

Biothermodynamics
Free Energy Concepts in Biochemistry;
Chemical Equilibria; Organic Phosphates

CHEM 420 – Principles of Biochemistry
Instructor – Anthony S. Serianni

Chapters 3/16: Voet/Voet, *Biochemistry*, 2011
Fall 2015

September 2 & 4

Joule (J)

$$1 \text{ J} = 1 \text{ kg} \cdot \text{m}^2 \cdot \text{s}^{-2} \quad 1 \text{ J} = 1 \text{ C} \cdot \text{V} (\text{coulomb volt})$$

$$1 \text{ J} = 1 \text{ N} \cdot \text{m} (\text{newton meter})$$

Calorie (cal)

1 cal heats 1 g of H₂O from 14.5 to 15.5°C

$$1 \text{ cal} = 4.184 \text{ J}$$

Large calorie (Cal)

$$1 \text{ Cal} = 1 \text{ kcal} \quad 1 \text{ Cal} = 4184 \text{ J}$$

Avogadro's number (N)

$$N = 6.0221 \times 10^{23} \text{ molecules} \cdot \text{mol}^{-1}$$

Coulomb (C)

$$1 \text{ C} = 6.241 \times 10^{18} \text{ electron charges}$$

Faraday (F)

$$1 \text{ F} = N \text{ electron charges}$$

$$1 \text{ F} = 96,485 \text{ C} \cdot \text{mol}^{-1} = 96,485 \text{ J} \cdot \text{V}^{-1} \cdot \text{mol}^{-1}$$

Kelvin temperature scale (K)

$$0 \text{ K} = \text{absolute zero} \quad 273.15 \text{ K} = 0^\circ\text{C}$$

Boltzmann constant (k_B)

$$k_{\text{B}} = 1.3807 \times 10^{-23} \text{ J} \cdot \text{K}^{-1}$$

Gas constant (R)

$$R = Nk_{\text{B}} \quad R = 1.9872 \text{ cal} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$$

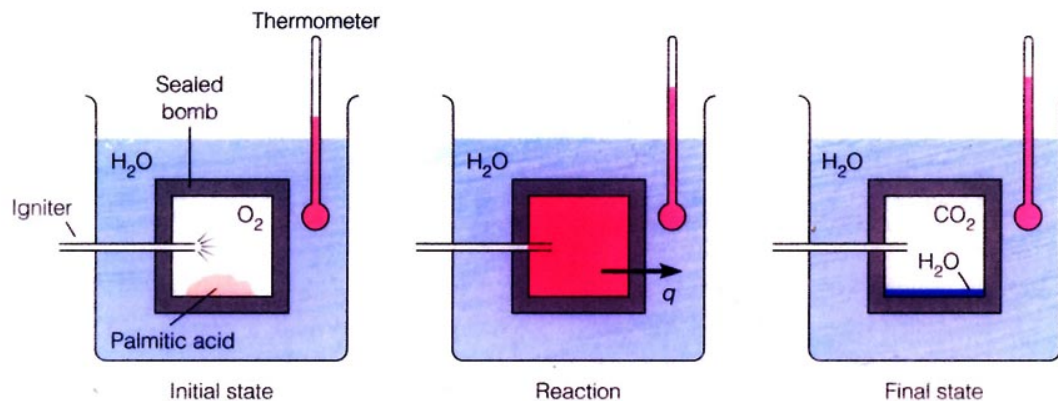
$$R = 8.3145 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1} \quad R = 0.08206 \text{ L} \cdot \text{atm} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$$

