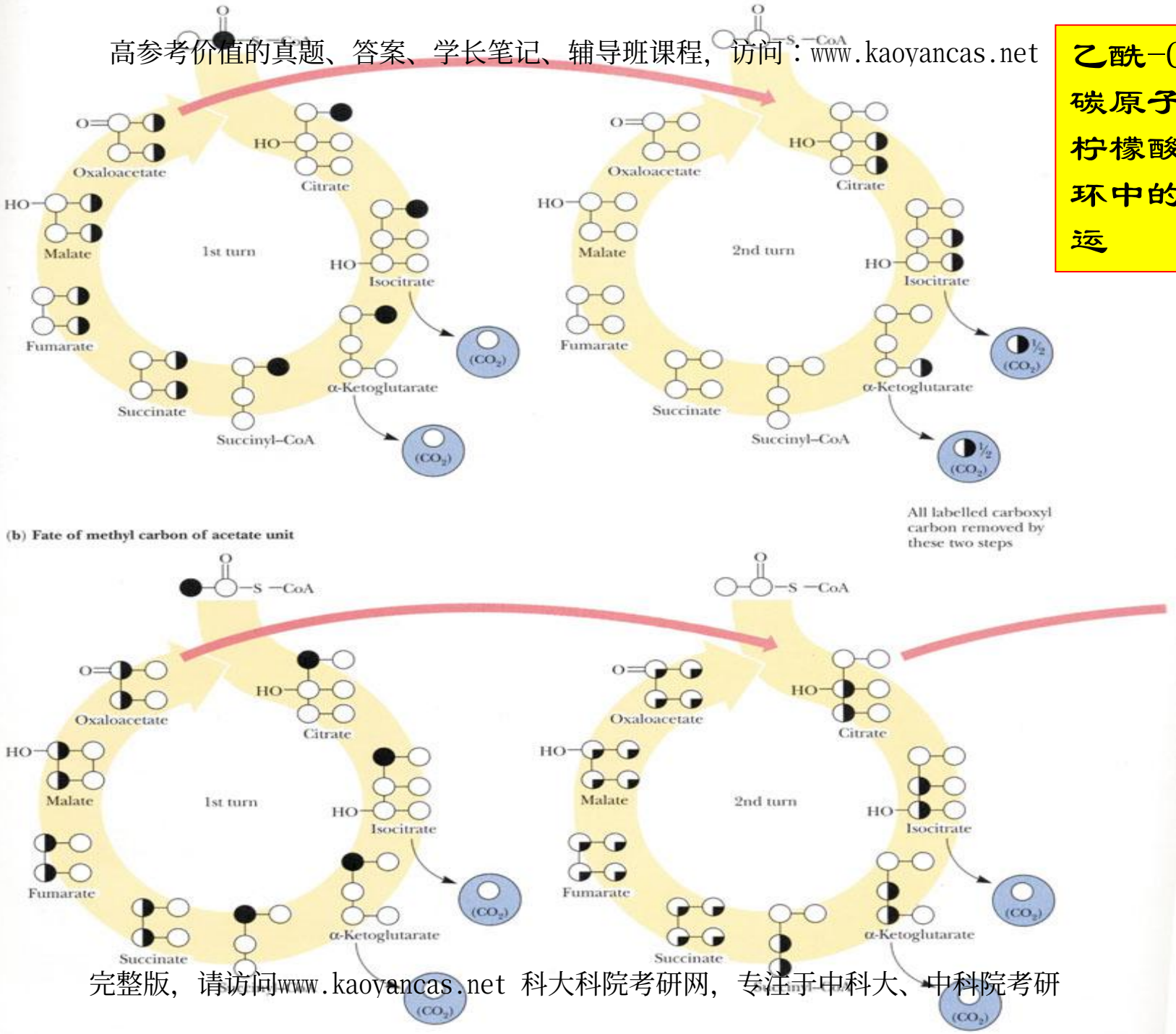
脱氢酶的作用

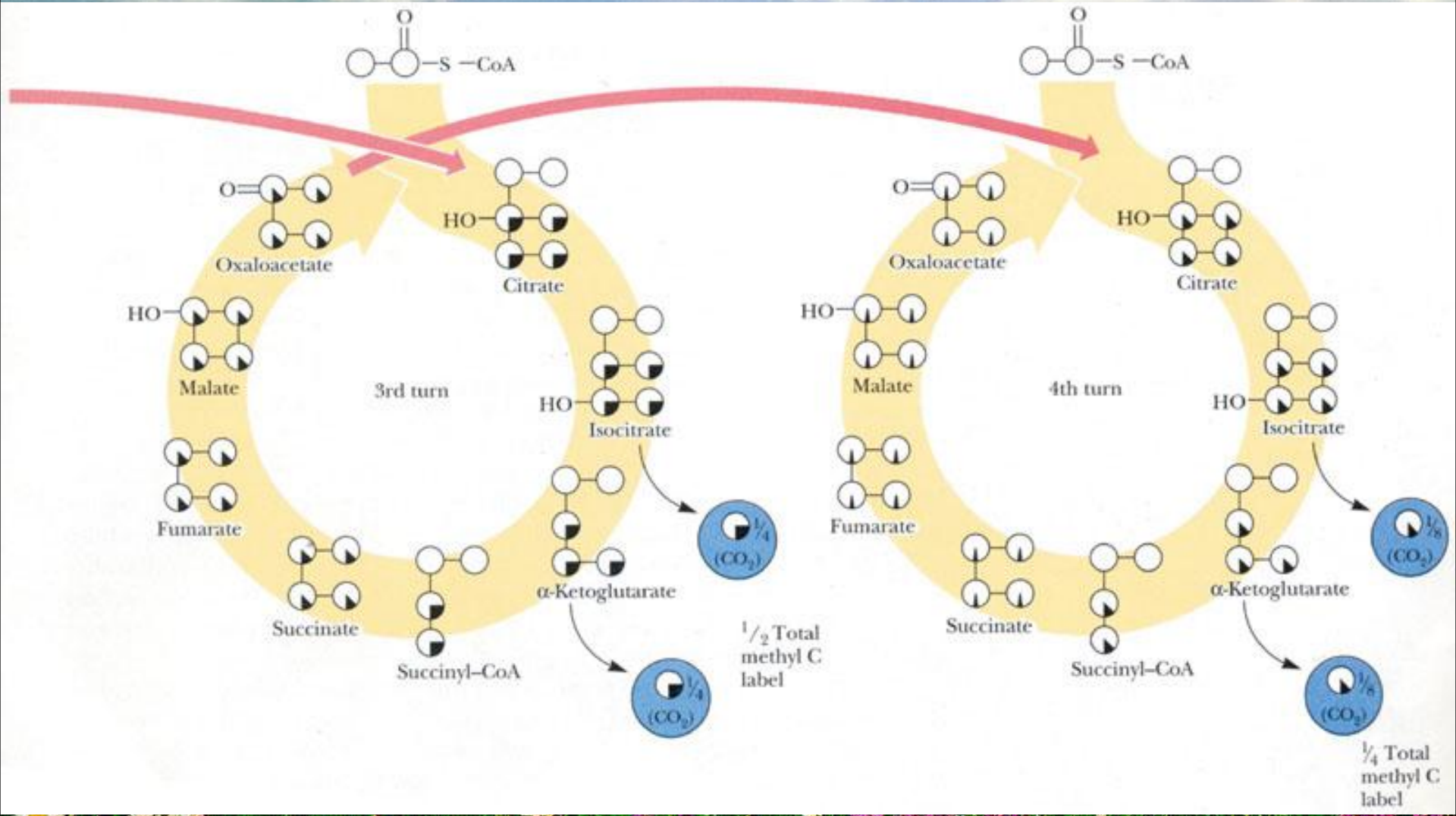




脱氢酶的活性中心

乙酰-CoA碳原子在柠檬酸循环中的命运





四、柠檬酸循环的化学总结算

柠檬酸循环有4个脱氢步骤，其中3对电子经NADH传递给氧，每对电子产生2.5个ATP，一对电子经$FADH_2$传递给氧，产生1.5个ATP，柠檬酸循环本身产生1个ATP，每次循环产生

7.5+1.5+1=10个ATP.

过去的计算是9+2+1=12个ATP.

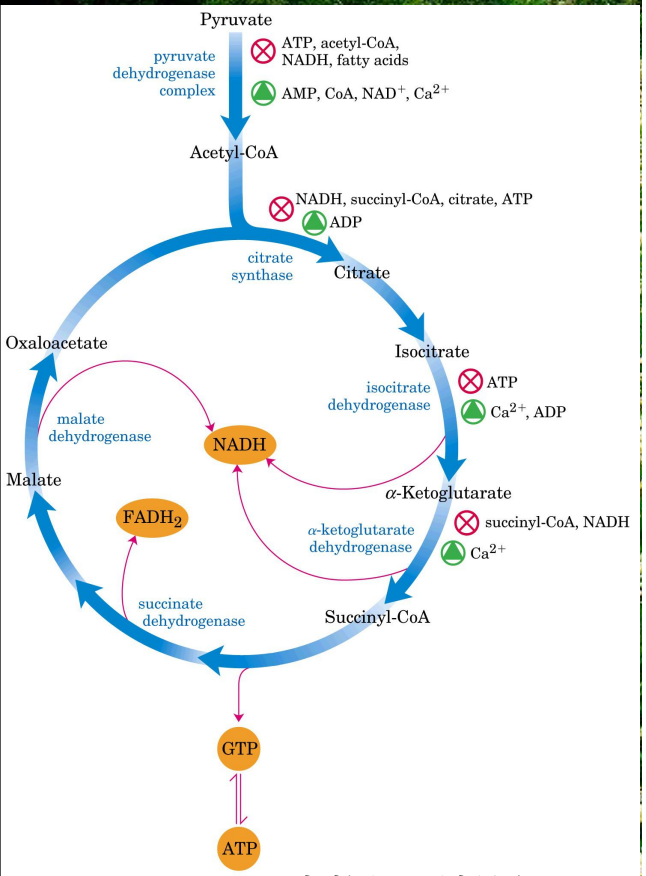
TABLE 16-1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

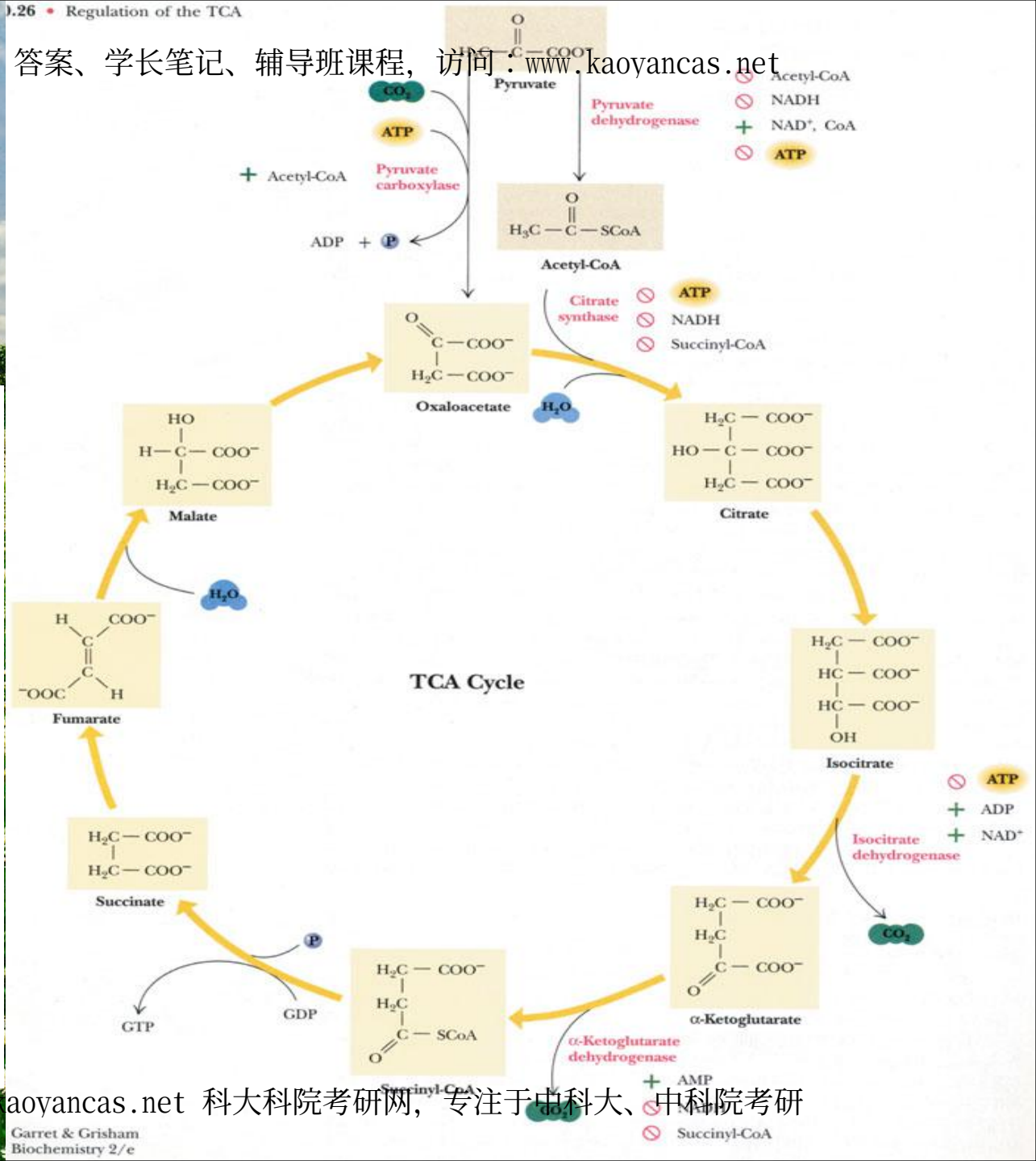
Reaction	*Number of ATP or reduced coenzyme directly formed*	*Number of ATP ultimately formed**
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30-32

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

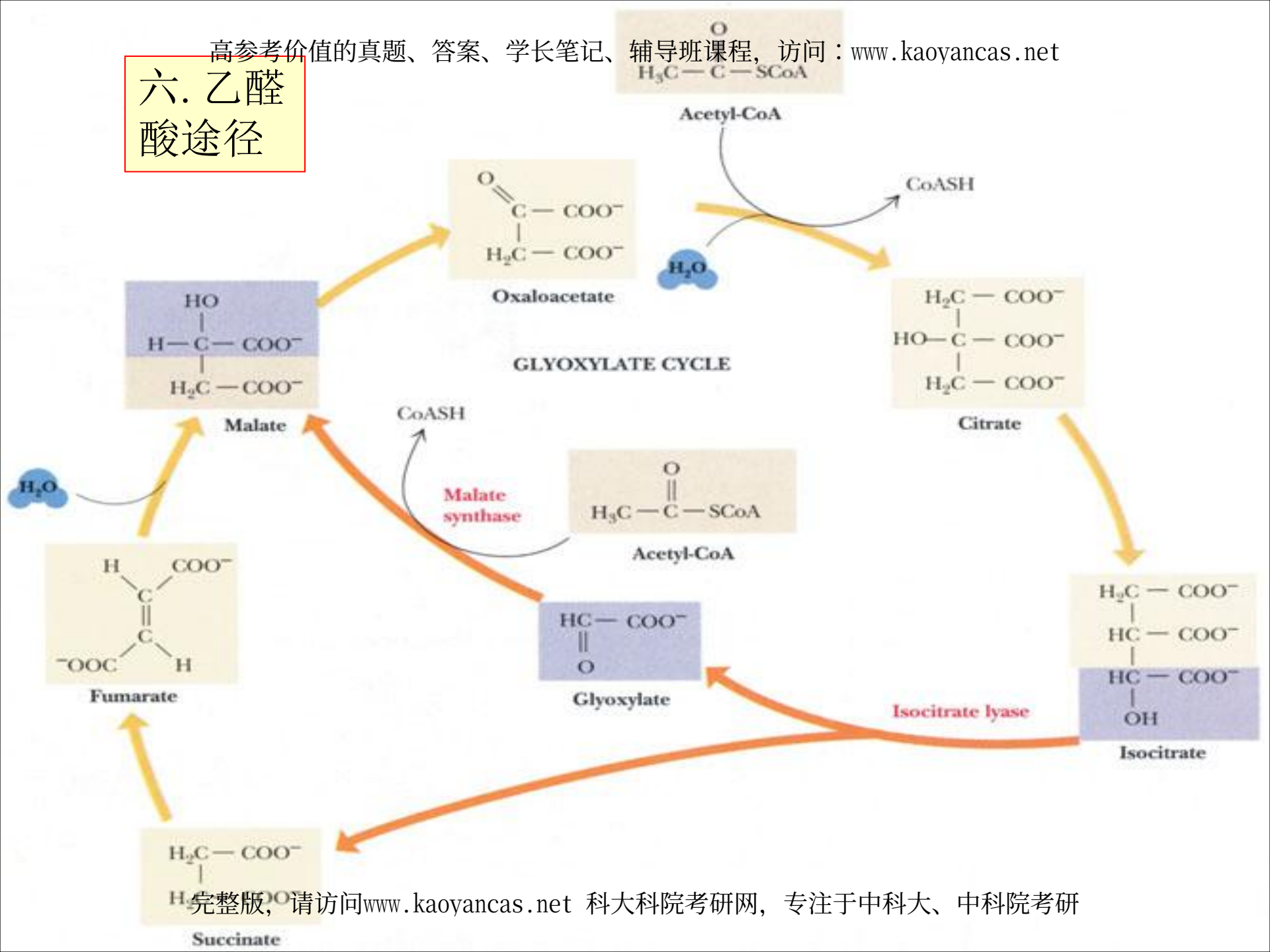
† This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19-27 and 19-28.

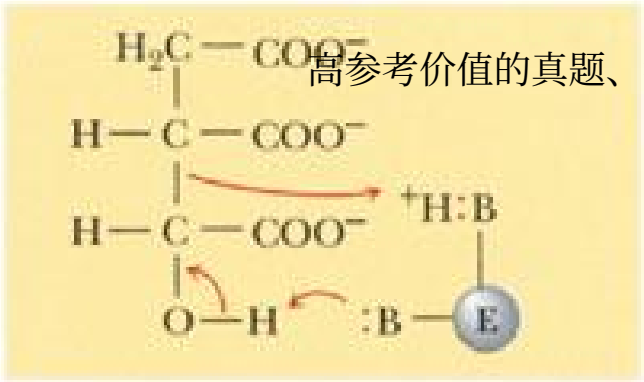
五、柠檬酸循环的调控

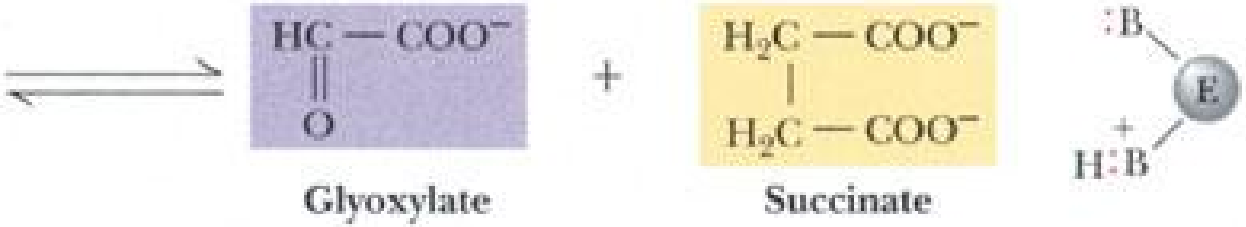




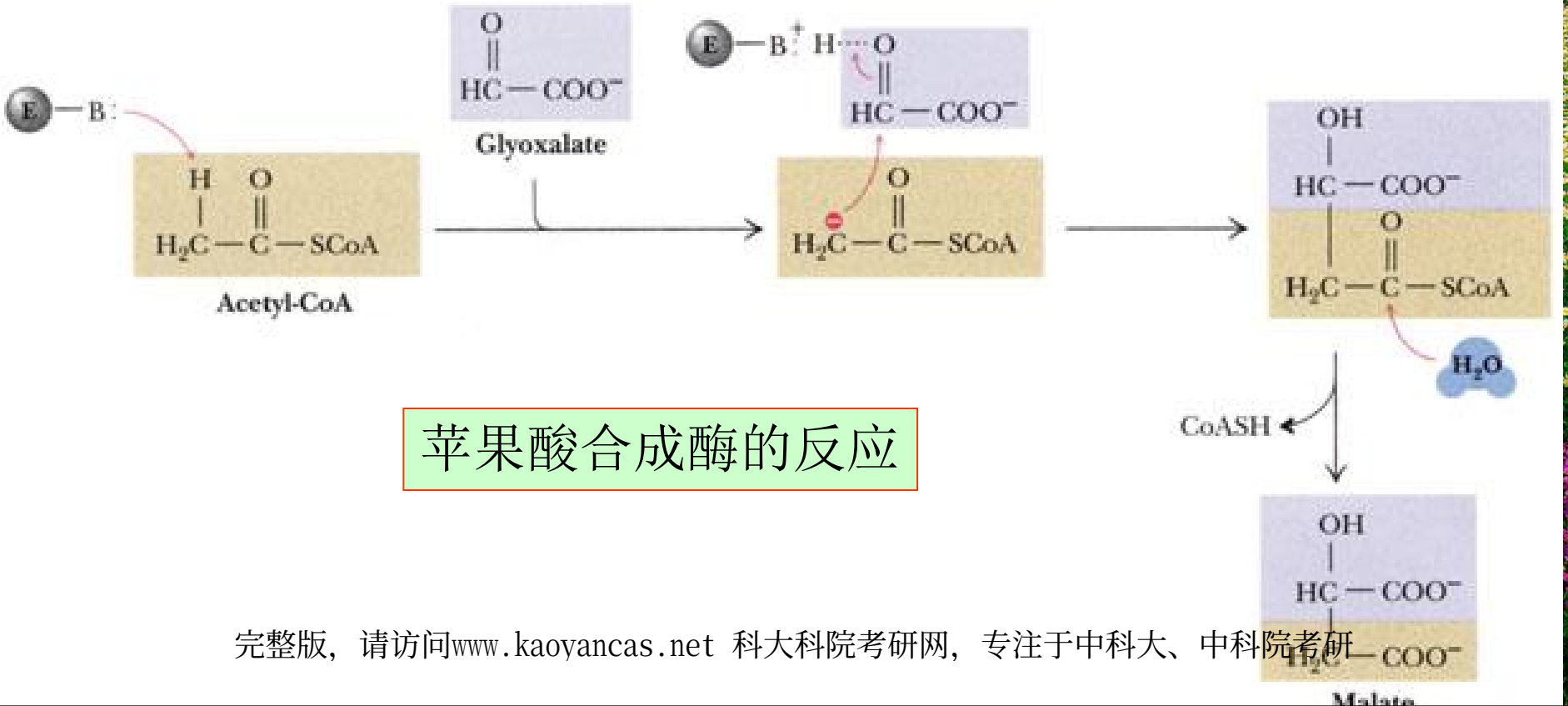
六. 乙醛酸途径



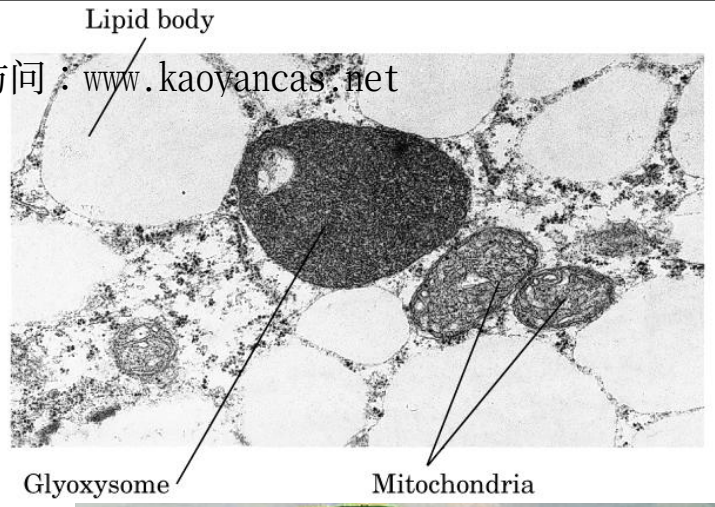


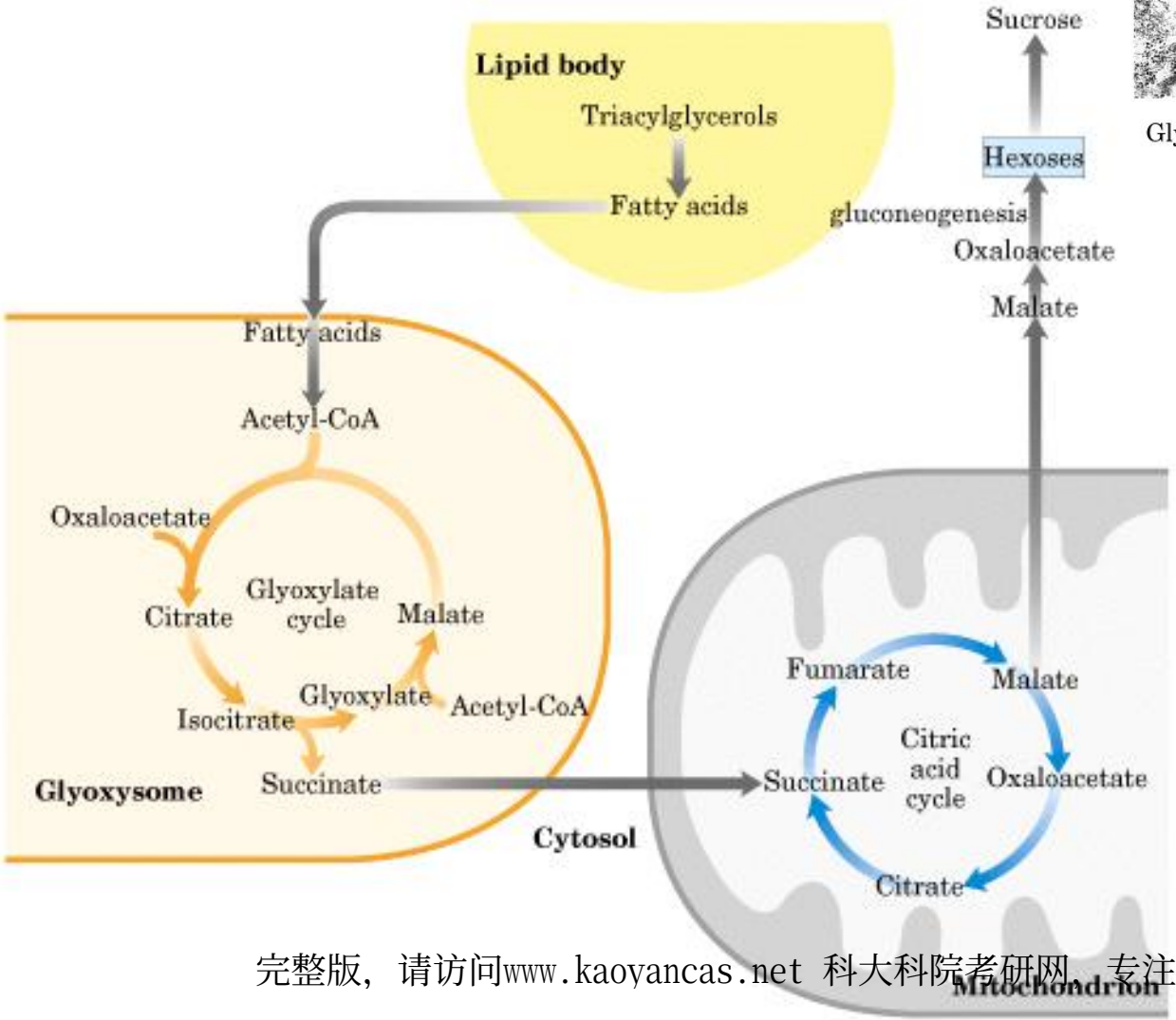


异柠檬酸裂解酶的反应



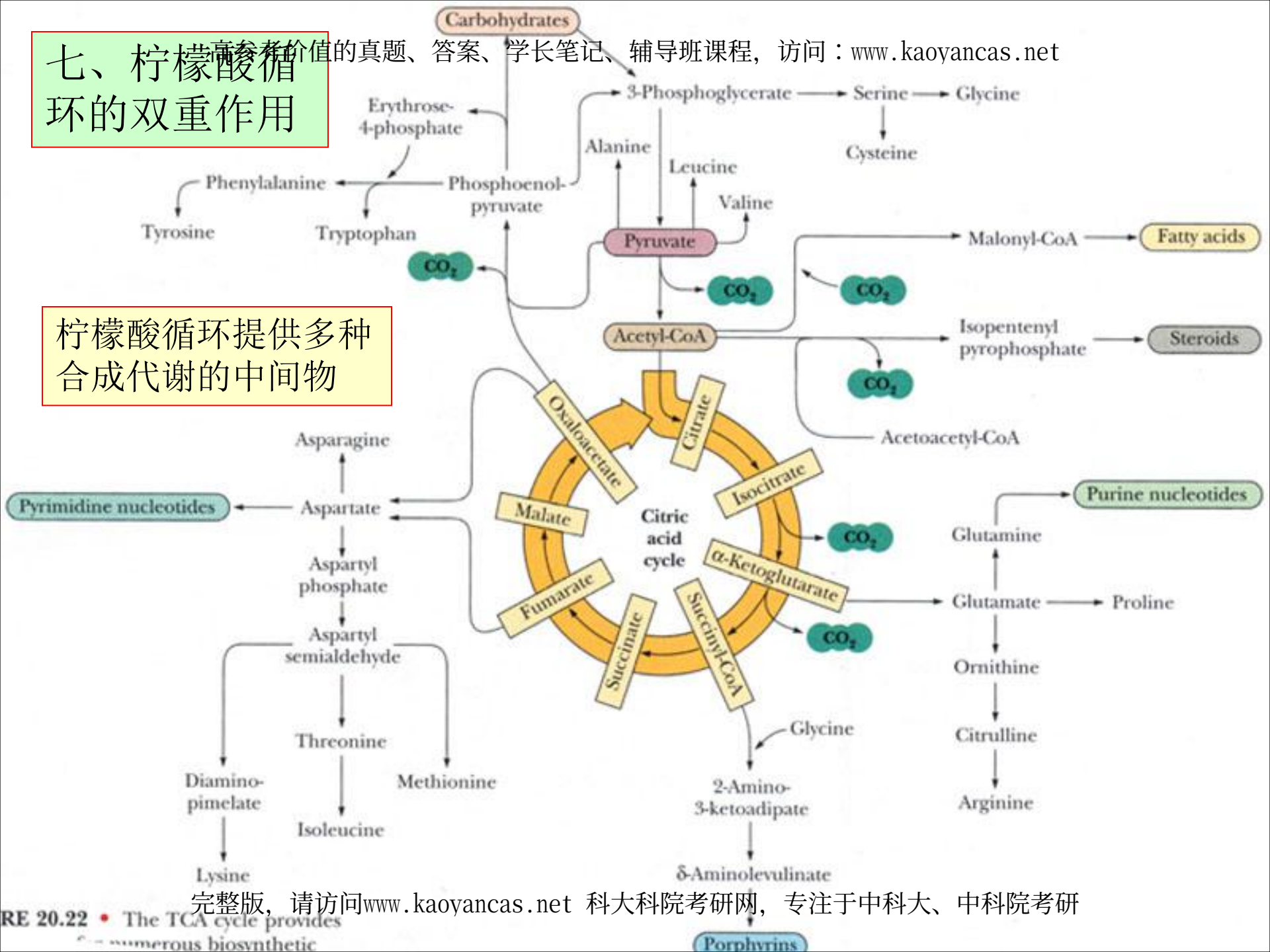
苹果酸合成酶的反应



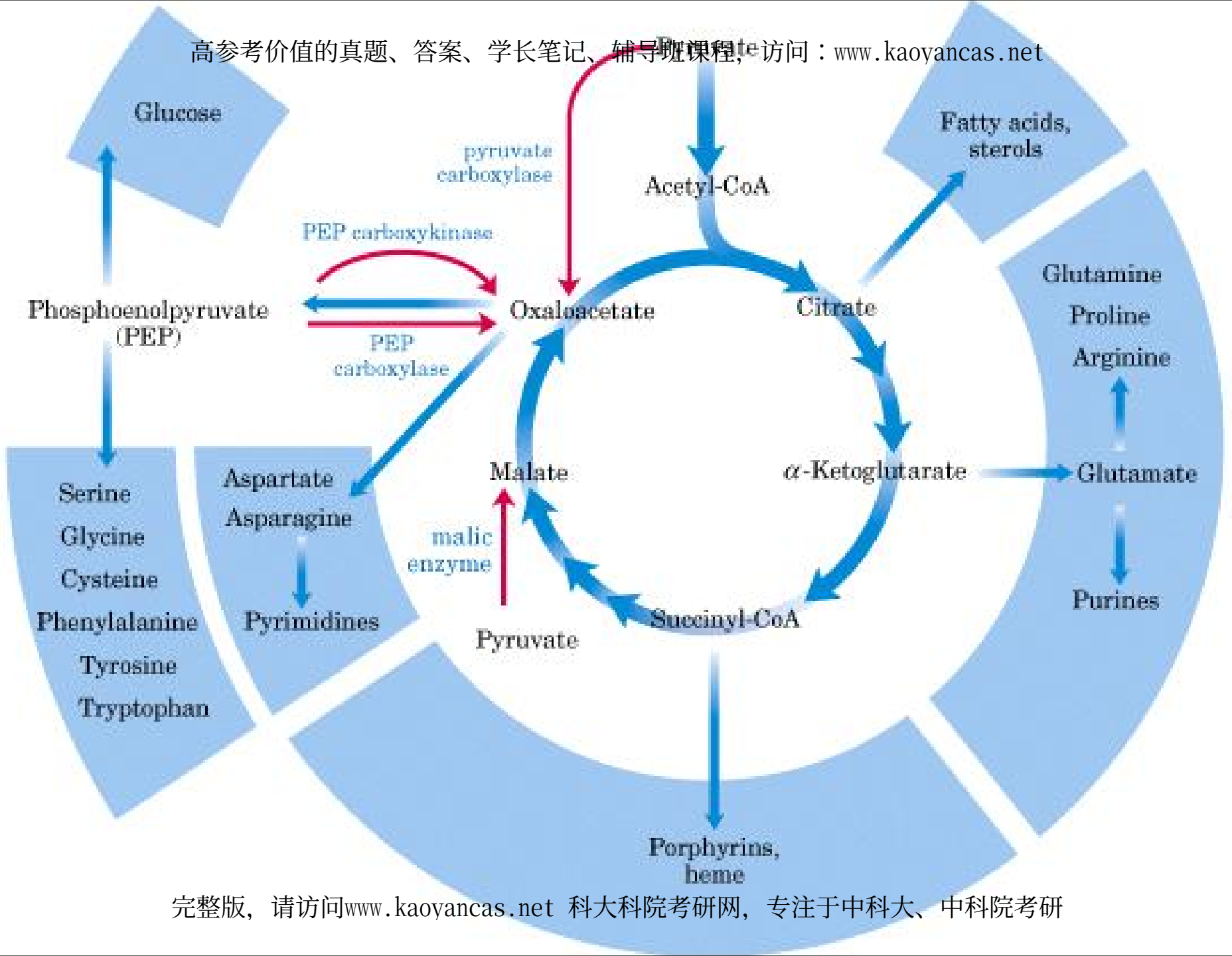


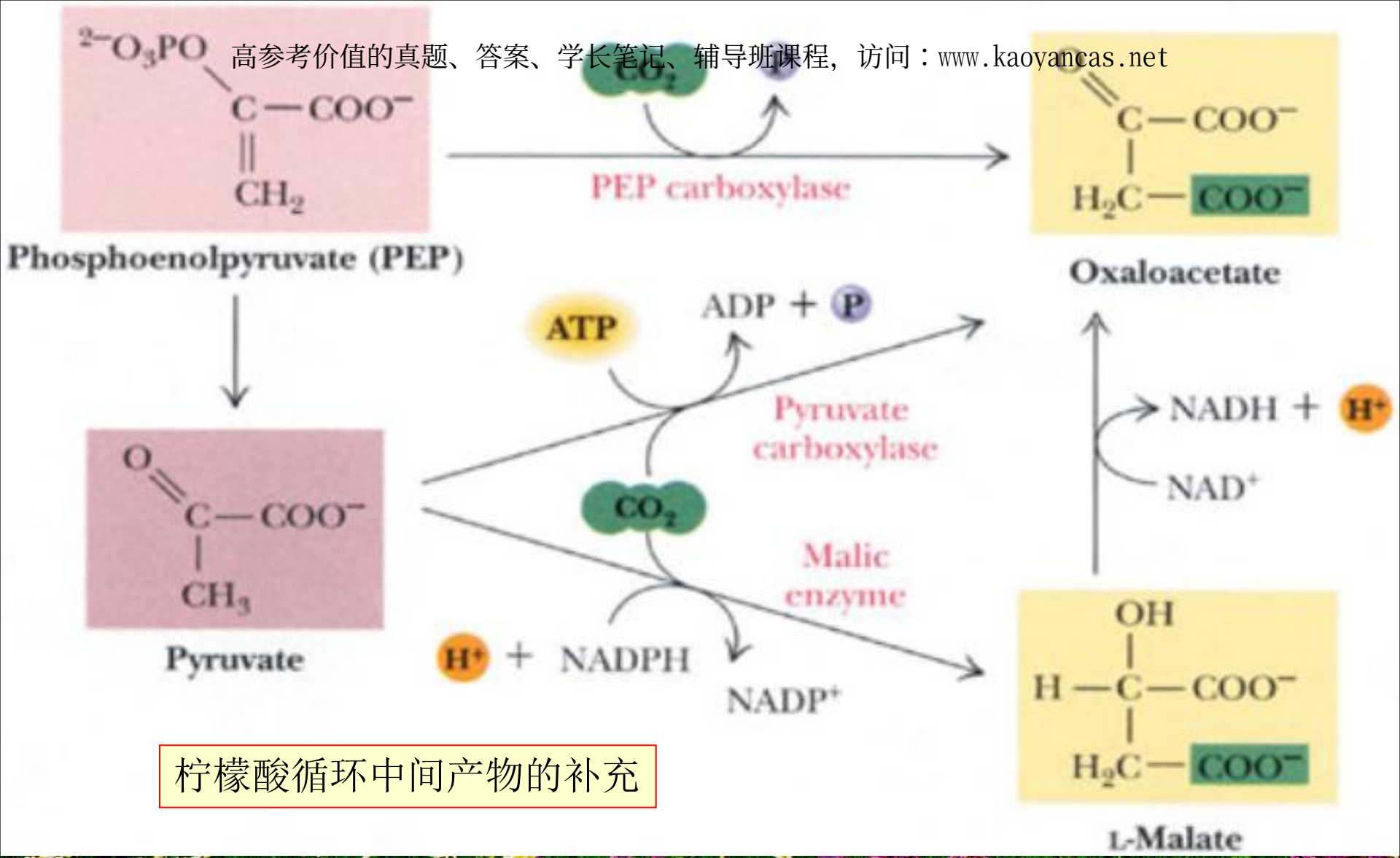
七、柠檬酸循环的双重作用

柠檬酸循环提供多种合成代谢的中间物



RE 20.22 • The TCA cycle provides numerous biosynthetic





柠檬酸循环中间产物的补充

此外，乙醛酸途径 和氨基酸脱氨基均可生成柠檬酸循环的中间产物。

八、柠檬酸循环的发现历史

1937年Hans Krebs 提出柠檬酸循环的反应机制，其主要的依据有：Krebs 于1932年发现乙酸，琥珀酸，延胡索酸，苹果酸，柠檬酸，草酰乙酸可以促进组织匀浆或切片的氧化作用；Albert Szent-Gyoryi发现少量的四碳二羧酸可以加快糖类氧化反应的速度，提出可能存在一个酶促的系列反应。他还发现了丙二酸对琥珀酸脱氢酶的抑制作用；Carl Martius 和Franz Knoop发现柠檬酸可以转化为图示的其他有机酸； Krebs发现草酰乙酸可以和活性乙酸反应生成柠檬酸，在反映体系中过量加入其中的任意一种有机酸可以很快转化为其他的有机酸，因而提出反应体系构成一个循环。

The Nobel Prize in Physiology or Medicine 1953

Presentation Speech

"for his discovery of the citric acid cycle"

Sir Hans Adolf Krebs

Great Britain

Sheffield University
Sheffield, Great Britain

1900 - 1981

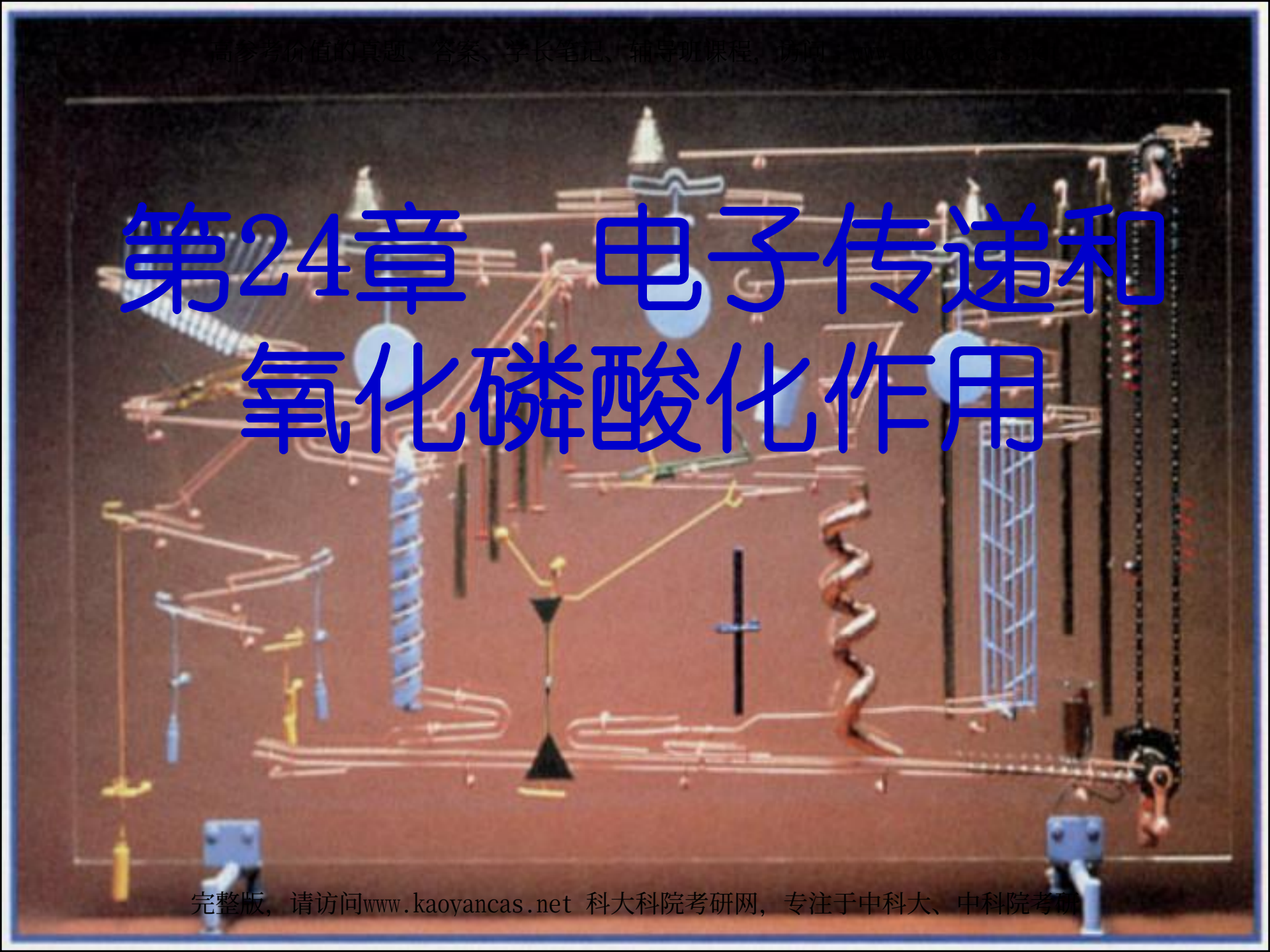
Biography

Hans Krebs, 1900–1981

Citrate
↓
Isocitrate
↓
α-Ketoglutarate
↓
Succinyl-CoA
↓
Succinate
↓
Fumarate
↓
Malate
↓
Oxaloacetate

基本要求

1. 熟悉形成乙酰-CoA 的过程。（难点）
2. 掌握柠檬酸循环的概况。（重点）
3. 熟悉柠檬酸循环的反应机制。（难点）
4. 掌握柠檬酸循环的能量计算。（重点）
5. 熟悉柠檬酸循环的调控和双重作用。（难点）
6. 熟悉柠檬酸循环的研究历史。



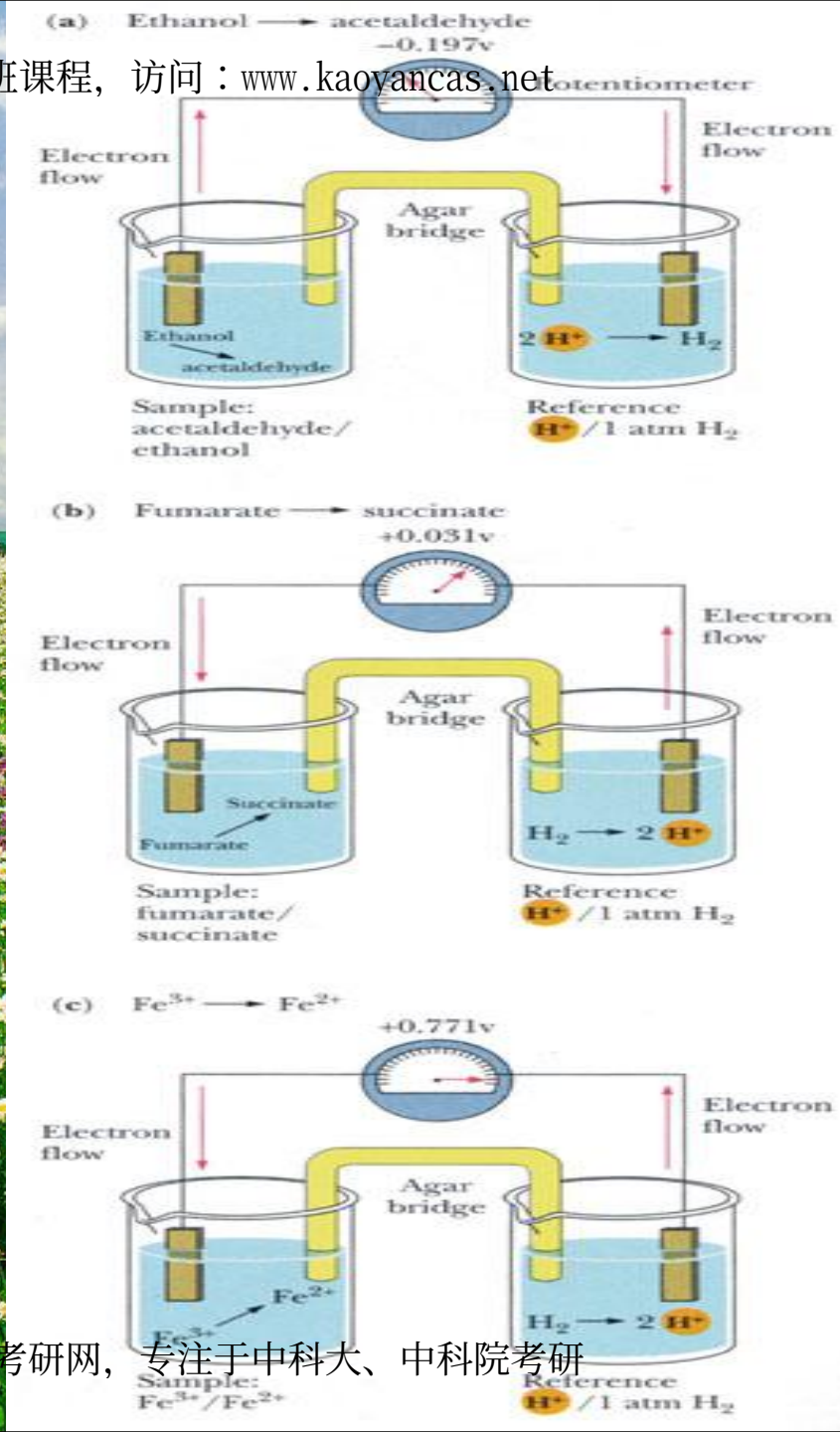
第24章　电子传递和氧化磷酸化作用

一、氧化-还原电势

（一）氧化-还原电势

生物氧化是通过加氧、脱氢或失电子而进行的，加氧反应较少见，氧原子通常是通过加水再脱氢引入代谢物的。代谢物中生成的羧基，可通过脱羧基作用生成二氧化碳。脱氢或失电子反应是生物氧化的主要方式，反应过程中伴随着氧化-还原电势的变化。

$$\varepsilon = E_{正极} - E_{负极}$$



(二) 生物体中某些重要的氧化-还原电势

TABLE 13.7 Standard Reduction Potentials of Some Biologically Important Half-Reactions, at pH 7.0 and 25 °C (298 K)

Half-reaction	E'° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome f $(Fe^{3+}) + e^- \longrightarrow$ cytochrome f (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) $+ e^- \longrightarrow Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome a $(Fe^{3+}) + e^- \longrightarrow$ cytochrome a (Fe^{2+})	0.29
Cytochrome c $(Fe^{3+}) + e^- \longrightarrow$ cytochrome c (Fe^{2+})	0.254
Cytochrome c_1 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b $(Fe^{3+}) + e^- \longrightarrow$ cytochrome b (Fe^{2+})	0.077
Ubiquinone $+ 2H^+ + 2e^- \longrightarrow$ ubiquinol $+ H_2$	0.045
$Fumarate^{2-} + 2H^+ + 2e^- \longrightarrow succinate^{2-}$	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA $+ 2H^+ + 2e^- \longrightarrow$ butyryl-CoA	−0.015
$Oxaloacetate^{2-} + 2H^+ + 2e^- \longrightarrow malate^{2-}$	−0.166
$Pyruvate^- + 2H^+ + 2e^- \longrightarrow lactate^-$	−0.185
Acetaldehyde $+ 2H^+ + 2e^- \longrightarrow$ ethanol	−0.197
$FAD + 2H^+ + 2e^- \longrightarrow FADH_2$	−0.219*
Glutathione $+ 2H^+ + 2e^- \longrightarrow$ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid $+ 2H^+ + 2e^- \longrightarrow$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
Acetoacetate $+ 2H^+ + 2e^- \longrightarrow \beta$-hydroxybutyrate	−0.346
α-Ketoglutarate $+ CO_2 + 2H^+ + 2e^- \longrightarrow$ isocitrate	−0.38
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Cytochrome a $(Fe^{3+}) + e^- \longrightarrow$ cytochrome a (Fe^{2+})	0.432

（三）电势和自由能的关系

$$\Delta G^0 = -nF\Delta E^0$$

$$\Delta G = -n\mathcal{F}\Delta E \quad \text{or} \quad \Delta G^\circ = -n\mathcal{F}\Delta E^\circ$$