Thermodynamic units and constants

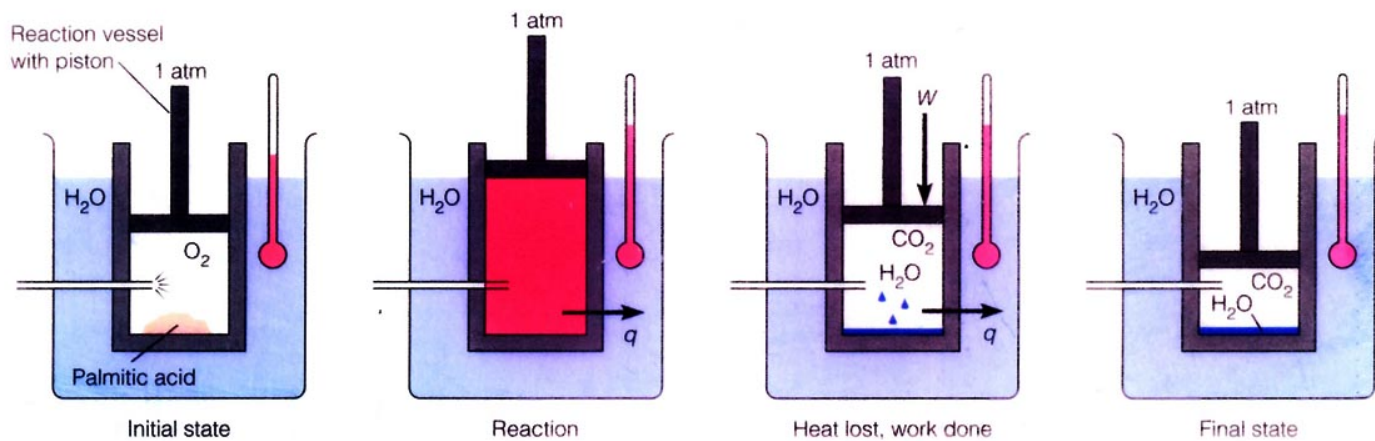
ΔE and ΔH

ENERGY, HEAT, AND WORK

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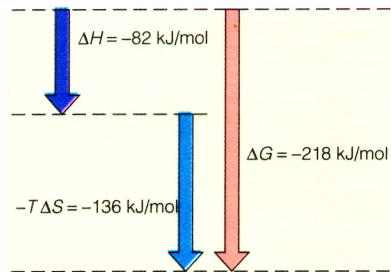
(a) Reaction at constant volume



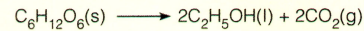
(b) Reaction at constant pressure

Variation of reaction spontaneity (sign of ΔG) with the signs of ΔH and ΔS

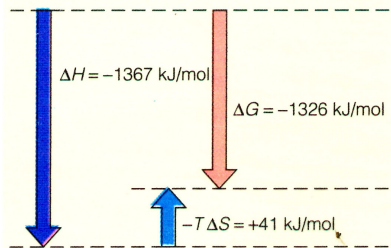
ΔH	ΔS	$\Delta G = \Delta H - T\Delta S$
-	+	The reaction is both enthalpically favored (exothermic) and entropically favored. It is spontaneous (exergonic) at all temperatures.
-	-	The reaction is enthalpically favored but entropically opposed. It is spontaneous only at temperatures <i>below</i> $T = \Delta H/\Delta S$.
+	+	The reaction is enthalpically opposed (endothermic) but entropically favored. It is spontaneous only at temperatures <i>above</i> $T = \Delta H/\Delta S$.
+	-	The reaction is both enthalpically and entropically opposed. It is <i>unspontaneous</i> (endergonic) at all temperatures.



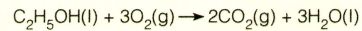
(a) Fermentation of glucose to ethanol



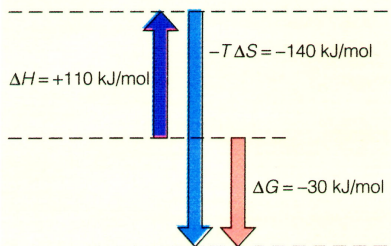
Both enthalpy and entropy changes favor the reaction.



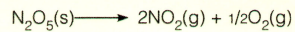
(b) Combustion of ethanol



Enthalpy favors this reaction, but entropy opposes it. We could call this an "enthalpy-driven" reaction. If water *vapor* were the product, an entropy increase would favor the reaction as well.