（四）标准电动势和平衡常数的关系

$$E^0 = (RT / nF)\ln k$$

$$= E^0 + (RT/nF)\ln\{[ox]/[red]\}$$

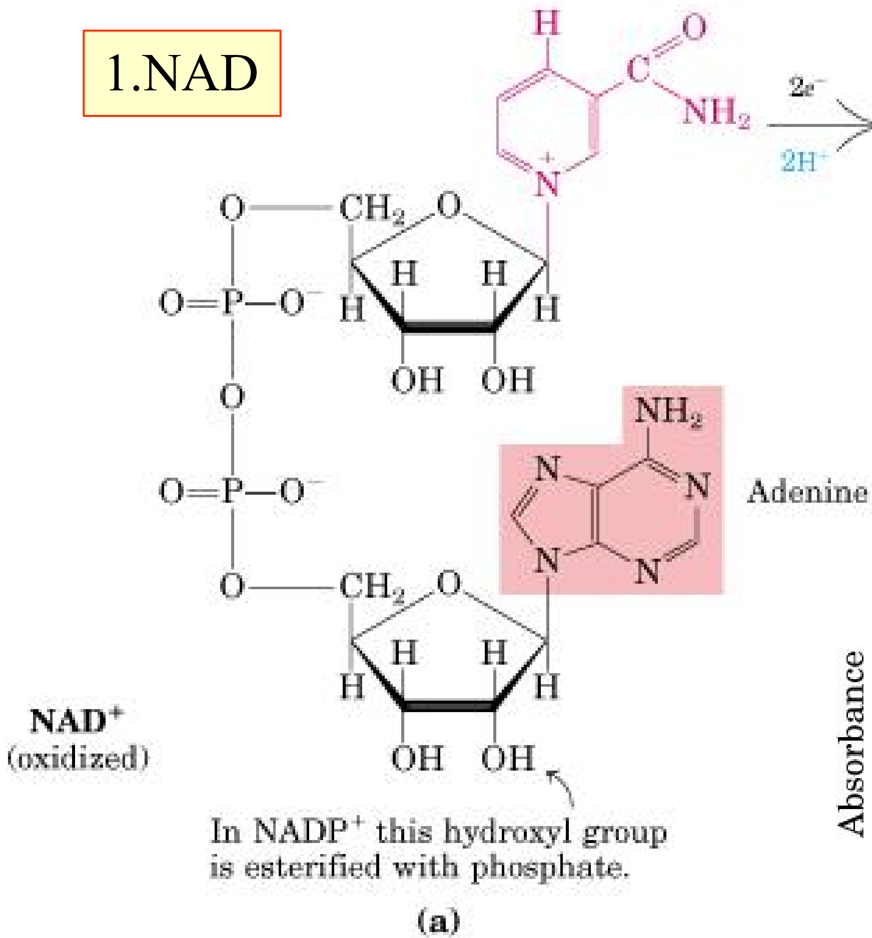
$$E = E^\circ + \frac{RT}{n\mathcal{F}} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]}$$

二、电子传递和氧化呼吸链

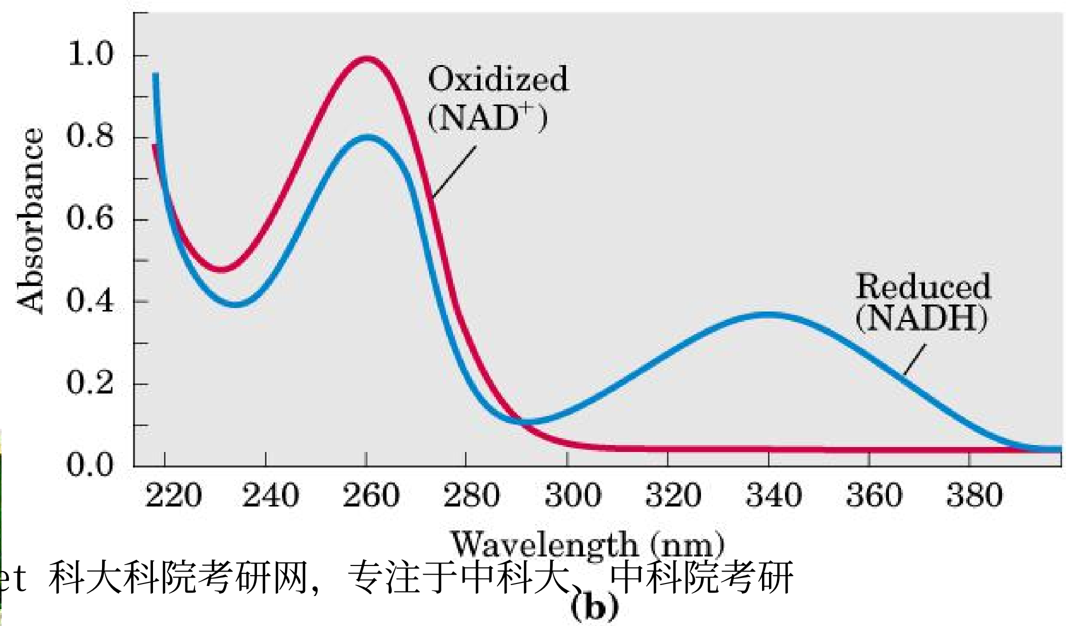
（一）呼吸链的概念

（二）呼吸链的各个成员

1.NAD



(a)



(b)

TABLE 19-1 Some Important Reactions Catalyzed by NAD(P)H-Linked Dehydrogenases

Reaction*	Location†
NAD-linked	
α-Ketoglutarate + CoA + NAD^+ ⇌ succinyl-CoA + CO_2 + NADH + H^+	M
L-Malate + NAD^+ ⇌ oxaloacetate + NADH + H^+	M and C
Pyruvate + CoA + NAD^+ ⇌ acetyl-CoA + CO_2 + NADH + H^+	M
Glyceraldehyde 3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + NADH + H^+	C
Lactate + NAD^+ ⇌ pyruvate + NADH + H^+	C
β-Hydroxyacyl-CoA + NAD^+ ⇌ β-ketoacyl-CoA + NADH + H^+	M
NADP-linked	
Glucose 6-phosphate + $NADP^+$ ⇌ 6-phosphogluconate + NADPH + H^+	C
NAD- or NADP-linked	
L-Glutamate + H_2O + $NAD(P)^+$ ⇌ α-ketoglutarate + NH_4^+ + NAD(P)H	M
Isocitrate + $NAD(P)^+$ ⇌ α-ketoglutarate + CO_2 + NAD(P)H + H^+	M and C

*These reactions and their enzymes are discussed in Chapters 14 through 18.
†M designates mitochondria; C, cytosol.

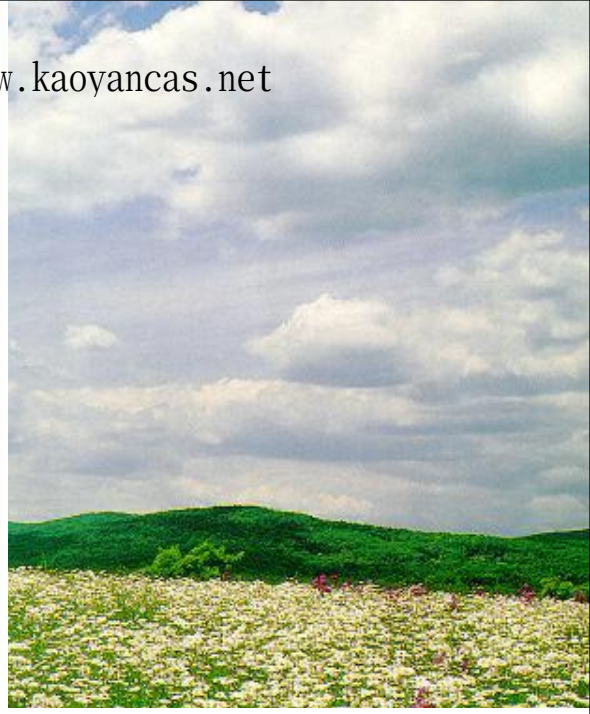
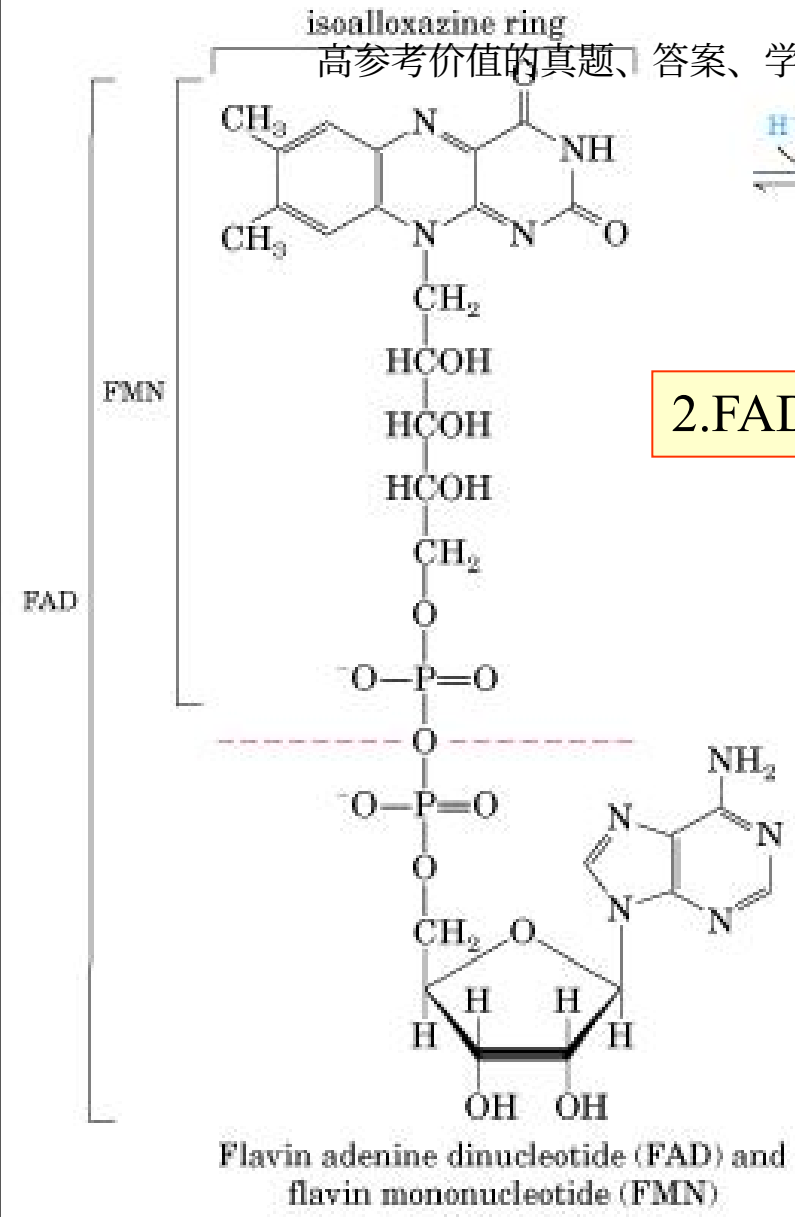


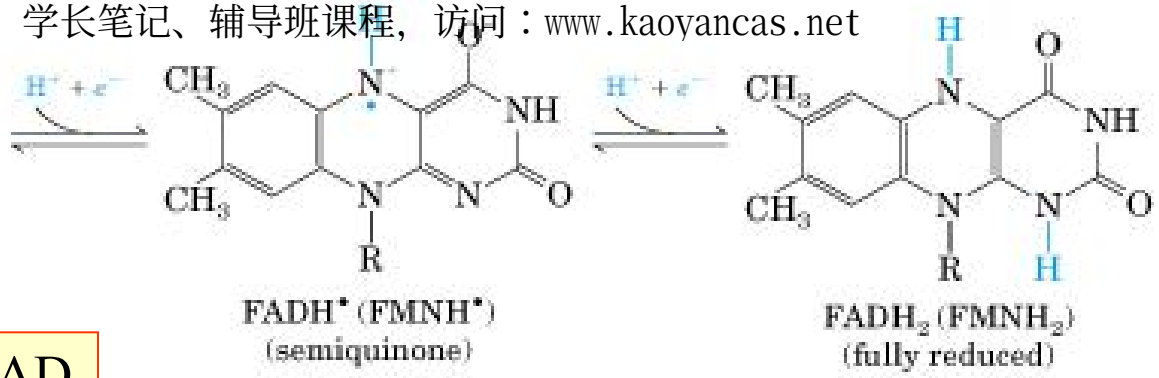
table 14–8

Stereospecificity of Dehydrogenases That Employ NAD^+ or $NADP^+$ as Coenzymes

Enzyme	Coenzyme	Stereochemical specificity for nicotinamide ring (A or B)
Isocitrate dehydrogenase	NAD^+	A
α-Ketoglutarate dehydrogenase	NAD^+	B
Glucose 6-phosphate dehydrogenase	$NADP^+$	B
Malate dehydrogenase	NAD^+	A
Glutamate dehydrogenase	NAD^+ or $NADP^+$	B
Glyceraldehyde 3-phosphate dehydrogenase	NAD^+	B
Lactate dehydrogenase	NAD^+	A
Alcohol dehydrogenase	NAD^+	A



Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)



FADH• (FMNH•) (semiquinone)

$FADH_2$ ($FMNH_2$) (fully reduced)

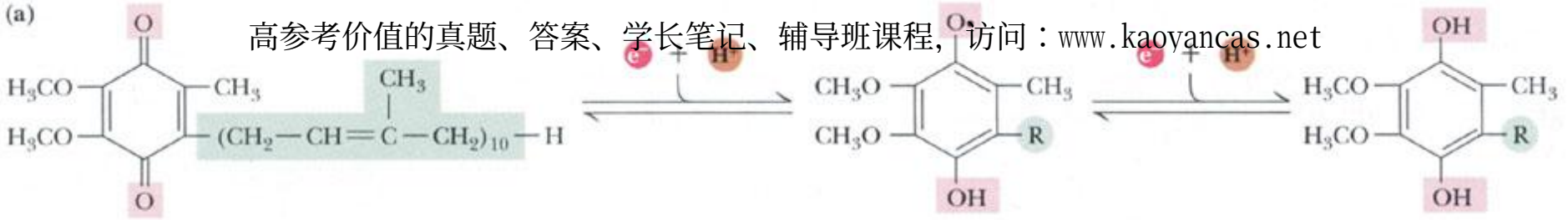
2.FAD

table 14–9

Some Enzymes (Flavoproteins) That Employ Flavin Nucleotide Coenzymes

Enzyme	Flavin nucleotide
Fatty acyl–CoA dehydrogenase	FAD
Dihydrolipoyl dehydrogenase	FAD
Succinate dehydrogenase	FAD
Glycerol 3-phosphate dehydrogenase	FAD
Thioredoxin reductase	FAD
NADH dehydrogenase (Complex I)	FMN
Glycolate dehydrogenase	FMN

(a)



3.CoQ

(b)

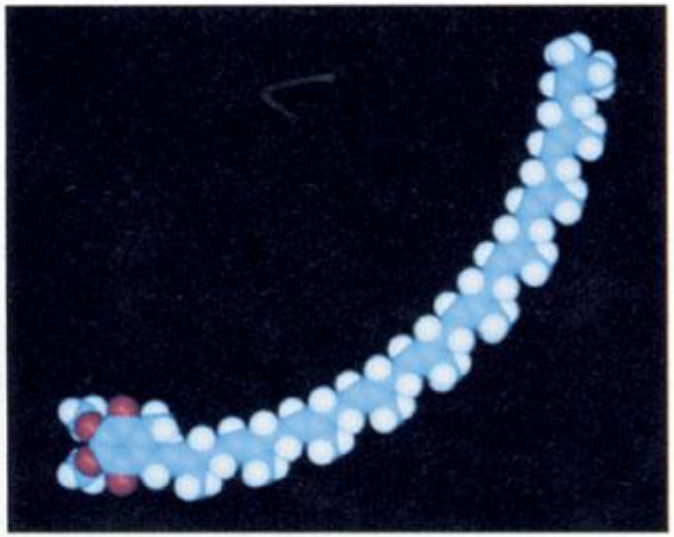
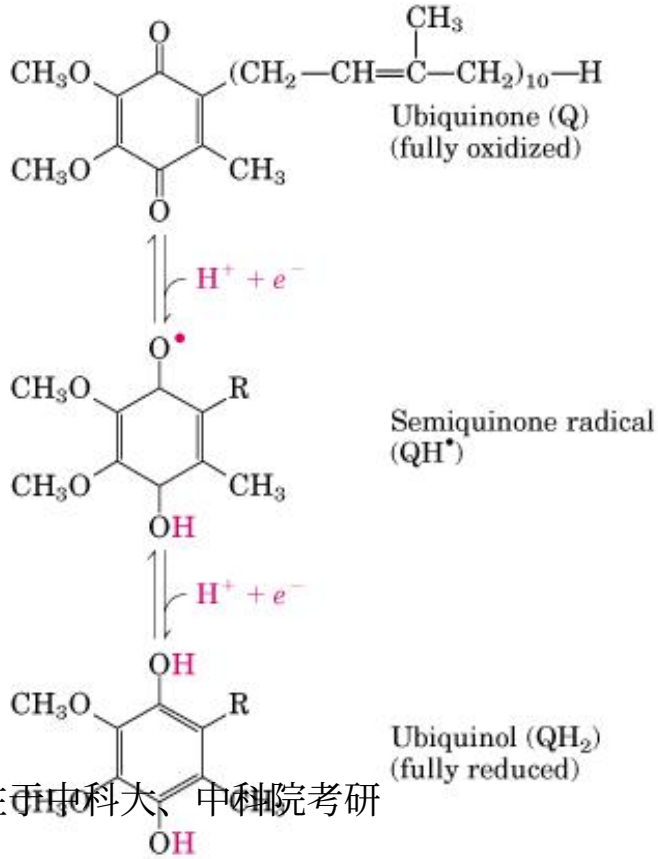
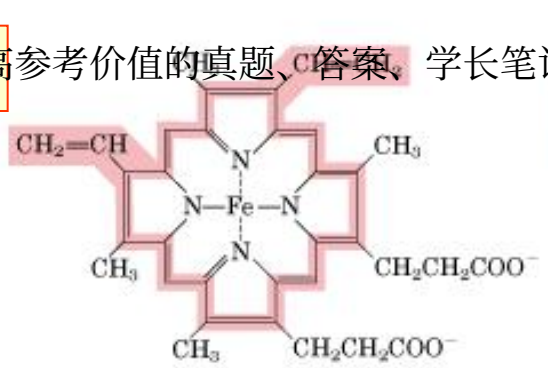


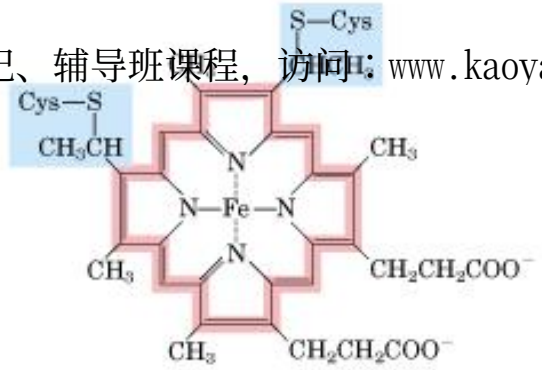
FIGURE 21.5 • (a) The three
(b) A space-filling model of coe



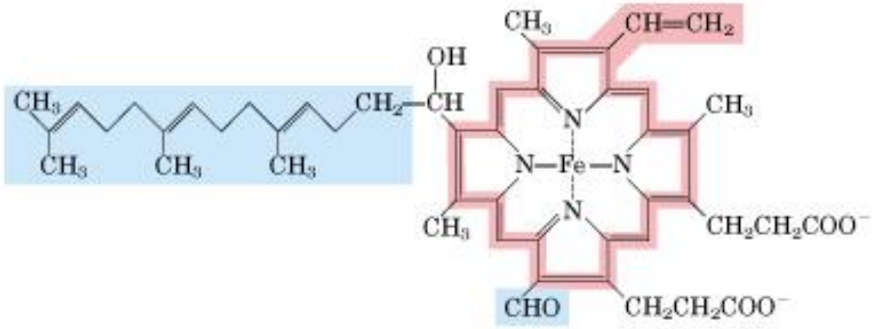
4.细胞色素



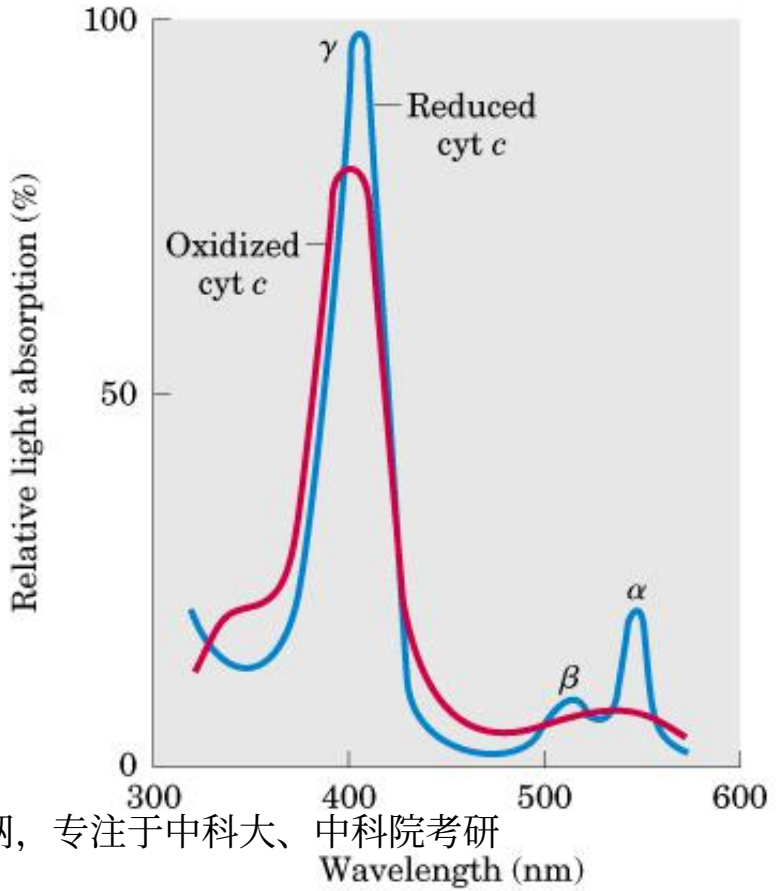
Iron protoporphyrin IX
(in *b*-type cytochromes)

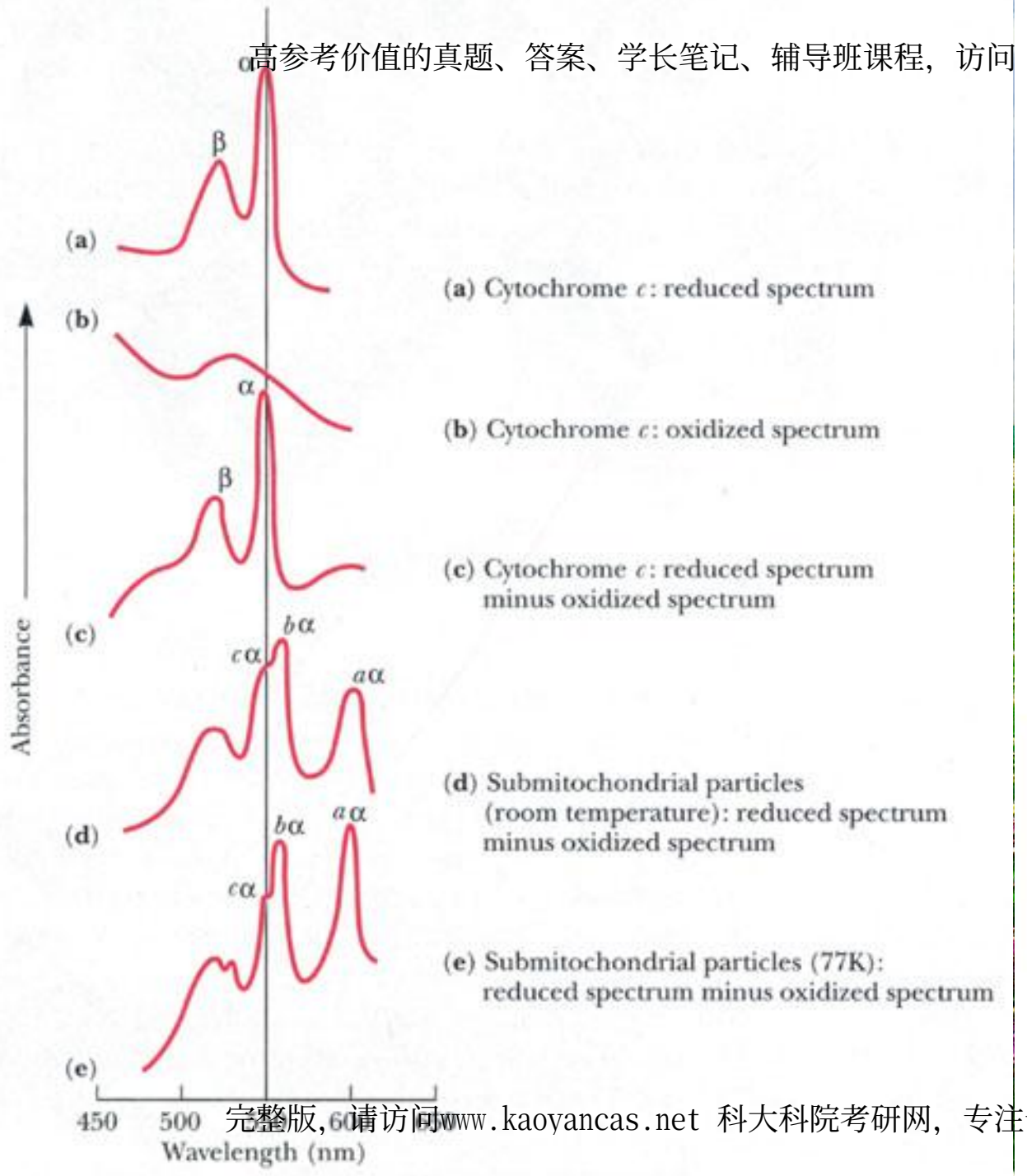


Heme C
(in *c*-type cytochromes)



Heme A
(in *a*-type cytochromes)





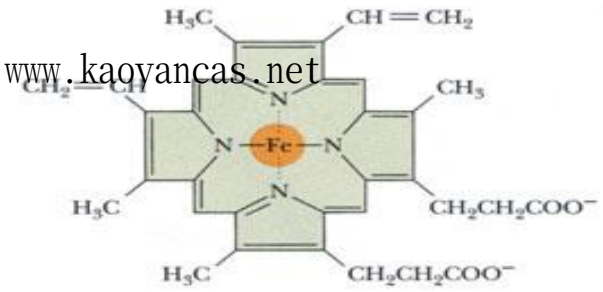
(a) Cytochrome *c*: reduced spectrum

(b) Cytochrome *c*: oxidized spectrum

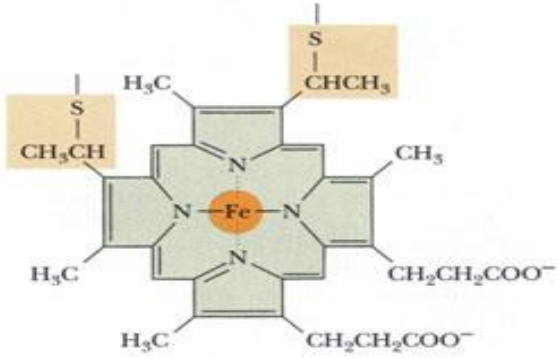
(c) Cytochrome *c*: reduced spectrum minus oxidized spectrum

(d) Submitochondrial particles (room temperature): reduced spectrum minus oxidized spectrum

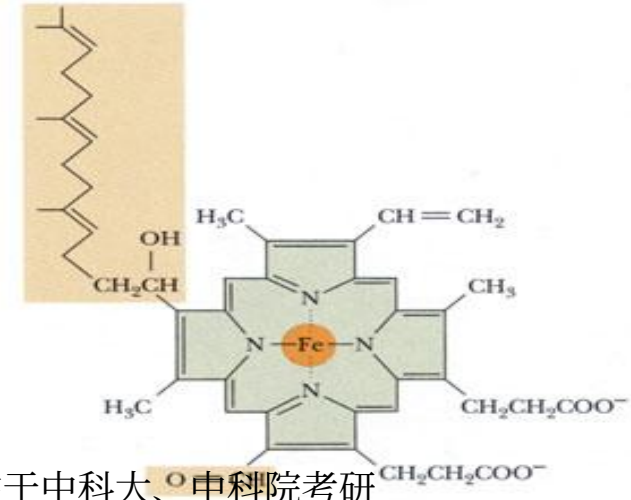
(e) Submitochondrial particles (77K): reduced spectrum minus oxidized spectrum



Iron protoporphyrin IX
(found in cytochrome *b*, myoglobin, and hemoglobin)



Heme C
(found in cytochrome *c*)



Heme A
(found in cytochrome *a*)

5.铁硫蛋白

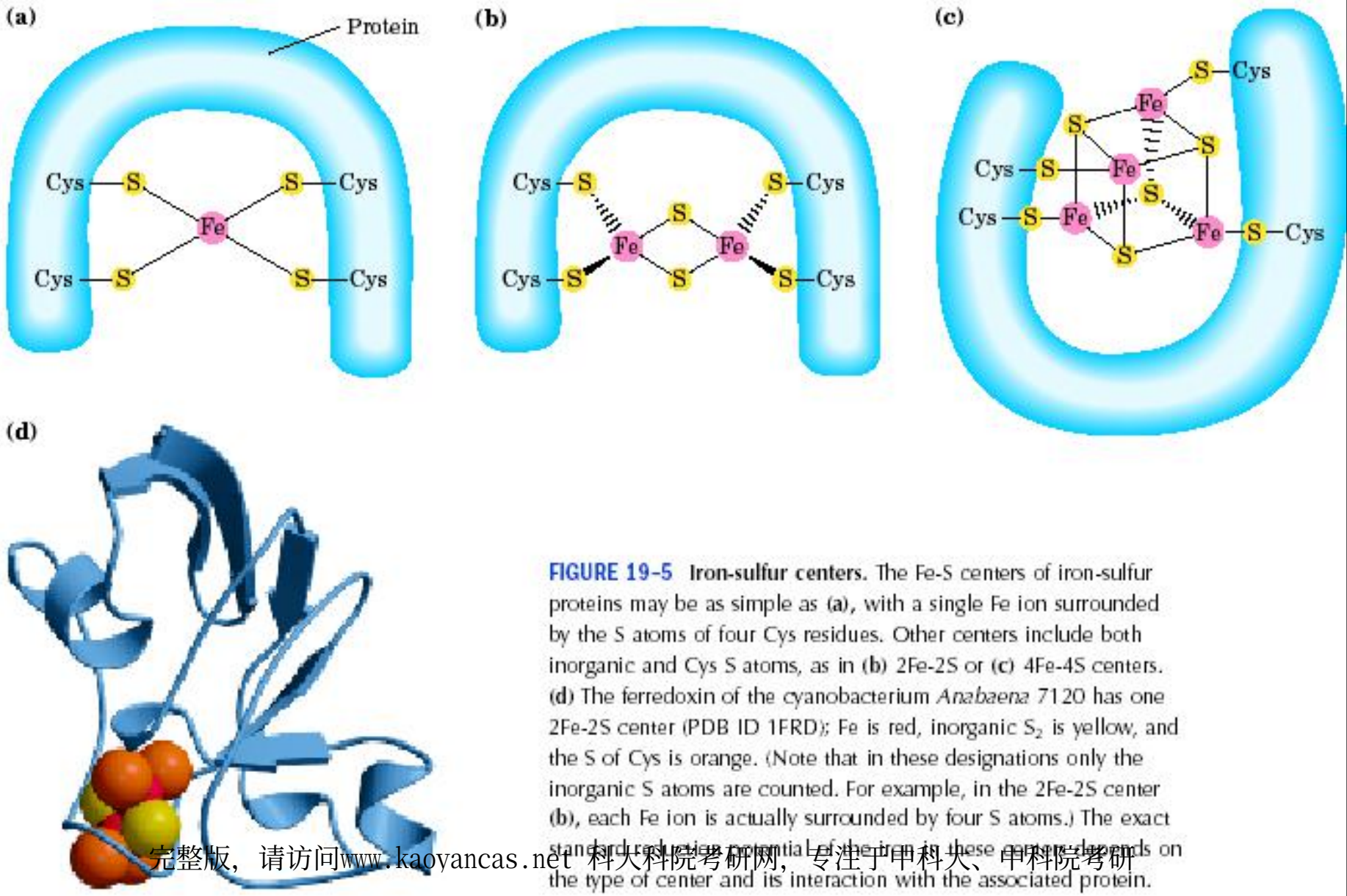


FIGURE 19–5 **Iron-sulfur centers.** The Fe-S centers of iron-sulfur proteins may be as simple as **(a)**, with a single Fe ion surrounded by the S atoms of four Cys residues. Other centers include both inorganic and Cys S atoms, as in **(b)** 2Fe-2S or **(c)** 4Fe-4S centers. **(d)** The ferredoxin of the cyanobacterium *Anabaena* 7120 has one 2Fe-2S center (PDB ID 1FRD); Fe is red, inorganic S_2 is yellow, and the S of Cys is orange. (Note that in these designations only the inorganic S atoms are counted. For example, in the 2Fe-2S center **(b)**, each Fe ion is actually surrounded by four S atoms.) The exact standard reduction potential of the iron in these centers depends on the type of center and its interaction with the associated protein.

table 19–2

Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

Redox reaction (half-reaction)	E'° (V)
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome *b* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *c* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.55
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816

（三）呼吸链的结构

1. 氧化还原电位
2. 阻断剂实验
3. 亚组分重组实验

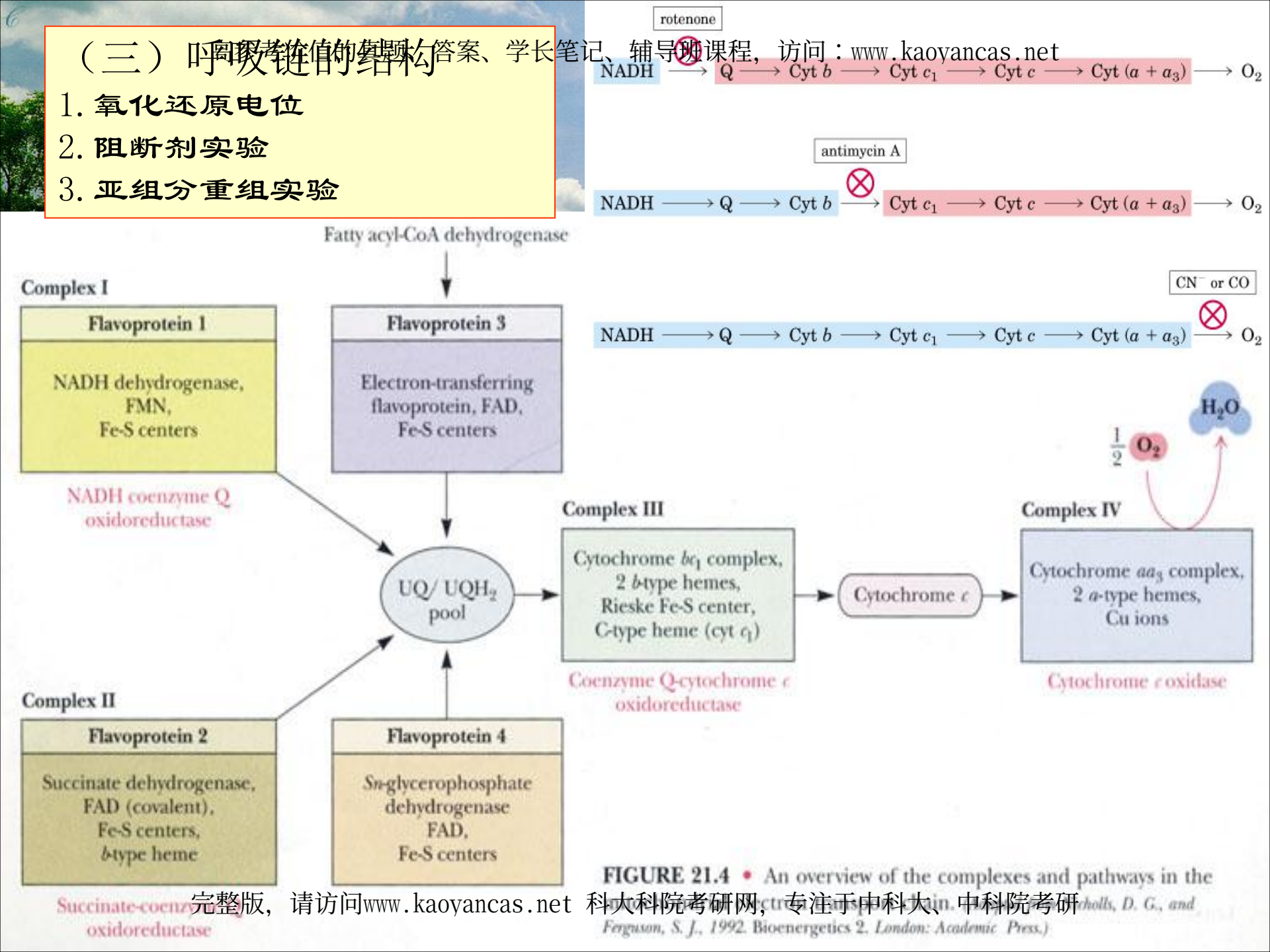


FIGURE 21.4 • An overview of the complexes and pathways in the [illegible] Holls, D. G., and Ferguson, S. J., 1992. Bioenergetics 2. London: Academic Press.)

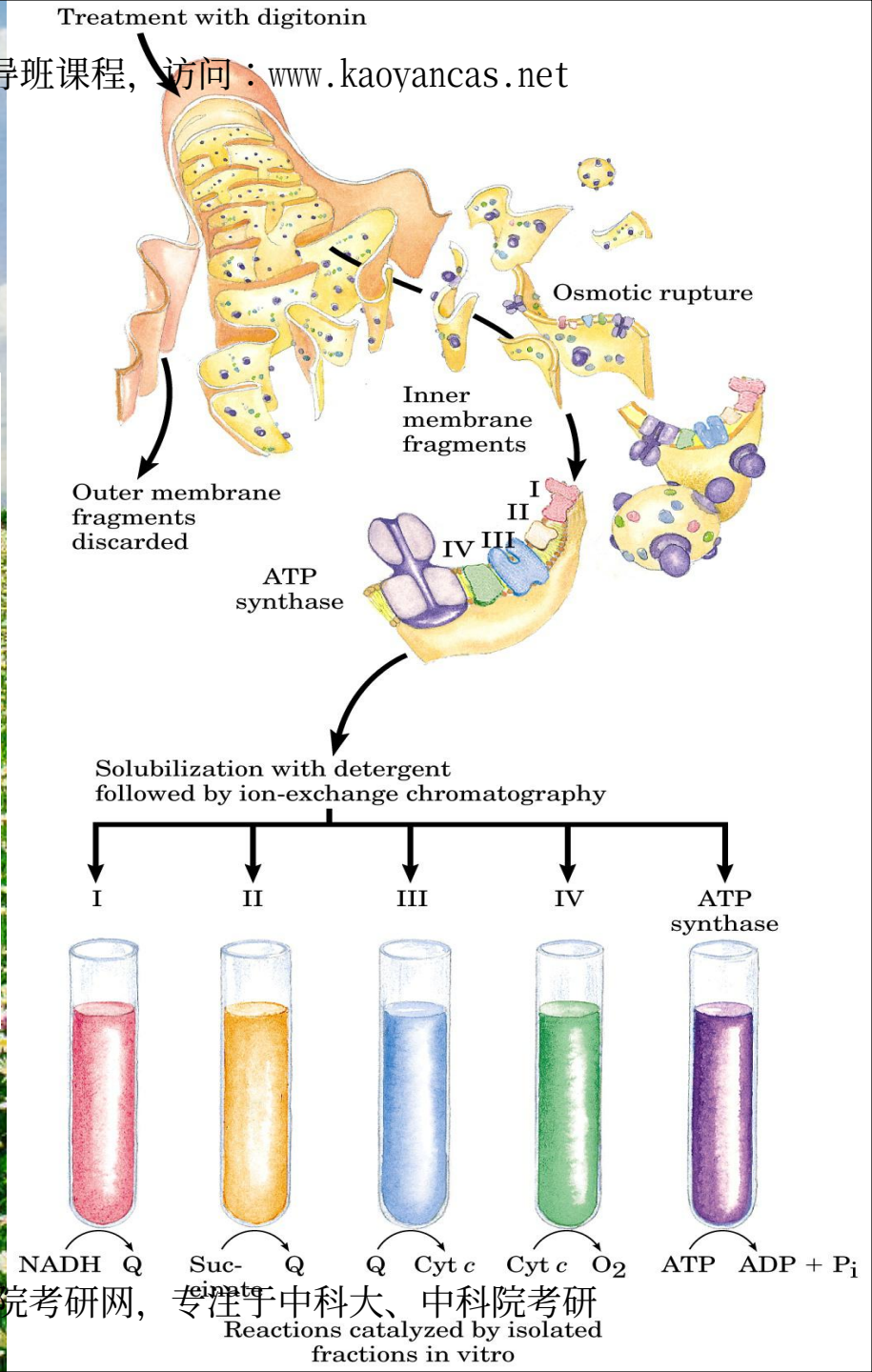
table 19–3

Protein Components of the Mitochondrial Electron-Transfer Chain

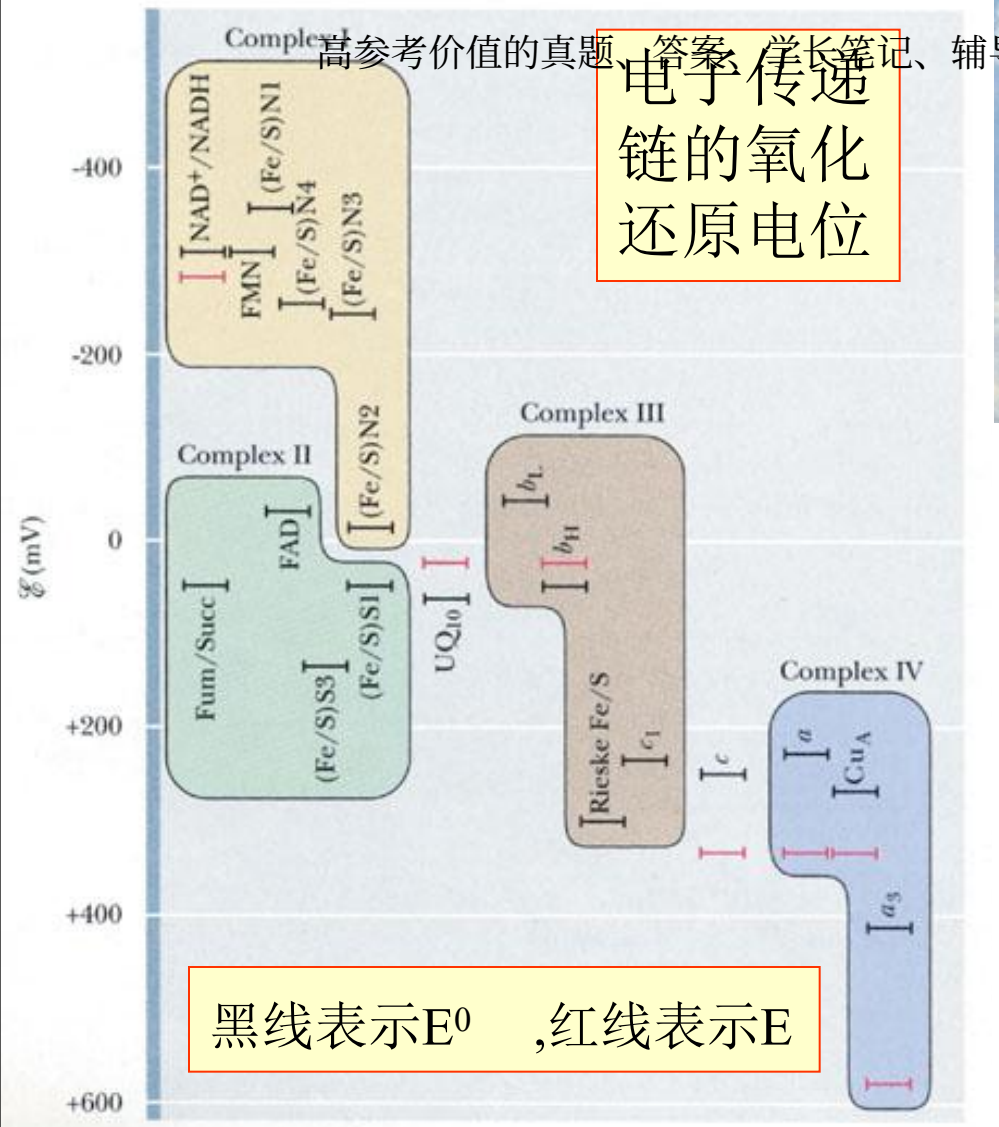
Enzyme complex	Mass (kDa)	Number of subunits*	Prosthetic group(s)
I NADH dehydrogenase	850	42 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	5	FAD, Fe-S
III Ubiquinone: cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome *c*†	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

*Numbers of subunits in the bacterial equivalents in parentheses.

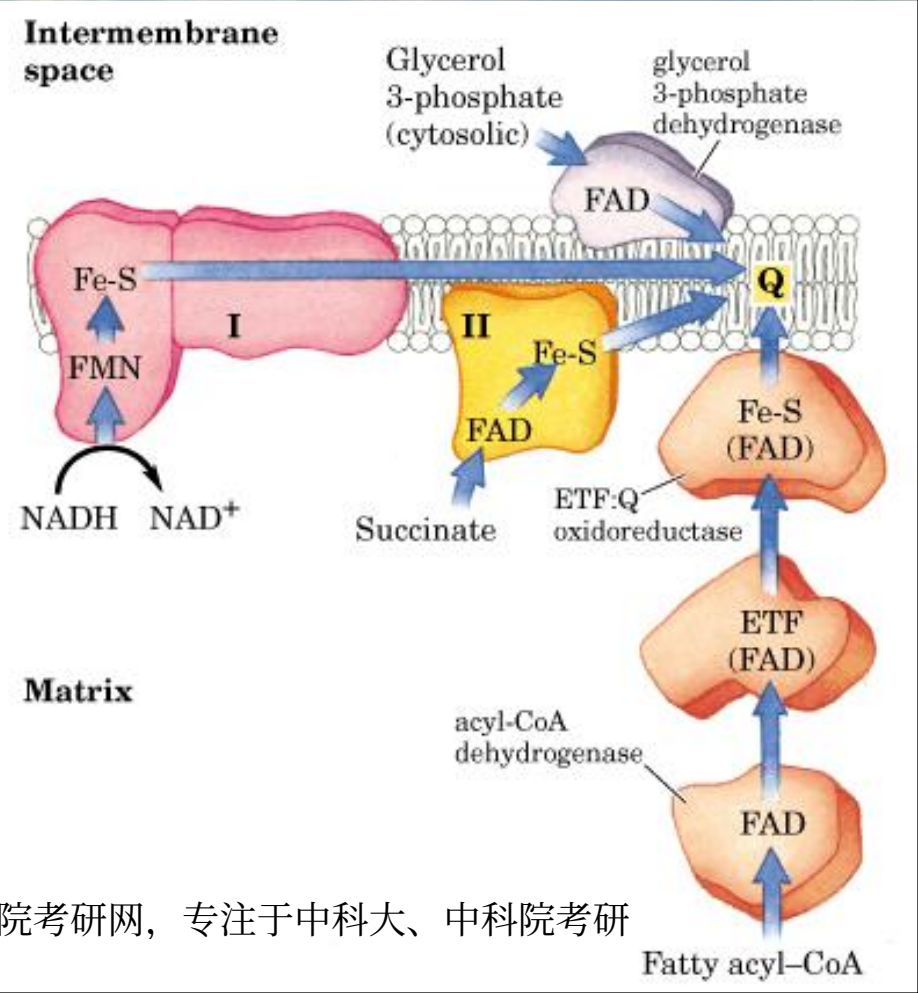
†Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.