(c) Decomposition of nitrogen pentoxide

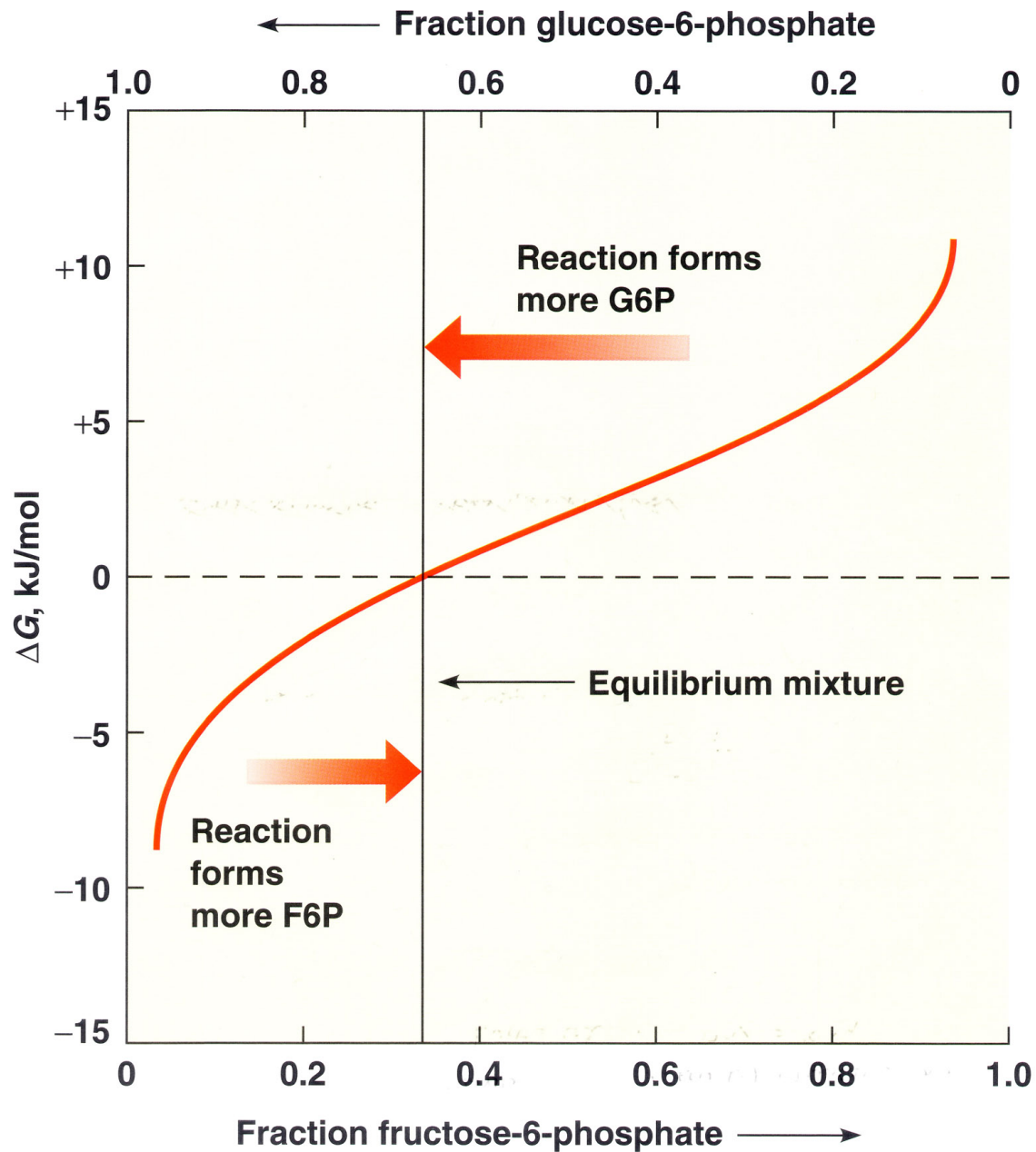


This is a somewhat unusual chemical reaction in that it is "entropy-driven." The reaction actually absorbs heat but is favored by the large entropy increase resulting from the formation of gaseous products.

Thermodynamic parameters
for three different
chemical reactions.