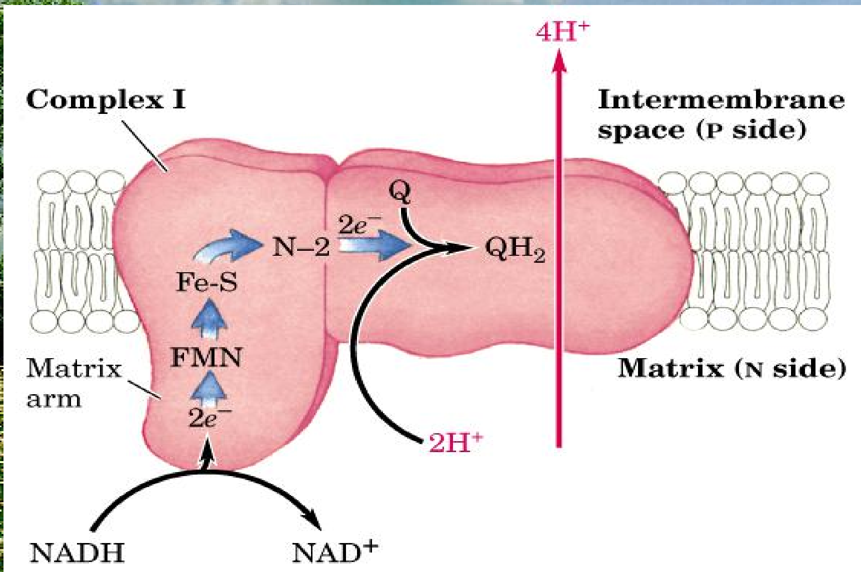
电子传递链的氧化还原电位



黑线表示E^0 ,红线表示E



4.线粒体内膜的电子传递途径



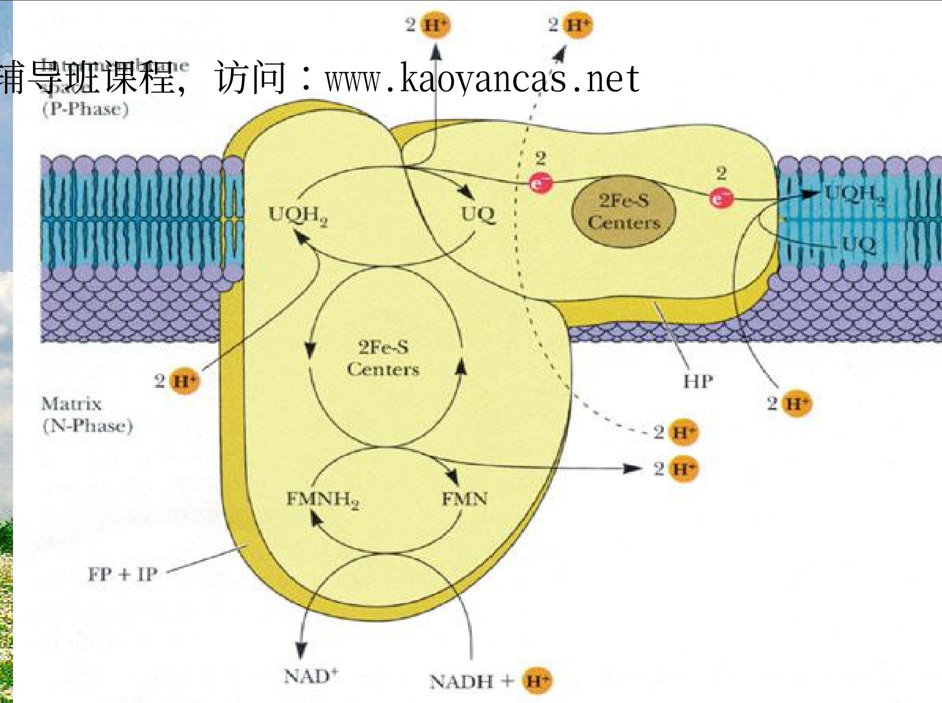
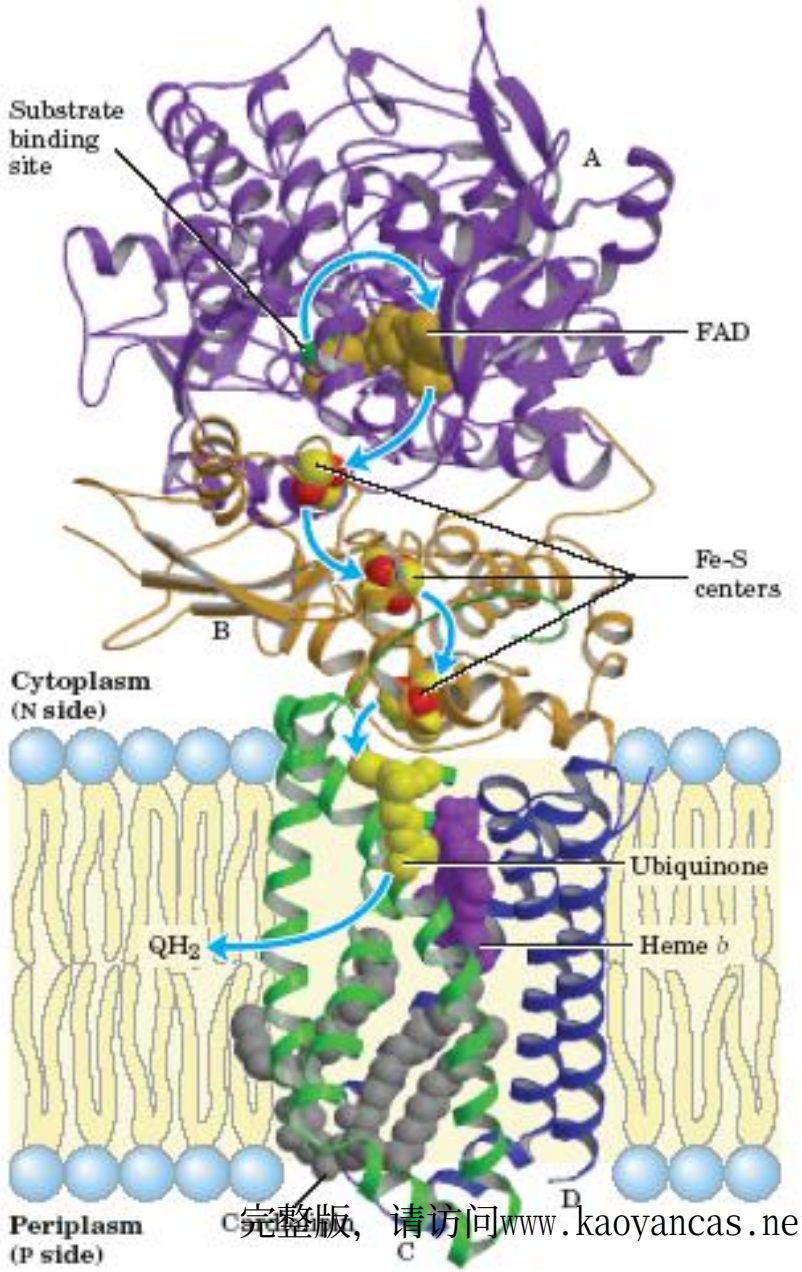


FIGURE 19–9 NADH:ubiquinone oxidoreductase (Complex I). Complex I catalyzes the transfer of a hydride ion from NADH to FMN, from which two electrons pass through a series of Fe-S centers to the iron-sulfur protein N-2 in the matrix arm of the complex. Electron transfer from N-2 to ubiquinone on the membrane arm forms QH_2, which diffuses into the lipid bilayer. This electron transfer also drives the expulsion from the matrix of four protons per pair of electrons. The detailed mechanism that couples electron and proton transfer in Complex I is not yet known, but probably involves a Q cycle similar to that in Complex III in which QH_2 participates twice per electron pair (see Fig. 19–12). Proton flux produces an electrochemical potential across the inner mitochondrial membrane (N side negative, P side positive), which conserves some of the energy released by the oxidation-reduction reactions. This electrochemical potential drives ATP synthesis.

复合体 的电子传递途径

复合体 的结构和电子传递途径



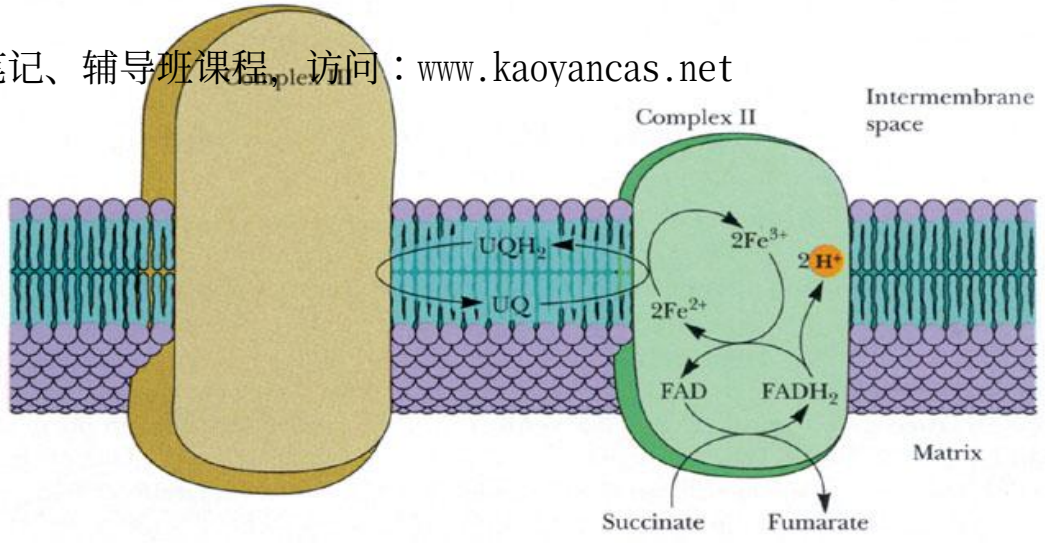
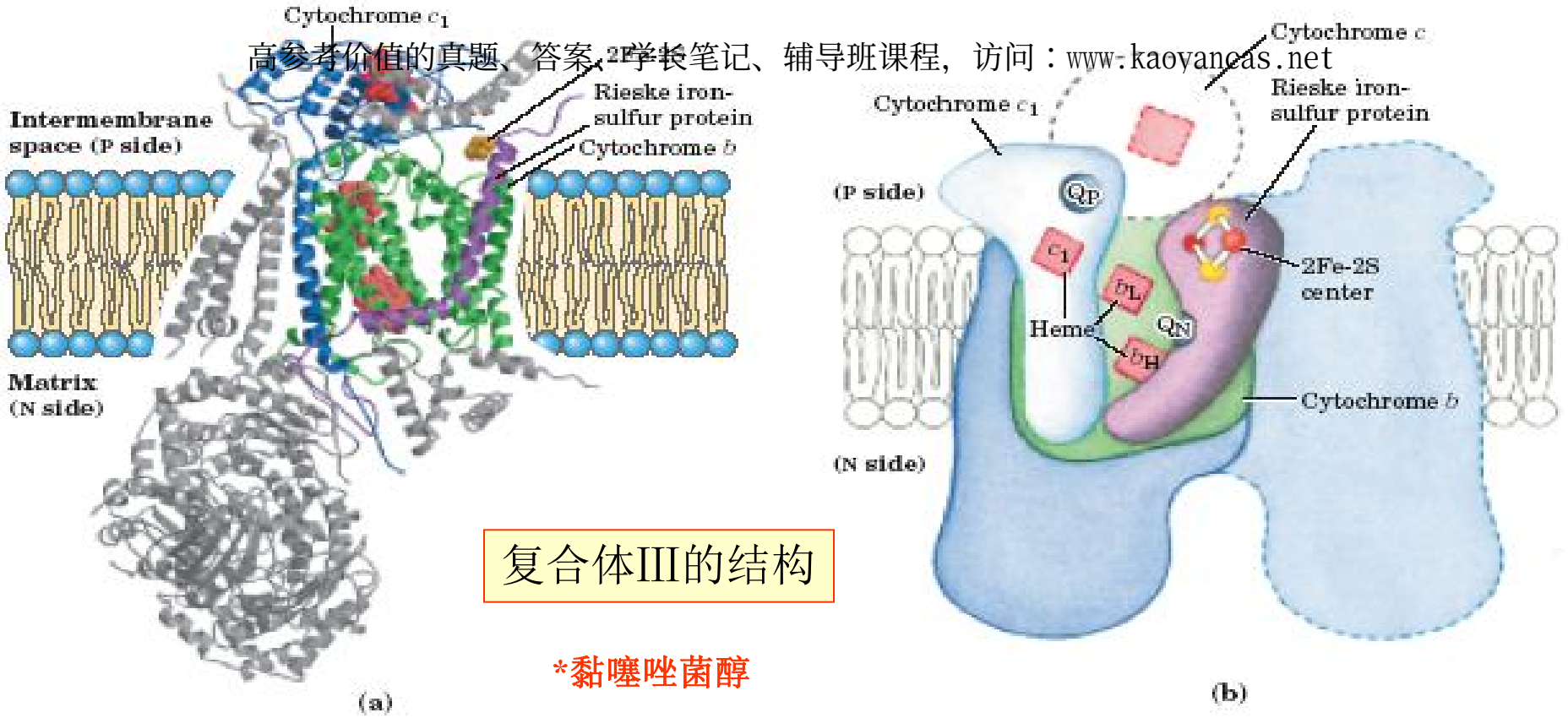


FIGURE 19–10 Structure of Complex II (succinate dehydrogenase) of *E. coli* (PDB ID 1NEK). The enzyme has two transmembrane subunits, C (green) and D (blue); the cytoplasmic extensions contain subunits B (orange) and A (purple). Just behind the FAD in subunit A (gold) is the binding site for succinate (occupied in this crystal structure by the inhibitor oxaloacetate, green). Subunit B has three sets of Fe-S centers (yellow and red); ubiquinone (yellow) is bound to subunit C; and heme *b* (purple) is sandwiched between subunits C and D. A cardiolipin molecule is so tightly bound to subunit C that it shows up in the crystal structure (gray spacefilling). Electrons move (blue arrows) from succinate to FAD, then through the three Fe-S centers to ubiquinone. The heme *b* is not on the main path of electron transfer but protects against the formation of reactive oxygen species (ROS) by electrons that go astray.



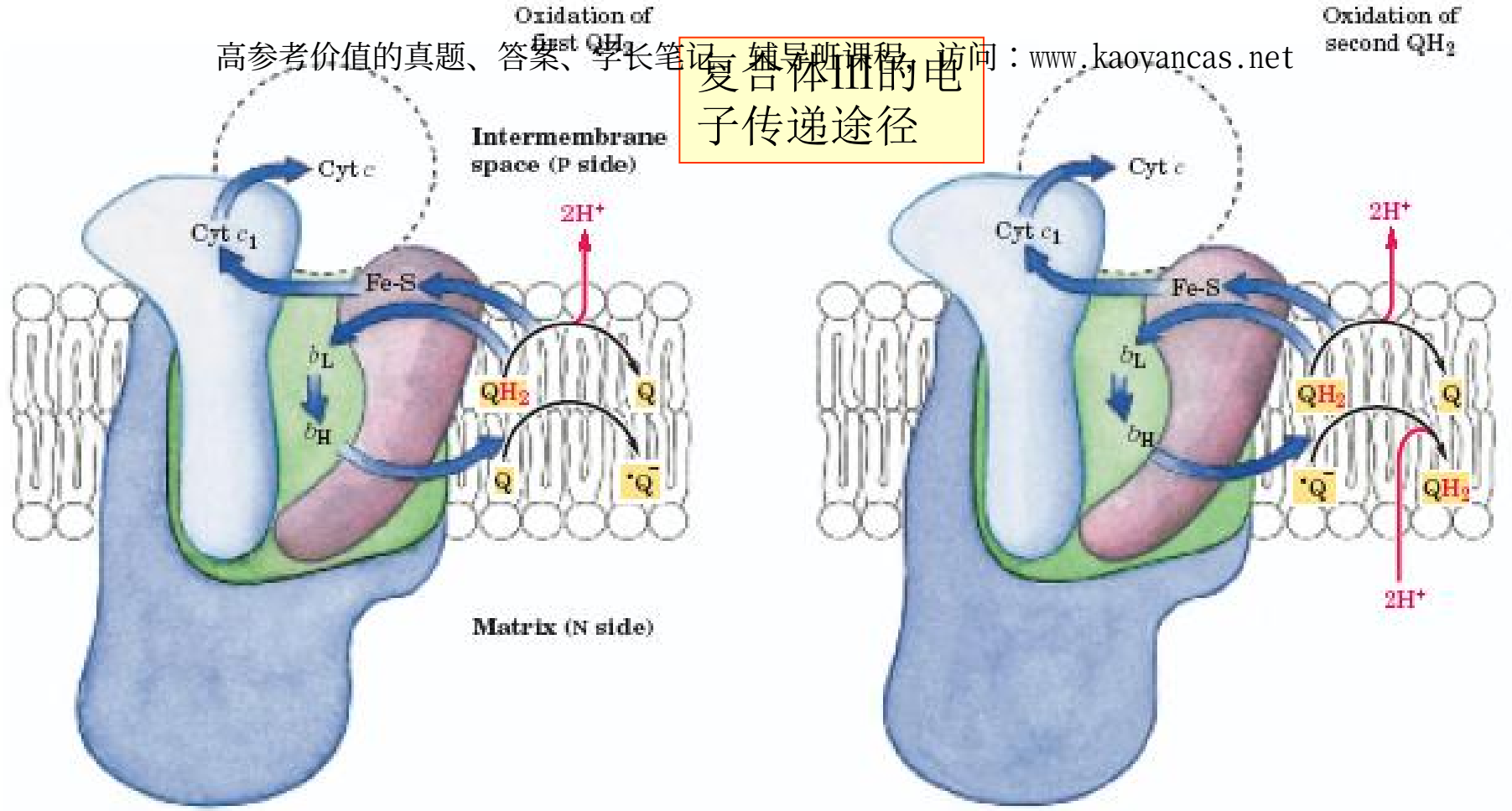
复合体III的结构

*黏噻唑菌醇

FIGURE 19-11 Cytochrome bc_1 complex (Complex III). The complex is a dimer of identical monomers, each with 11 different subunits. (a) Structure of a monomer. The functional core is three subunits: cytochrome *b* (green) with its two hemes (b_H and b_L, light red); the Rieske iron-sulfur protein (purple) with its 2Fe-2S centers (yellow); and cytochrome c_1 (blue) with its heme (red) (PDB ID 1BGY). (b) The dimeric functional unit. Cytochrome c_1 and the Rieske iron-sulfur protein project from the P surface and can interact with cytochrome *c* (not part of the functional complex) in the intermembrane space. The complex has two distinct binding sites for ubiquinone, Q_N and Q_P, which correspond to the sites of inhibition by two drugs that block oxidative phosphorylation. Antimycin A, which blocks electron flow from heme b_H to Q_N, inhibits the heme b_H to heme b_L step. Myxothiazol*, which prevents electron flow from QH_2 to the Rieske iron-sulfur protein, binds at Q_P, near the 2Fe-2S center and heme b_L on the P side. The dimeric structure is essential to the function of Complex III. The interface between monomers forms two pockets, each containing a Q_P site from one monomer and a Q_N site from the other. The ubiquinone intermediates move within these sheltered pockets.

Complex III crystallizes in two distinct conformations (not shown). In one, the Rieske Fe-S center is close to its electron acceptor, the heme of cytochrome c_1, but relatively distant from cytochrome *b* and the QH_2-binding site at which the Rieske Fe-S center receives electrons. In the other, the Fe-S center has moved away from cytochrome c_1 and toward cytochrome *b*. The Rieske protein is thought to oscil-

复合体III的电子传递途径



$$QH_2 + \text{cyt } c_1(\text{oxidized}) \longrightarrow {}^{\cdot}Q^- + 2H_P^+ + \text{cyt } c_1(\text{reduced})$$

$$QH_2 + {}^{\cdot}Q^- + 2H_N^+ + \text{cyt } c_1(\text{oxidized}) \longrightarrow QH_2 + 2H_P^+ + Q + \text{cyt } c_1(\text{reduced})$$

Net equation:

$$QH_2 + 2\ \text{cyt } c_1(\text{oxidized}) + 2H_N^+ \longrightarrow Q + 2\ \text{cyt } c_1(\text{reduced}) + 4H_P^+$$

FIGURE 19–12 The Q cycle. The path of electrons through Complex III is shown by blue arrows. On the P side of the membrane, two molecules of QH_2 are oxidized, one via the Rieske Fe-S center and one via cytochrome *b*, releasing a total of four protons per Q (four protons in all) into the intermembrane space. Each QH_2 donates one electron (via the Rieske Fe-S center) to cytochrome c_1, and one electron (via cytochrome *b*) to a molecule of Q near the N side, producing it in two steps to QH_2. This reduction also uses two protons per Q, which are taken up from the matrix.

复合体Ⅳ的结构

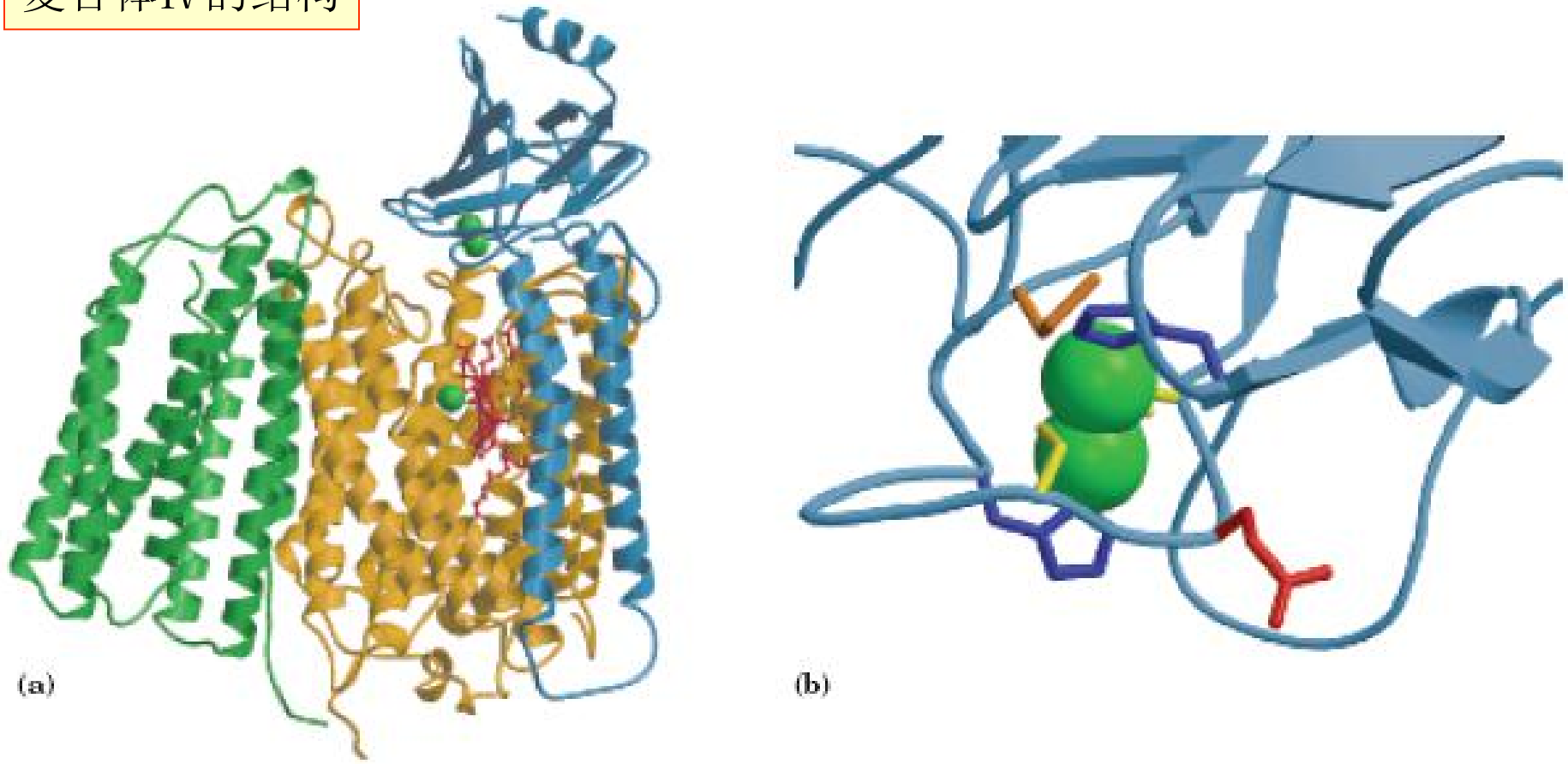


FIGURE 19-13 Critical subunits of cytochrome oxidase (Complex IV). The bovine complex is shown here (PDB ID 1OCC). (a) The core of Complex IV, with three subunits. Subunit I (yellow) has two heme groups, *a* and a_3 (red), and a copper ion, Cu_B (green sphere). Heme a_3 and Cu_B form a binuclear Fe-Cu center. Subunit II (blue) contains two Cu ions (green spheres) complexed with the —SH groups of two Cys residues in a binuclear center, Cu_A, that resembles the 2Fe-2S centers of iron-sulfur proteins. This binuclear center and the cytochrome *c*-binding site are located in a domain of subunit II that protrudes from the P side of the inner membrane (into the intermembrane space). Subunit III (green) seems to be essential for Complex IV function, but its role is not well understood. (b) The binuclear center of Cu_A. The Cu ions (green spheres) share electrons equally. When the center is reduced they have the formal charges $Cu^{1+}Cu^{1+}$; when oxidized, $Cu^{1.5+}Cu^{1.5+}$. Ligands around the Cu ions include two His (dark blue), two Cys (yellow), an Asp (red), and Met (orange) residues.

复合体Ⅳ的电子传递

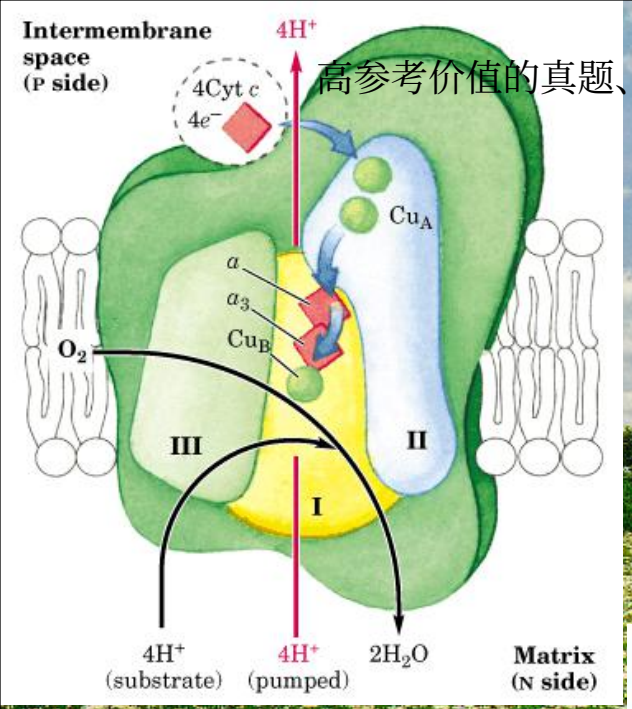
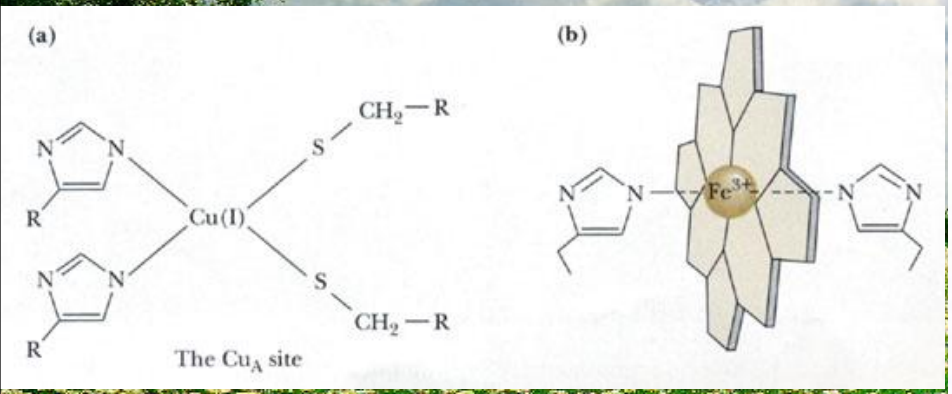
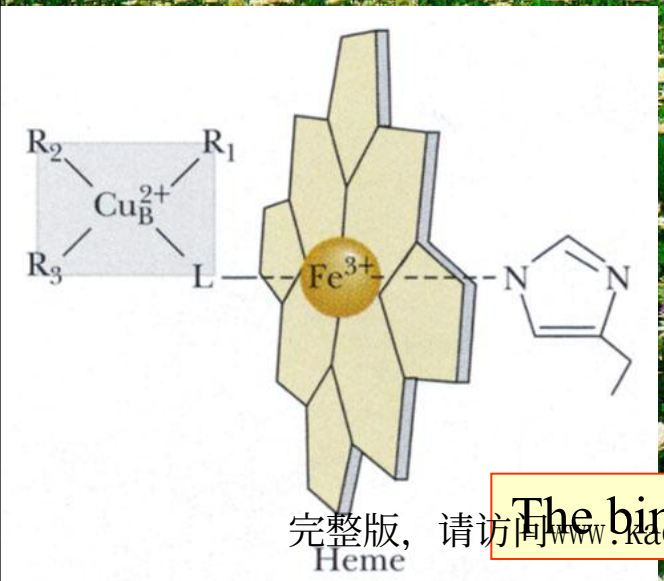


FIGURE 19–14 Path of electrons through Complex IV. The three proteins critical to electron flow are subunits I, II, and III. The larger green structure includes the other ten proteins in the complex. Electron transfer through Complex IV begins when two molecules of reduced cytochrome *c* (top) each donate an electron to the binuclear center Cu_A. From here electrons pass through heme a to the Fe-Cu center (cytochrome a_3 and Cu_B). Oxygen now binds to heme a_3 and is reduced to its peroxy derivative (O_2^{2-}) by two electrons from the Fe-Cu center. Delivery of two more electrons from cytochrome *c* (making four electrons in all) converts the O_2^{2-} to two molecules of water, with consumption of four "substrate" protons from the matrix. At the same time, four more protons are pumped from the matrix by an as yet unknown mechanism.

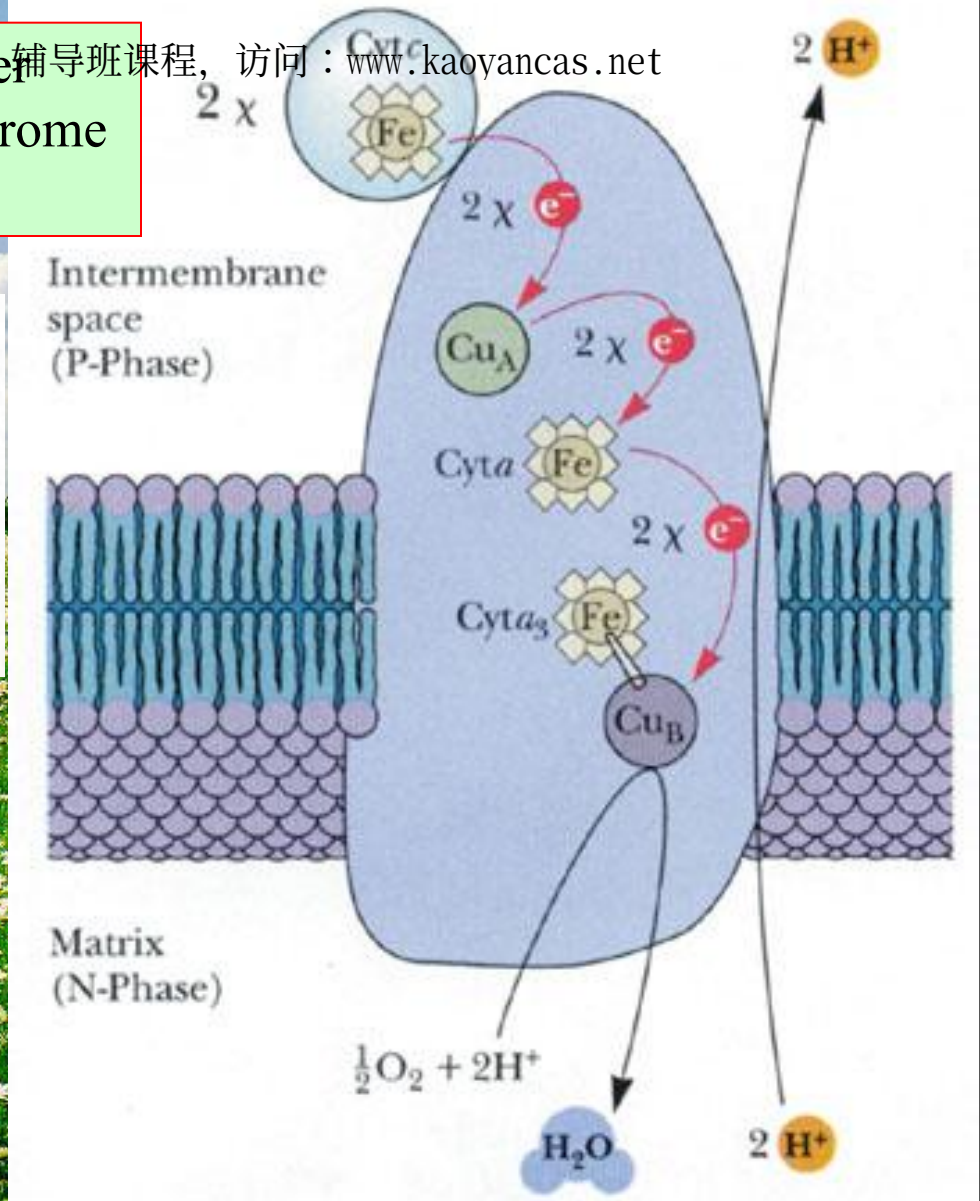
The electron transfer pathway for cytochrome oxidase.

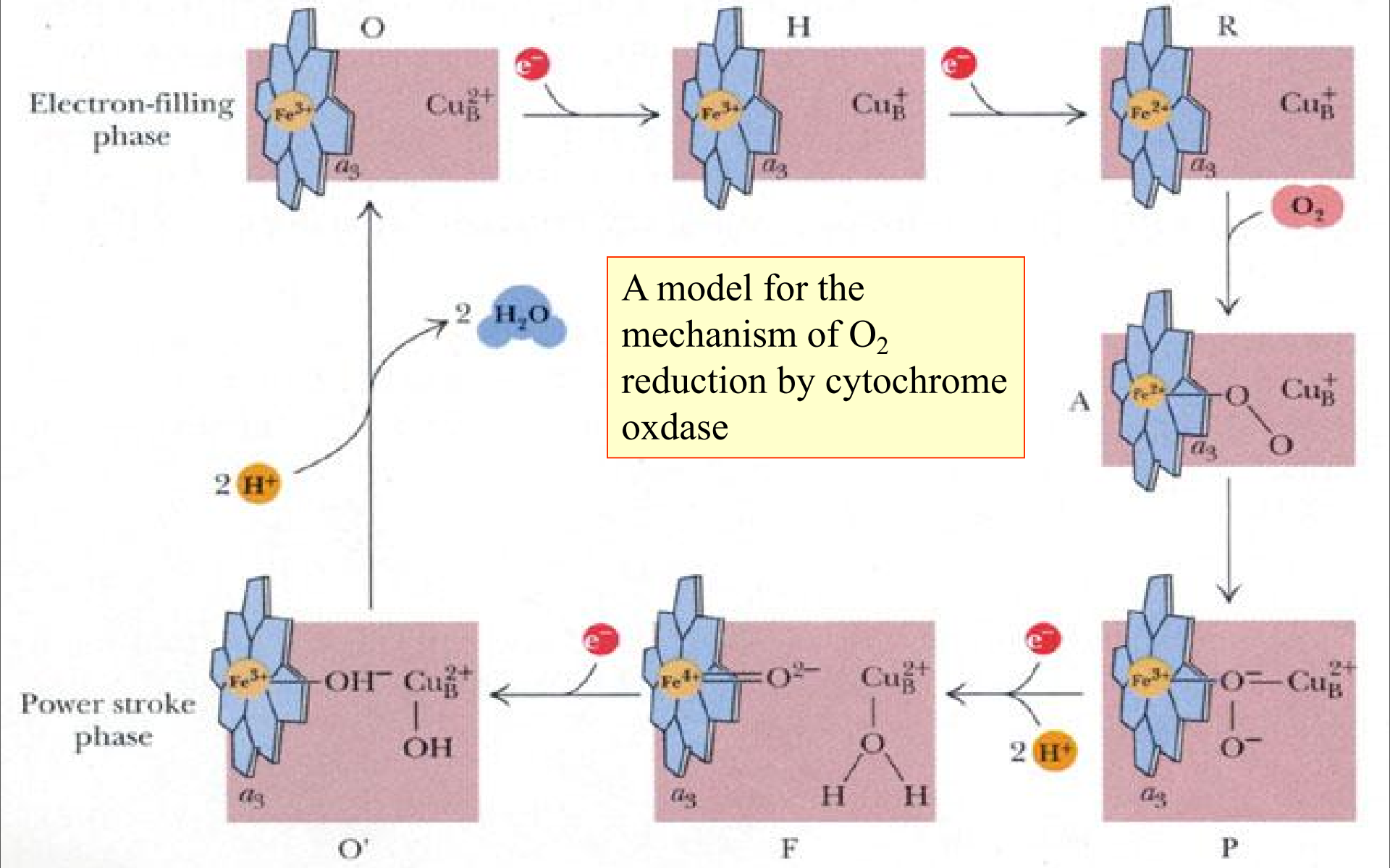


The CuA site of cytochrome oxdase.

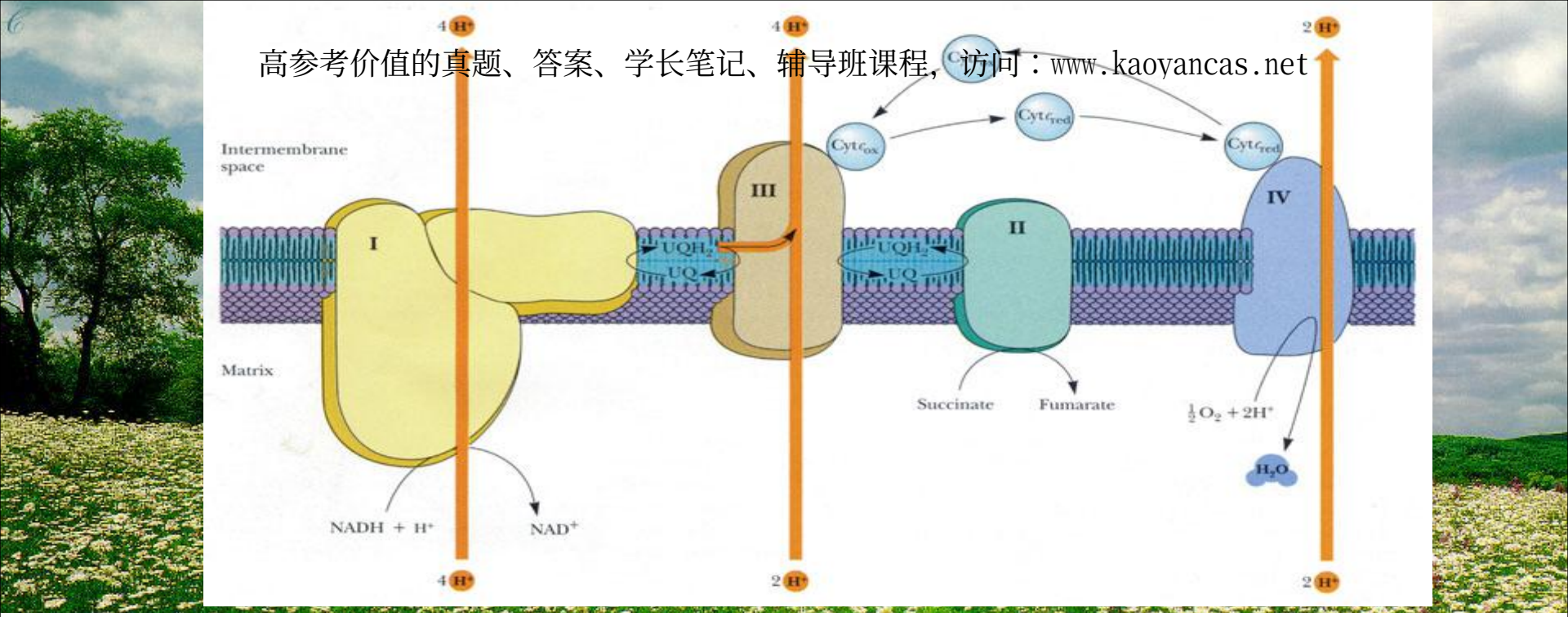


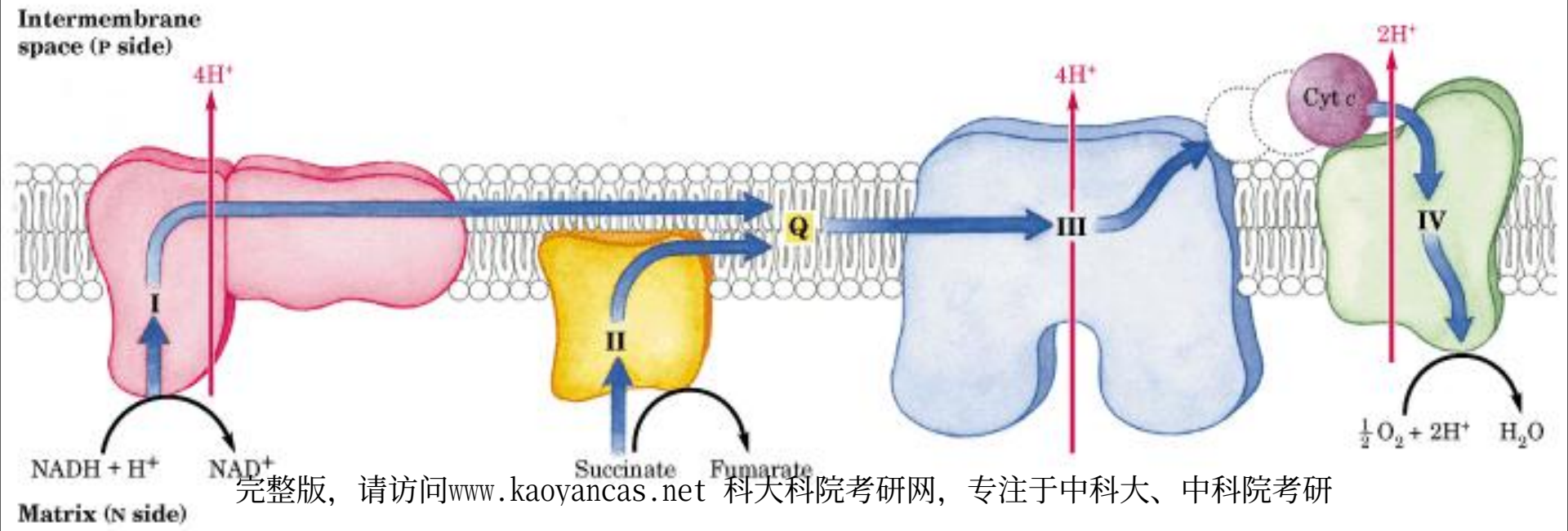
The binuclear center of cytochrome oxdase





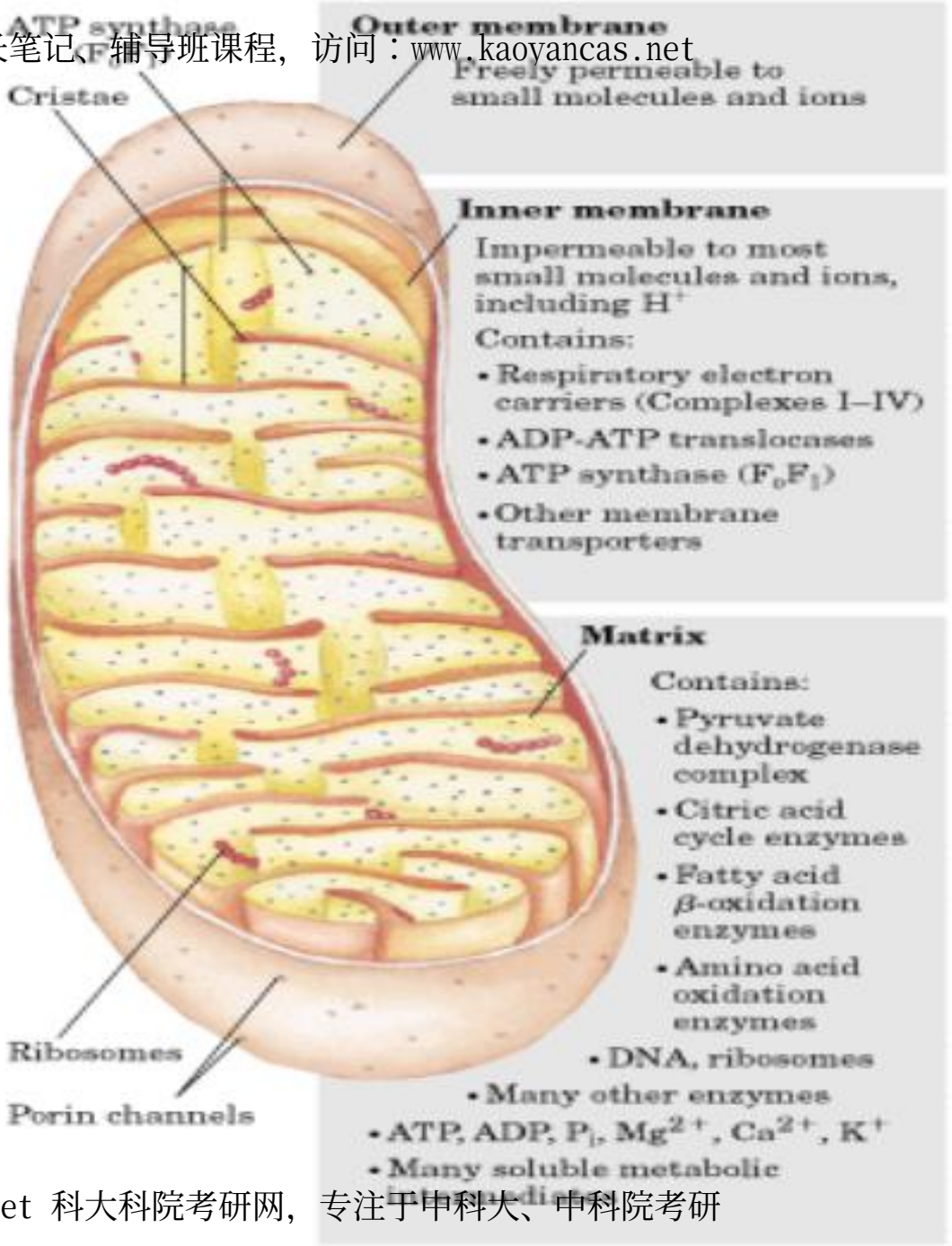
A model for the mechanism of O_2 reduction by cytochrome oxdase



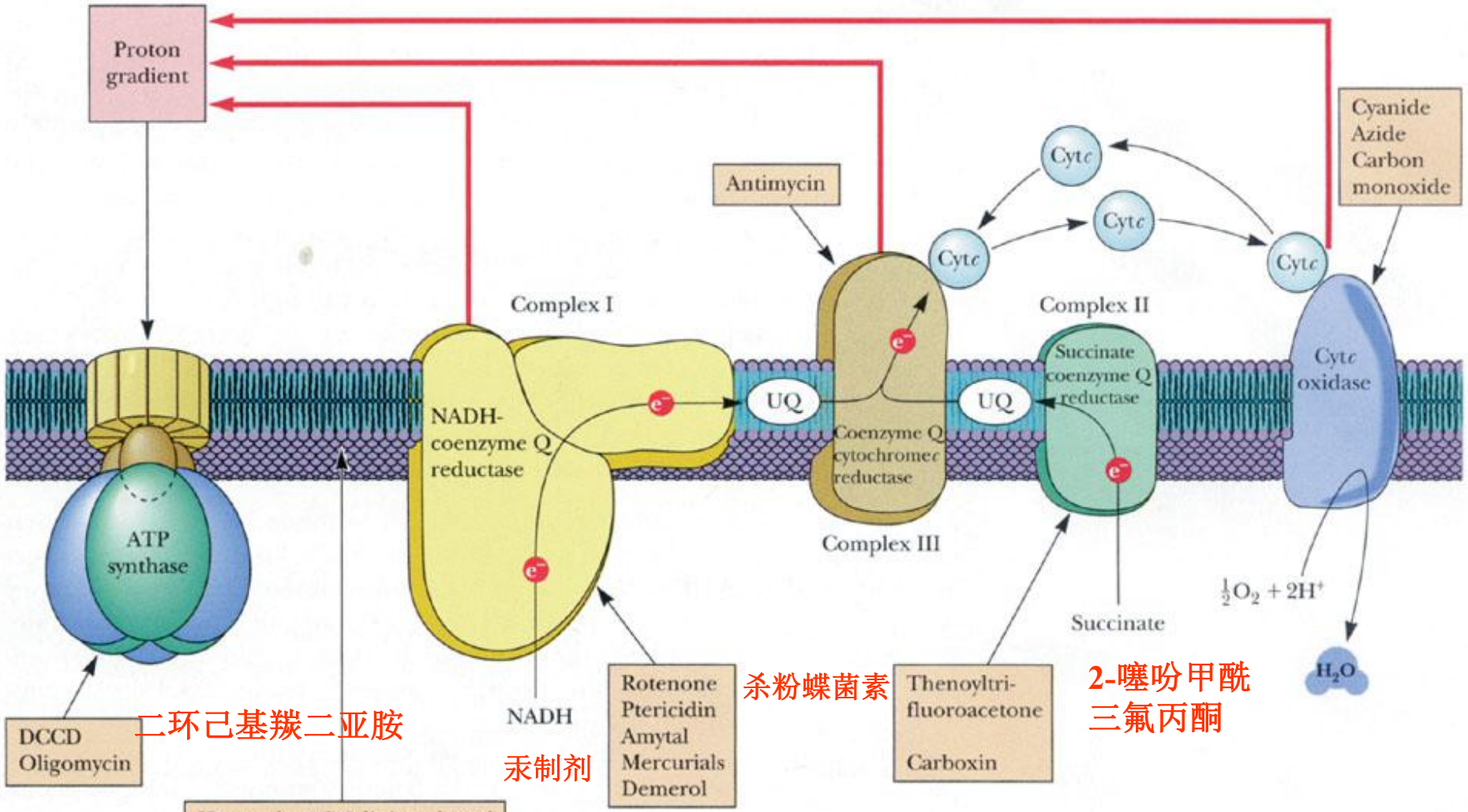


三、氧化磷酸化作用

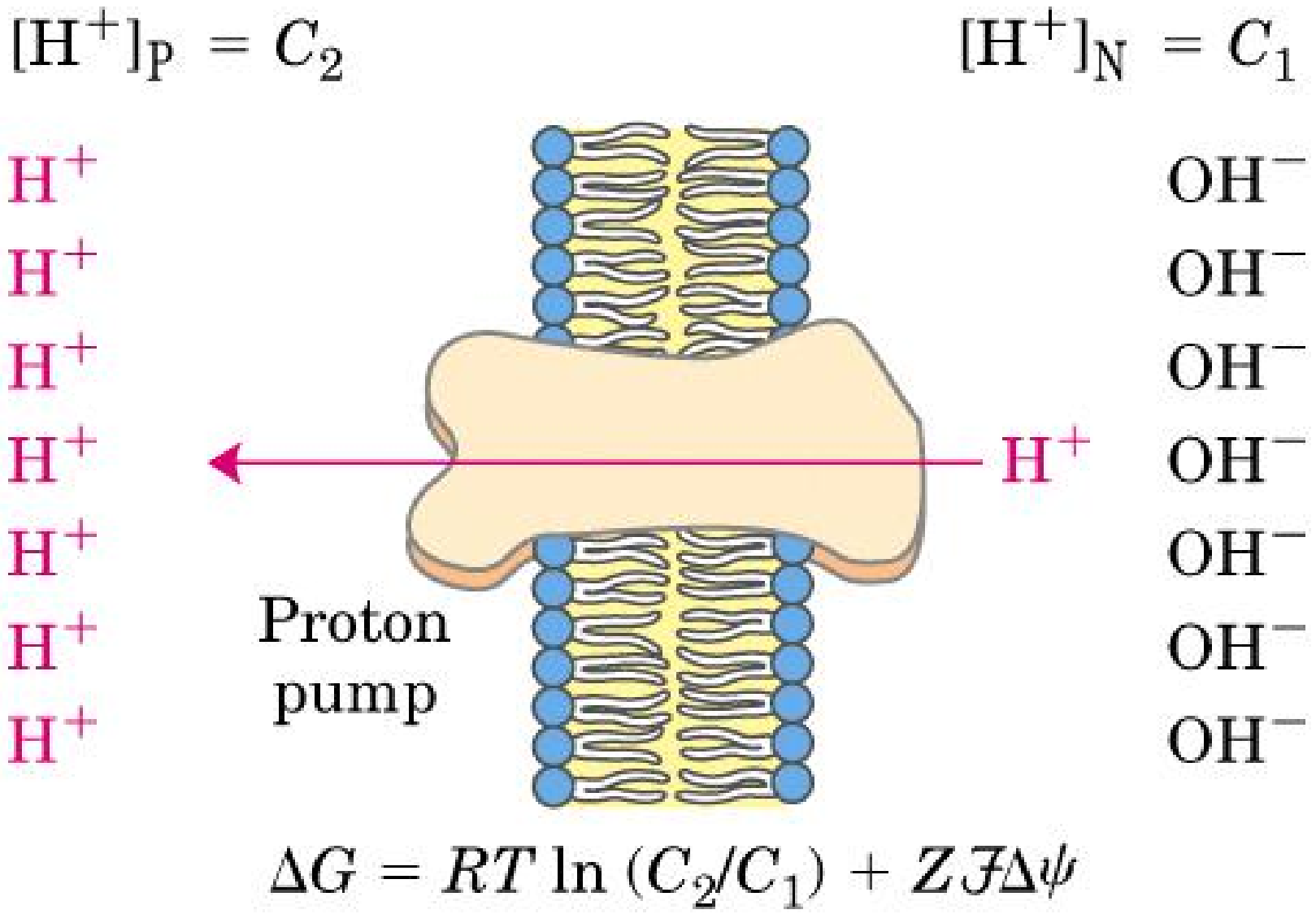
（一）线粒体的结构



（二）氧化磷酸化作用机制

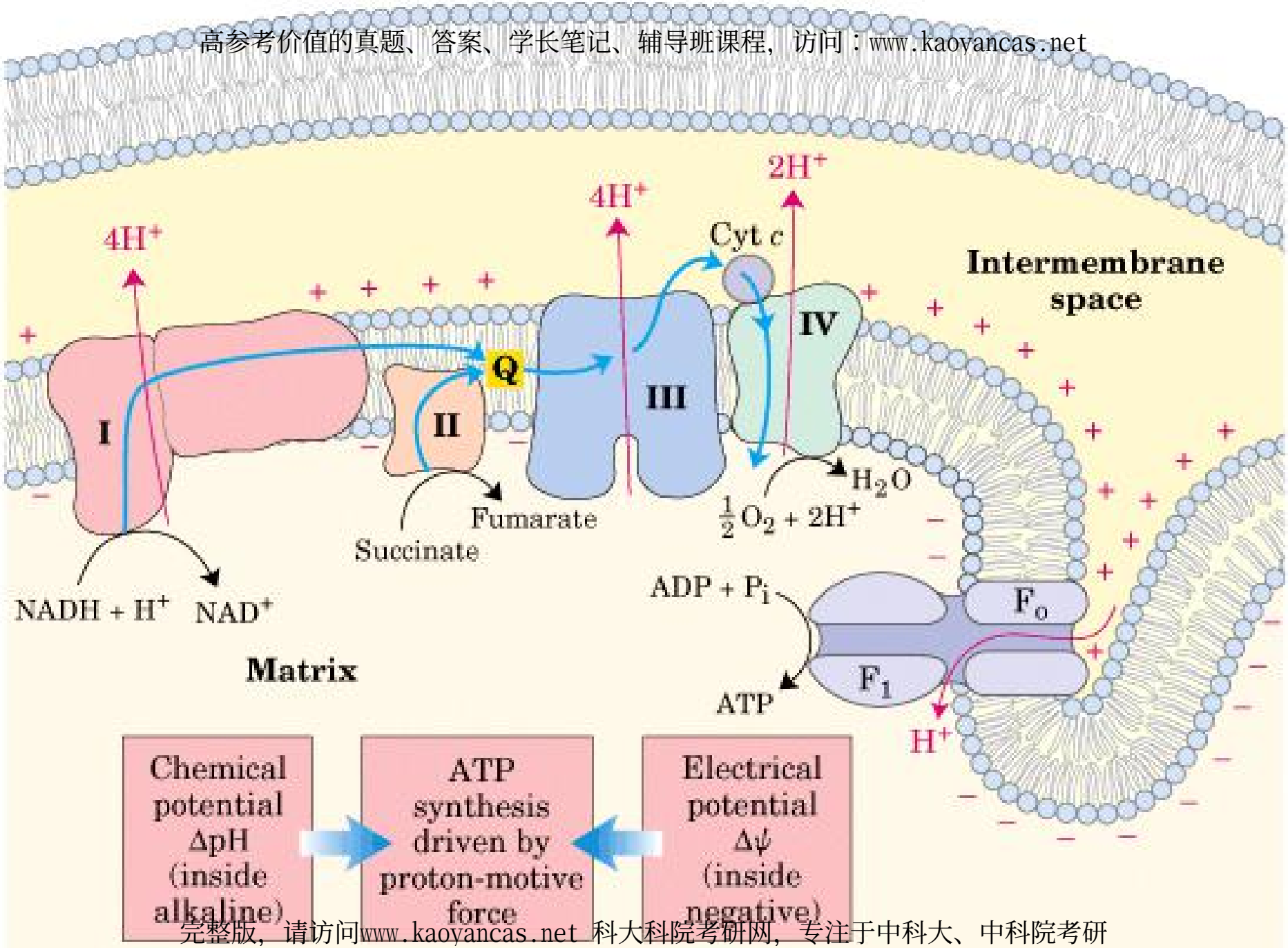


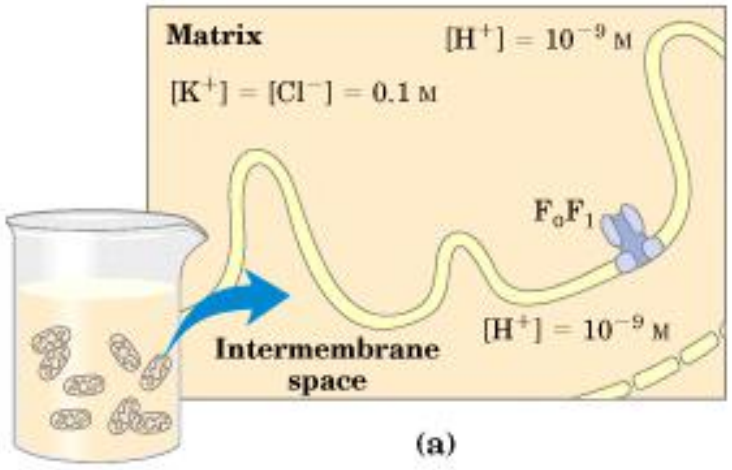
（三）质子梯度的形式



$$\Delta G = RT \ln (C_2/C_1) + Z\mathcal{F}\Delta\psi$$

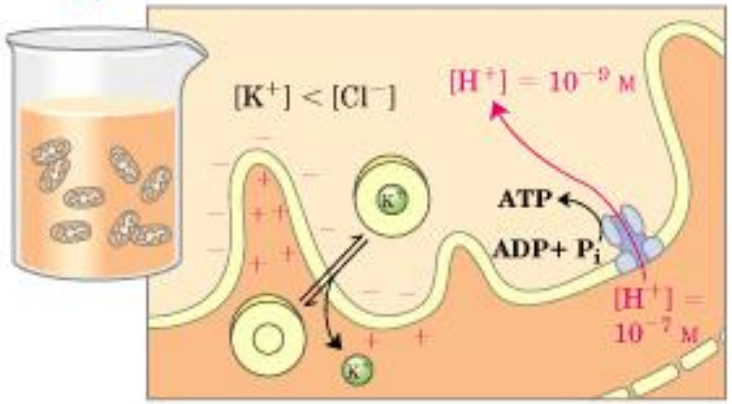
$$= 2.3RT\,\Delta pH + \mathcal{F}\Delta\psi$$





(a)

pH lowered from 9 to 7;
valinomycin present; no K^+



(b)

The Nobel Prize in Chemistry 1978

"for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory"

Press release

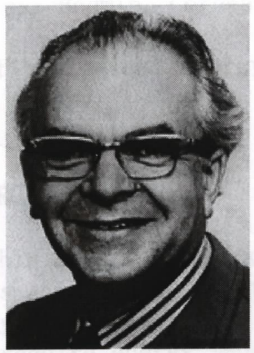
Peter D. Mitchell

Great Britain

Glynn Research Laboratories
Bodmin, Great Britain

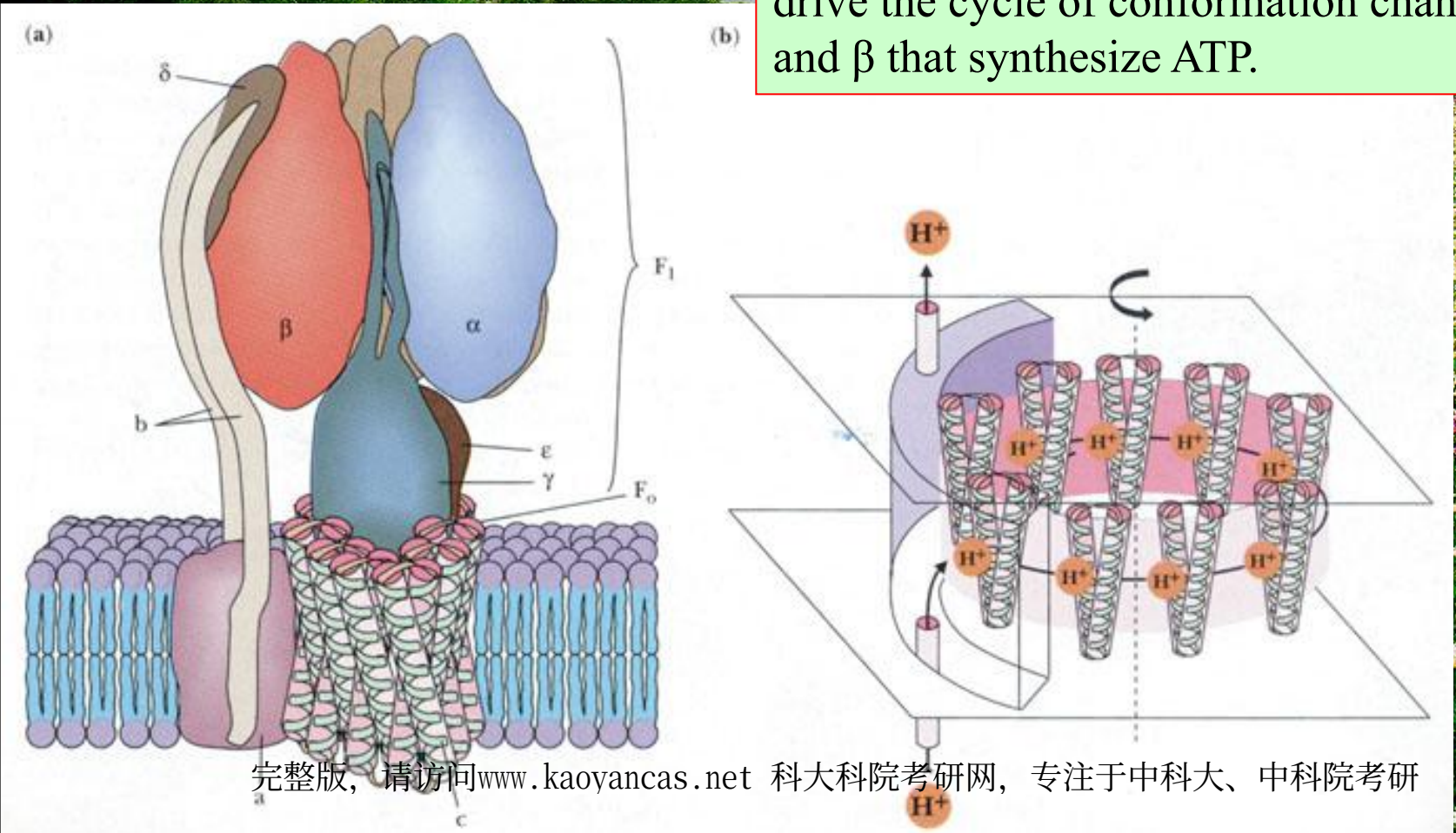
1920 - 1992

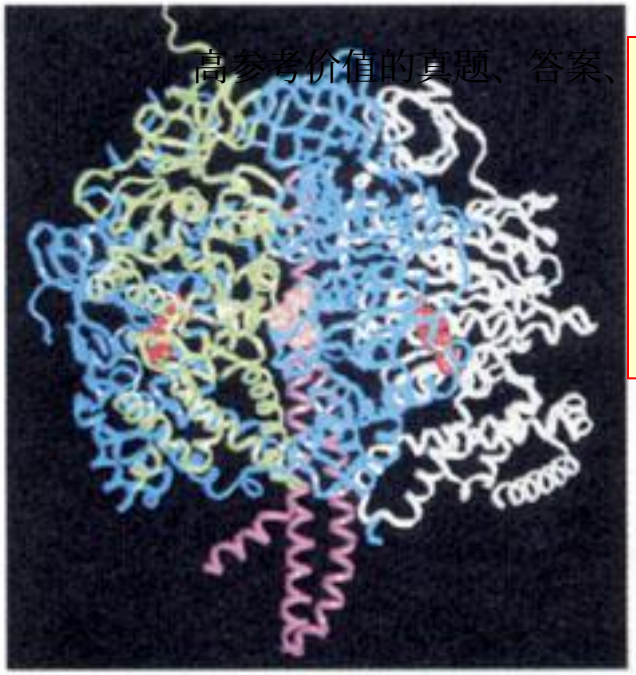
Biography



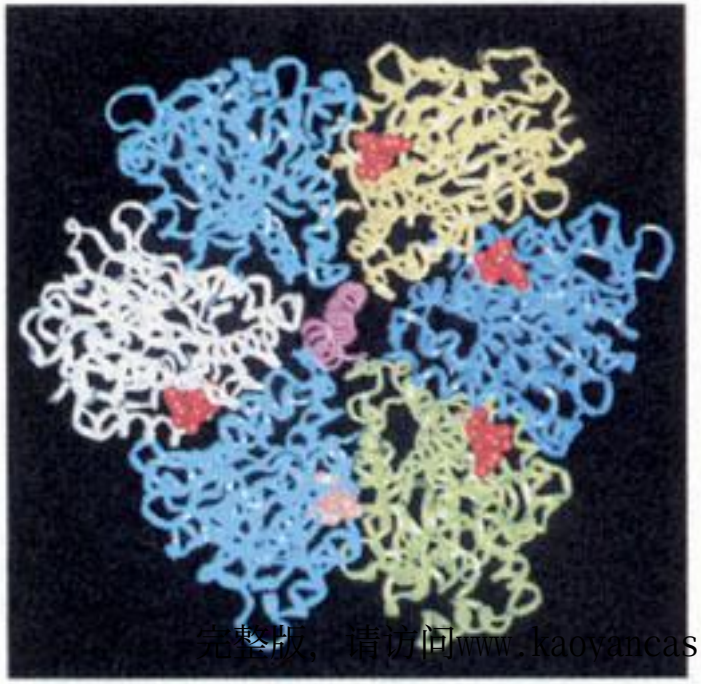
（四）ATP合成机制

A model of the F_1 and F_0 components of the ATP synthase .a rotation molecular motor.The a,b,α,β,and δ subunits constiture the stator of the motor,and the c,γ,and ε subunits form the rotor.Flow of protons through the structure turns the rotor and drive the cycle of conformation changes in α and β that synthesize ATP.





(a)

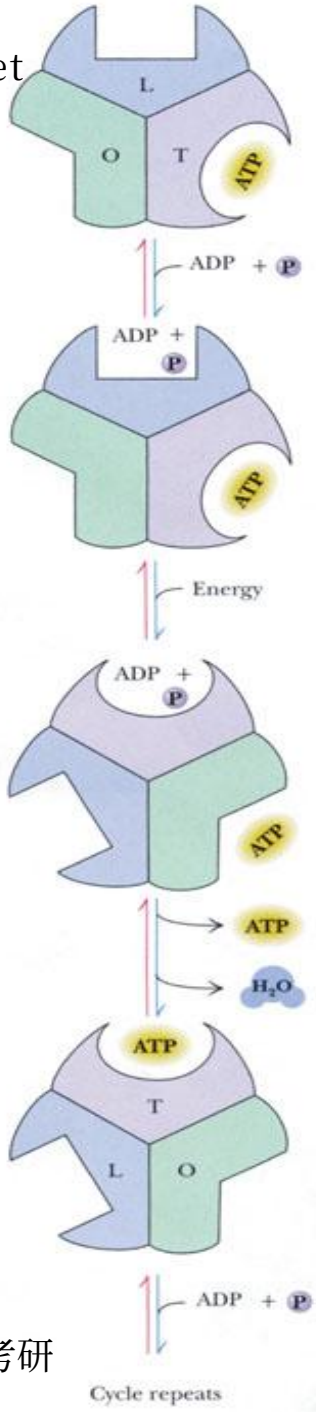


(b)

Molecular graphic images (a) side view and (b) top view of F1-ATP synthase.The γ-subunit is the pink structure visible in the center of view (b).

The binding change mechanism for ATP synthesis by ATP synthase

实验证明3个质子通过离子通道可以合成一个ATP



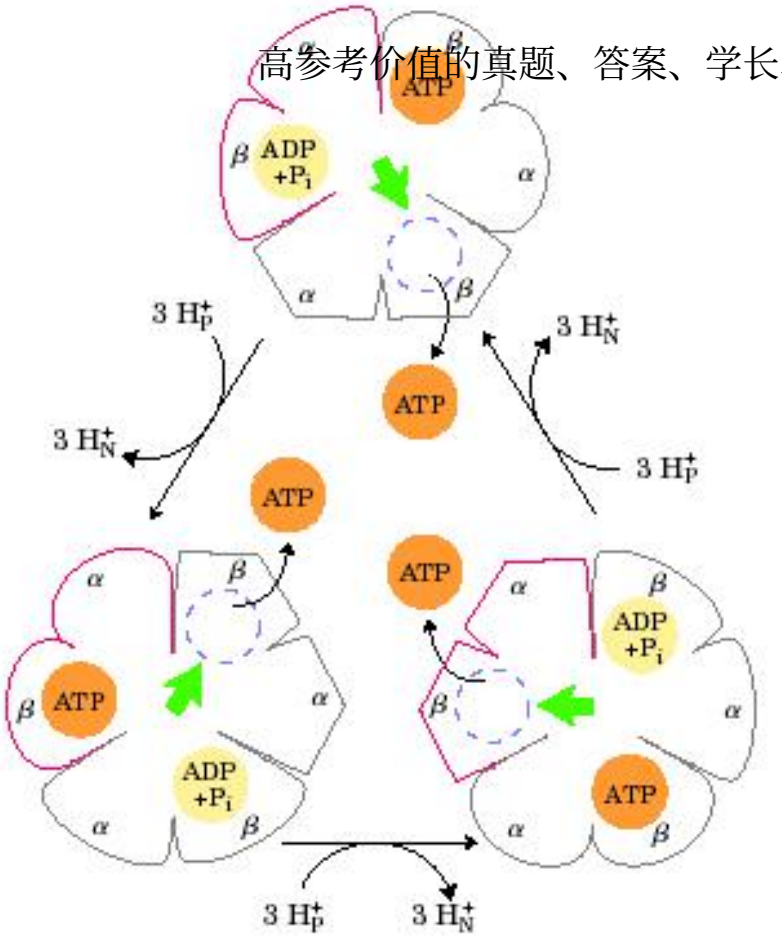
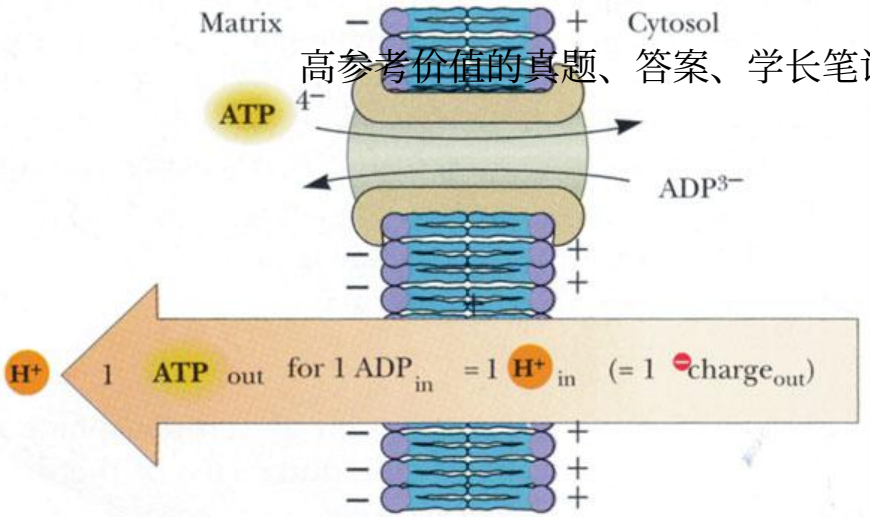


FIGURE 19–24 Binding-change model for ATP synthase. The F_1 complex has three nonequivalent adenine nucleotide–binding sites, one for each pair of α and β subunits. At any given moment, one of these sites is in the β-ATP conformation (which binds ATP tightly), a second is in the β-ADP (loose-binding) conformation, and a third is in the β-empty (very-loose-binding) conformation. The proton-motive force causes rotation of the central shaft—the γ subunit, shown as a green arrowhead—which comes into contact with each $\alpha\beta$ subunit pair in succession. This produces a cooperative conformational change in which the β-ATP site is converted to the β-empty conformation, and ATP dissociates; the β-ADP site is converted to the β-ATP conformation, which promotes condensation of bound ADP + P_i to form ATP; and the β-empty site becomes a β-ADP site, which loosely binds ADP + P_i entering from the solvent. This model, based on experimental findings, requires that at least two of the three catalytic sites alternate in activity; ATP cannot be released from one site unless and until ADP and P_i are bound at the other.



The Nobel Prize in Chemistry 1997

Press release

"for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)"

Outward trasport of ATP(via the ATP/ADP translocase) is favored by the membrane electrochemical potential

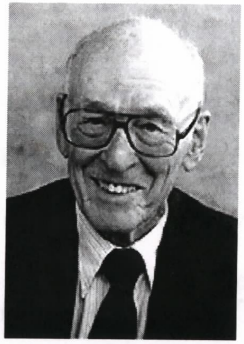
Paul D. Boyer

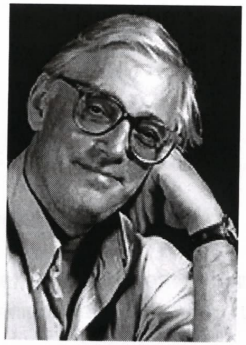
USA

University of California, Los Angeles, USA

1918 -

Autobiography
Nobel Diploma
Prize Award
Ceremony





John E. Walker

U.K.

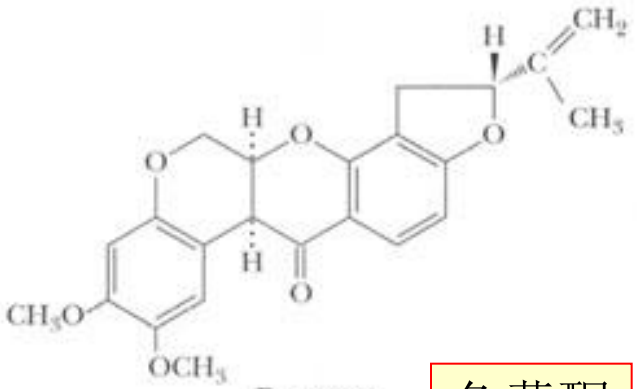
Medical Research Council Laboratory of Molecular Biology, Cambridge, U.K.

1941 -

Autobiography
Nobel Diploma
Prize Award
Ceremony

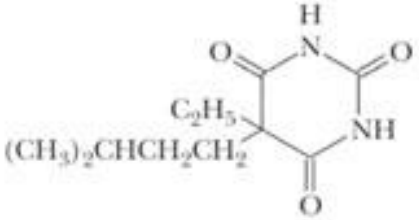
(五）氧化磷酸化的解偶联和抑制剂

电子传递链的抑制剂



Rotenone

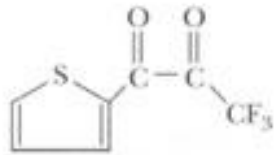
鱼藤酮



Amytal (amobarbital)

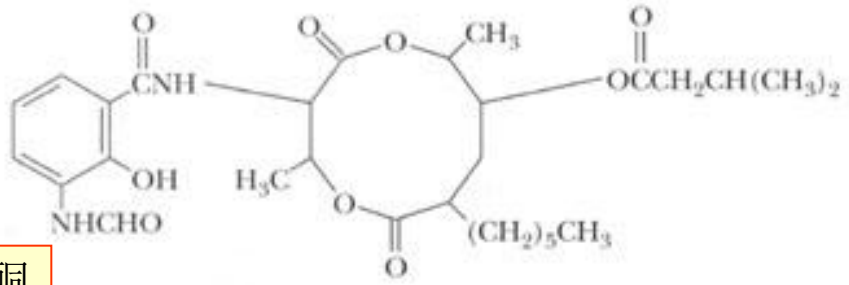
安密妥

Demerol (meperdine)

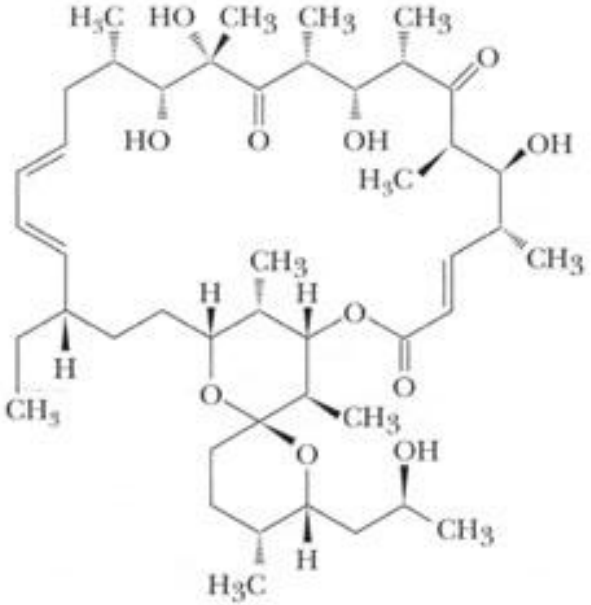


2-Thenoyltrifluoroacetone

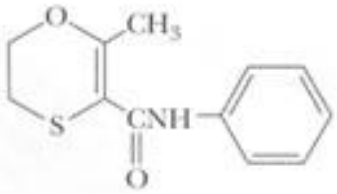
2-噻吩甲酰三氟丙酮



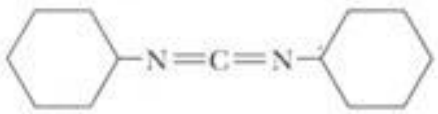
Antimycin A_1



Oligomycin A



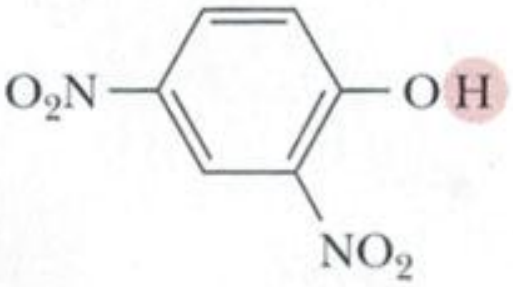
Carboxin



Dicyclohexylcarbodiimide (DCCD)

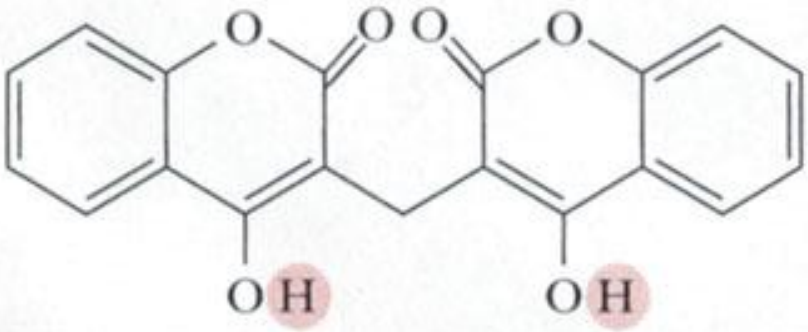
二环己基
羰二亚胺

Dinitrophenol



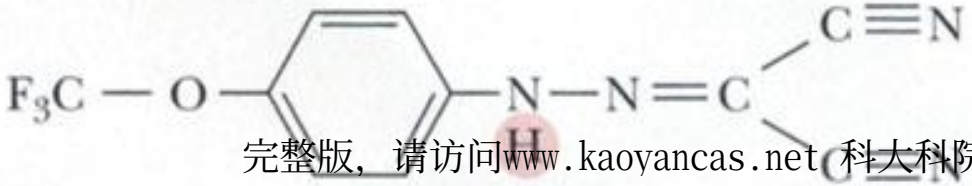
解偶联剂

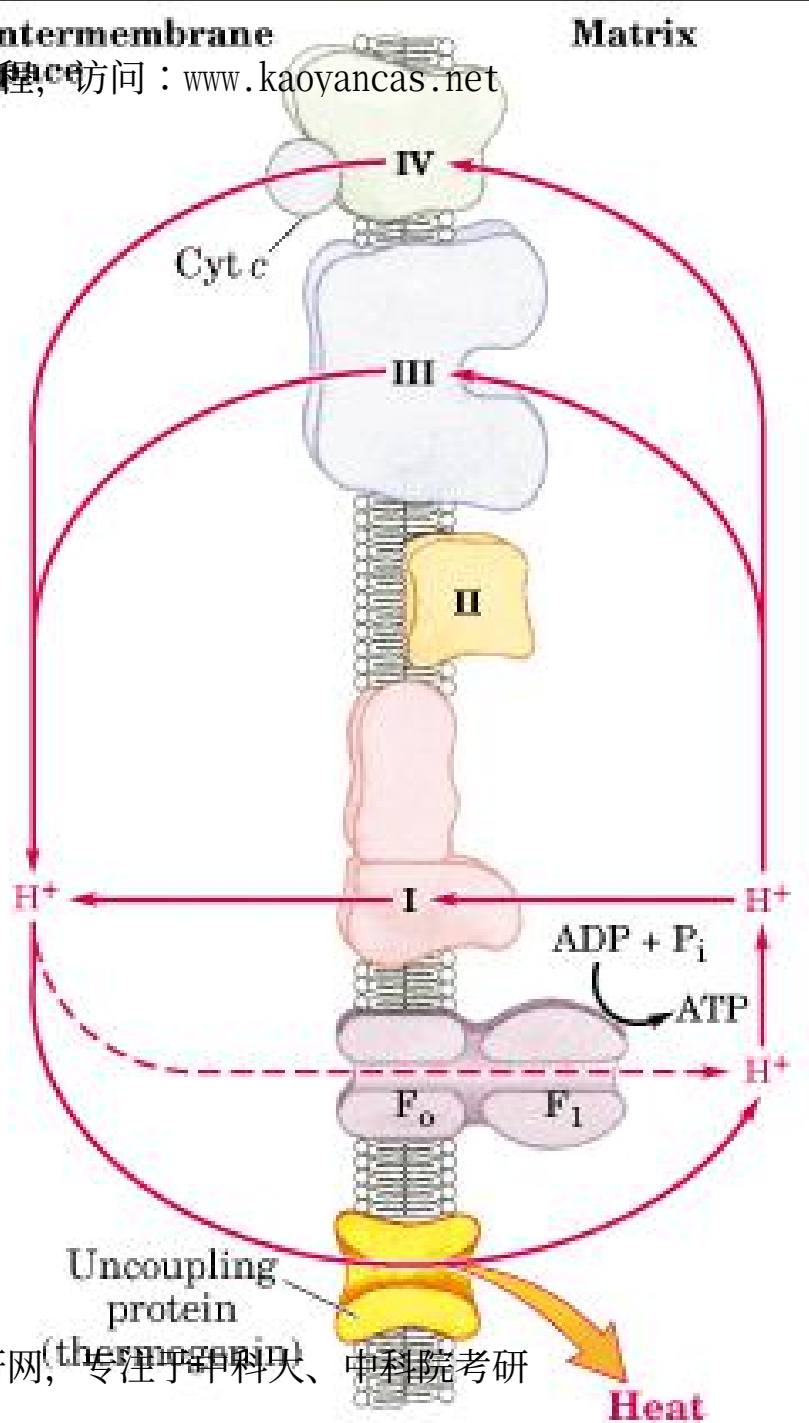
Dicumarol 双香豆素



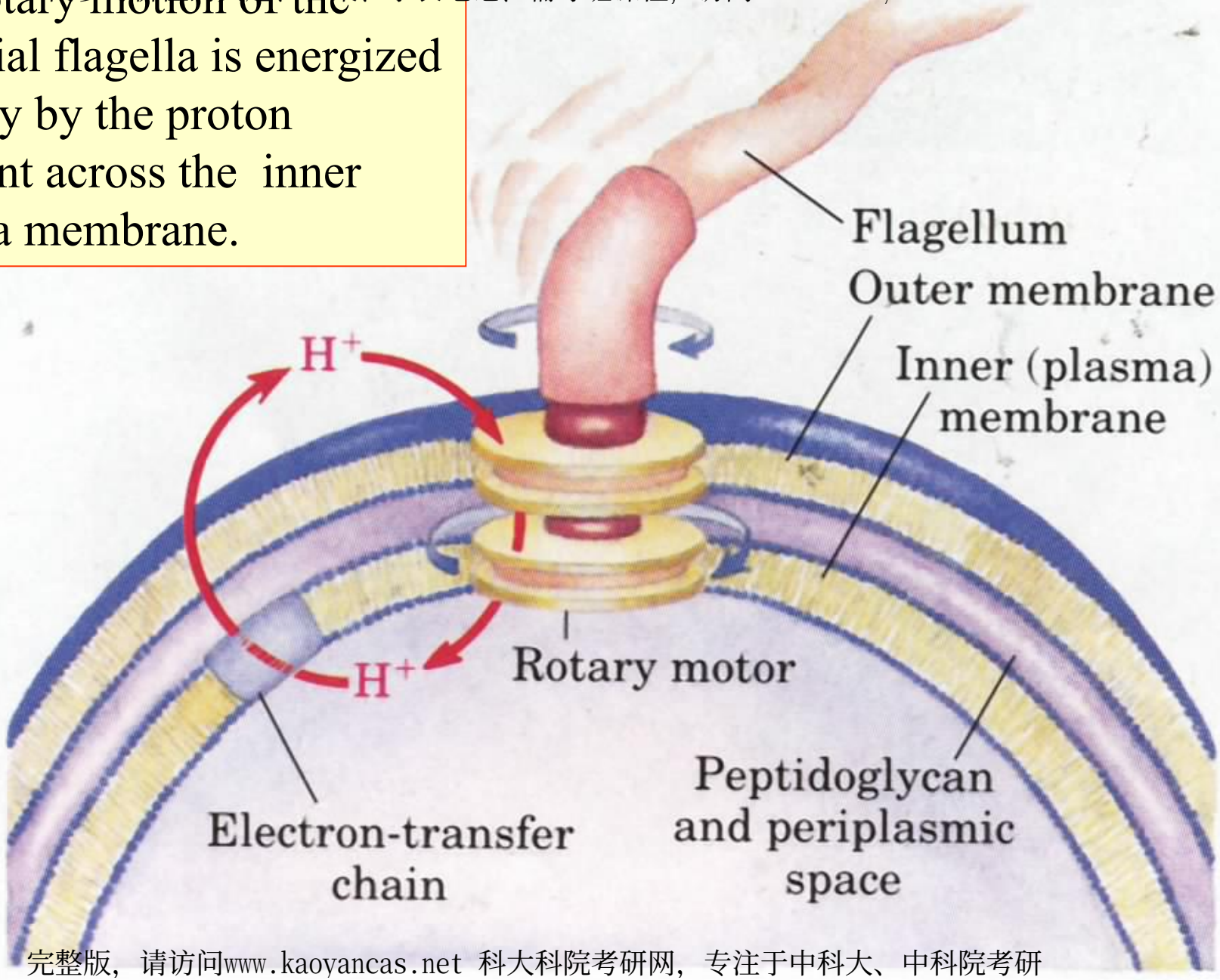
对三氟甲氧基苯腙羰基氰化物

Carbonyl cyanide-p-trifluoro-methoxyphenyl hydrazone
—best known as **FCCP**; for **F**luoro **C**arbonyl **C**yanide **P**henylhydrazone



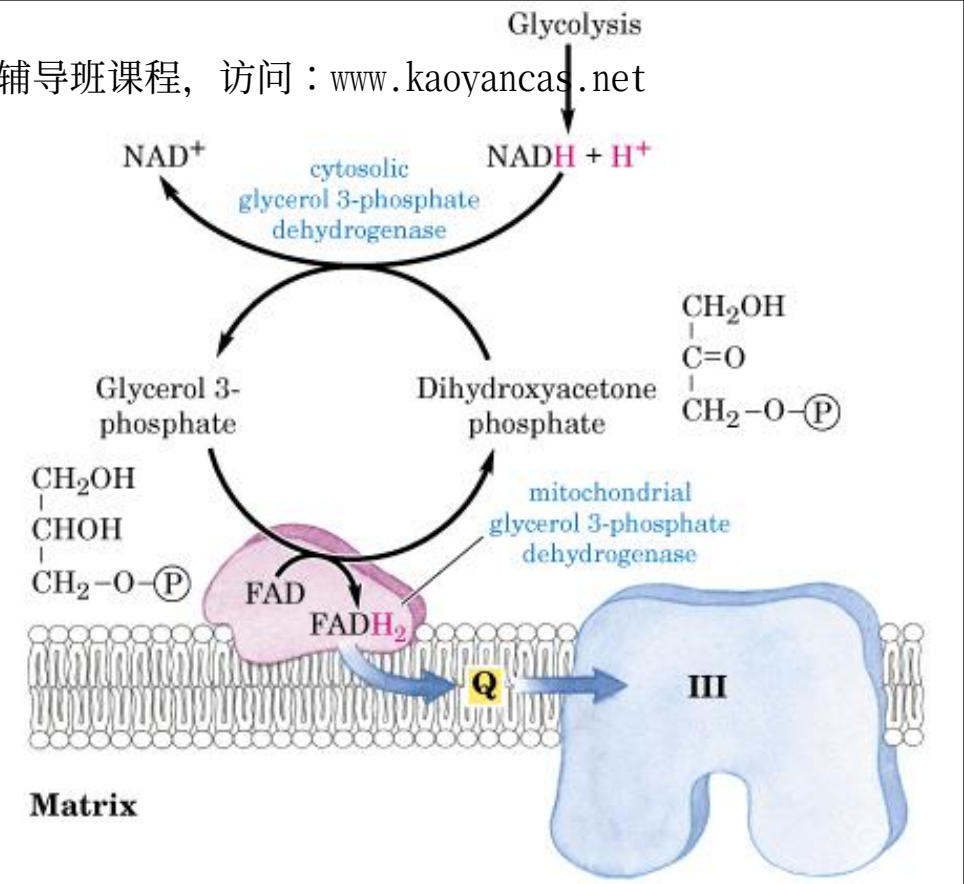


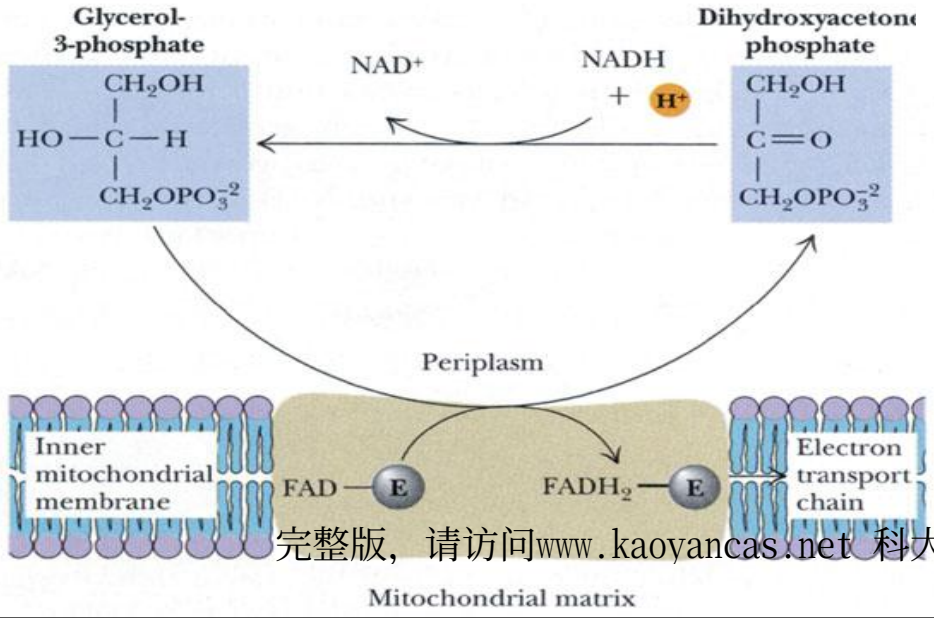
The rotary motion of the bacterial flagella is energized directly by the proton gradient across the inner plasma membrane.



（六）细胞溶胶内NADH的再氧化

甘油-3-磷酸穿梭

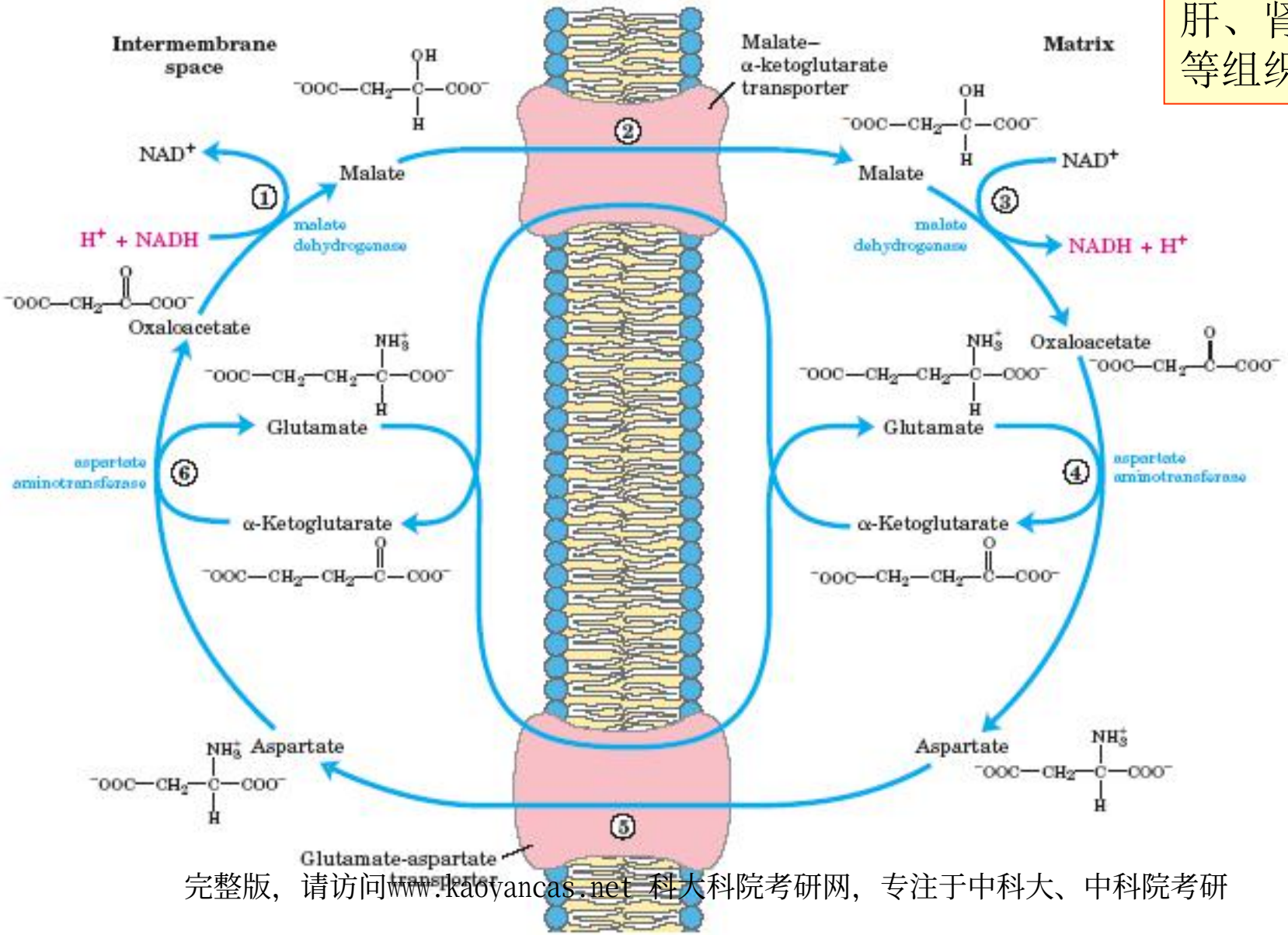




主要存在于肌肉和神经组织

苹果酸-天冬氨酸穿梭

主要存在于肝、肾、心等组织



(七)氧化磷酸化的调控

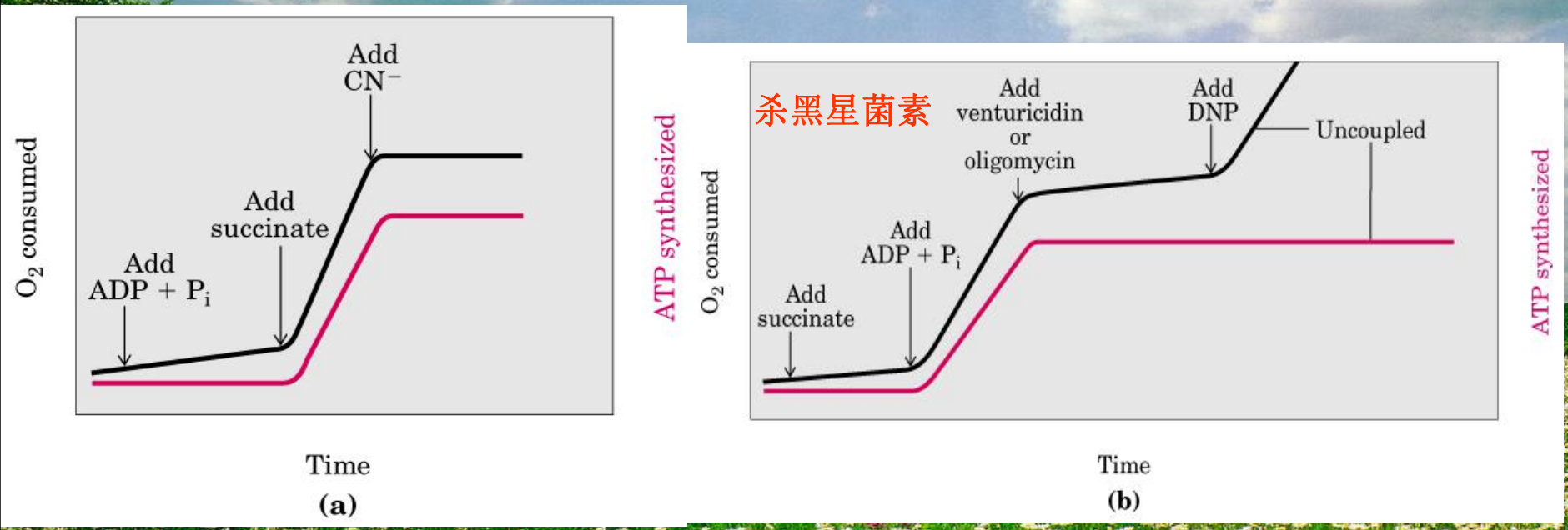
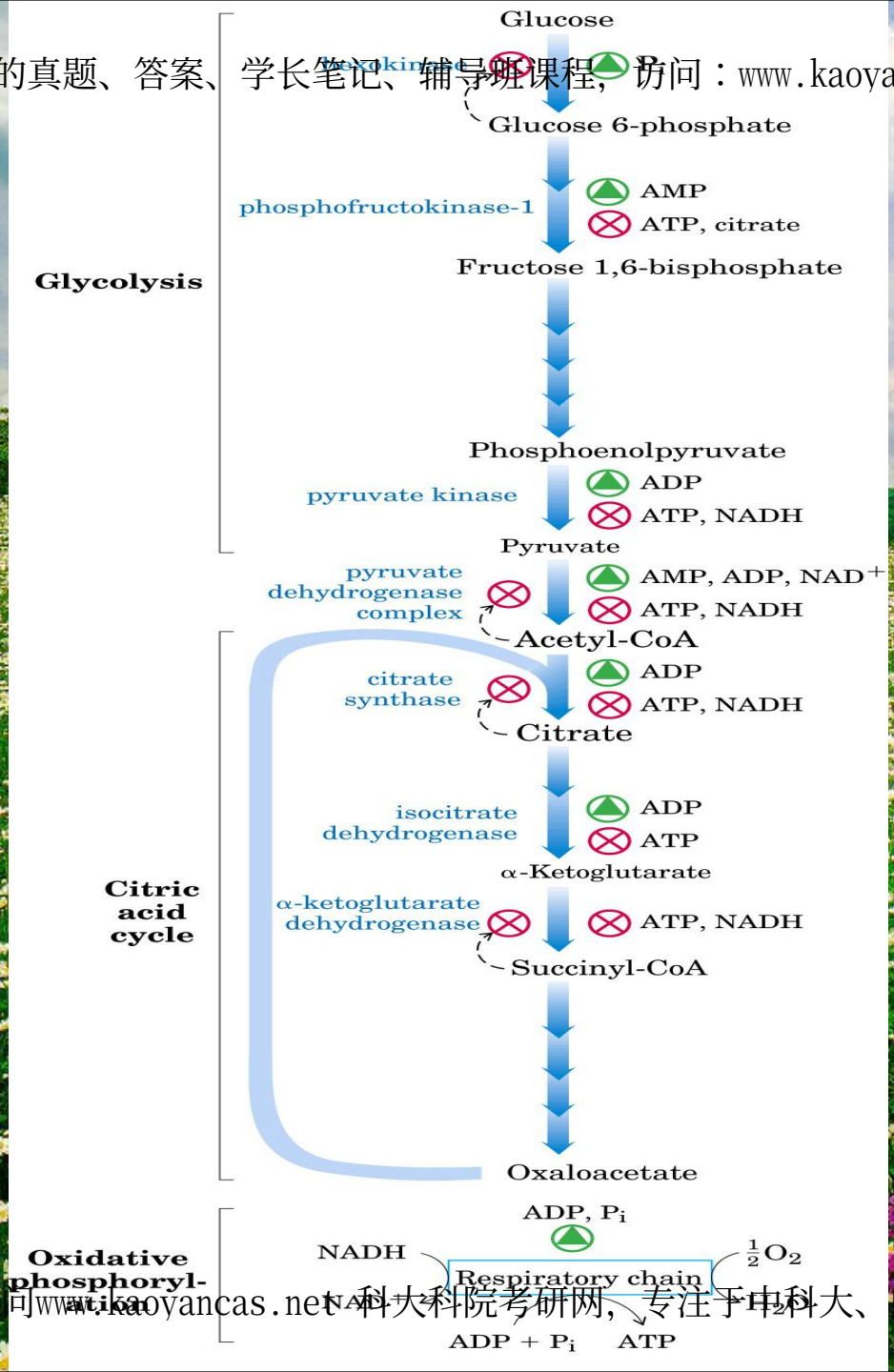


FIGURE 19–18 Coupling of electron transfer and ATP synthesis in mitochondria. In experiments to demonstrate coupling, mitochondria are suspended in a buffered medium and an O_2 electrode monitors O_2 consumption. At intervals, samples are removed and assayed for the presence of ATP. (a) Addition of ADP and P_i alone results in little or no increase in either respiration (O_2 consumption; black) or ATP synthesis (red). When succinate is added, respiration begins immediately and ATP is synthesized. Addition of cyanide (CN^-), which blocks electron transfer between cytochrome oxidase and O_2, inhibits both respiration and ATP synthesis. (b) Mitochondria provided with succinate respire and synthesize ATP only when ADP and P_i are added. Subsequent addition of venturicidin or oligomycin, inhibitors of ATP synthase, blocks both ATP synthesis and respiration. Dinitrophenol (DNP) is an uncoupler, allowing respiration to continue without ATP synthesis.



(八) 葡萄糖彻底氧化的总结算

table 19–5

ATP Yield from Complete Oxidation of Glucose

Process	Direct product	Final ATP
Glycolysis	2 NADH (cytosolic)	3 or 5*
	2 ATP	2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 $FADH_2$	3
	2 ATP or 2 GTP	2
Total yield per glucose		30 or 32

*The number depends on which shuttle system transfers reducing equivalents into mitochondria.

(九) 氧的不完全还原

氧的彻底还原需要4个电子，1个电子使氧还原成超氧化物负离子，2个电子使氧还原成过氧化氢，3个电子使氧还原成羟自由基。

超氧化物歧化酶可清除超氧化物自由基，可能的机制如图24-33所示。

过氧化氢可被过氧化氢酶或谷胱甘肽过氧化物酶清除。

清除超氧化物自由基和过氧化氢可防止羟自由基的生成。

抗氧化剂有助于预防疾病和延缓衰老。

基本要求

1. 熟悉氧化-还原电势的基本概念。（难点）
2. 掌握呼吸链的结构和有关抑制剂。（重点、难点）
3. 掌握氧化磷酸化作用的机制。（重点、难点）