Variation of K_{eq} with ΔG° at 25 °C

K_{eq}	$\Delta G^\circ(\text{kJ} \cdot \text{mol}^{-1})$
10^6	-34.3
10^4	-22.8
10^2	-11.4
10^1	-5.7
10^0	0.0
10^{-1}	5.7
10^{-2}	11.4
10^{-4}	22.8
10^{-6}	34.3



The difference
 between ΔG and
 ΔG° for an
 enzyme-catalyzed
 reaction:
 Phosphoglucoisomerase
 (PGI)

Chemical vs biological oxidation of a saccharide

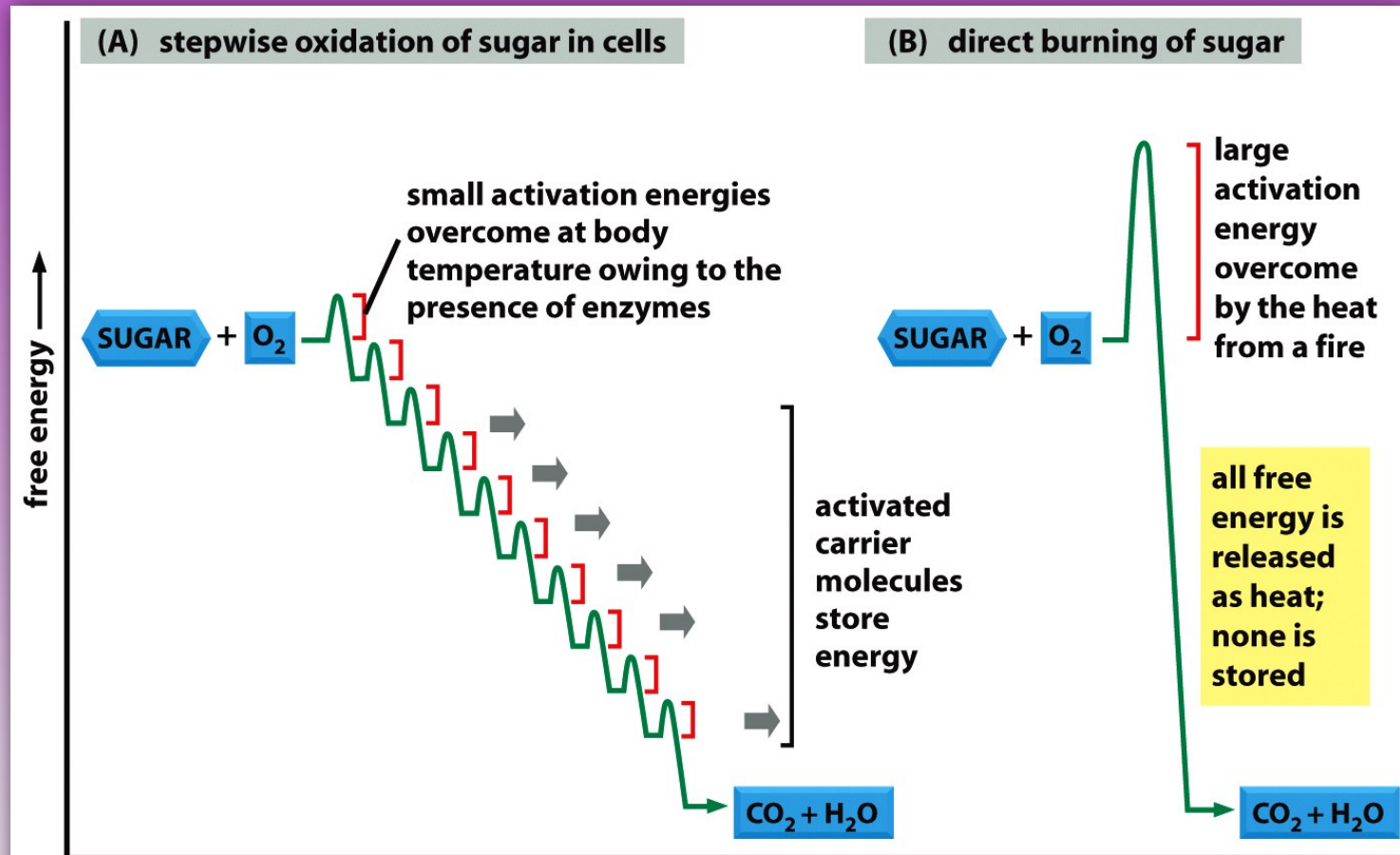
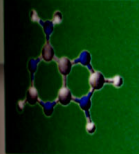


Figure 2-69 Molecular Biology of the Cell 5/e (© Garland Science 2008)



BOX 14-1 PERSPECTIVES IN BIOCHEMISTRY

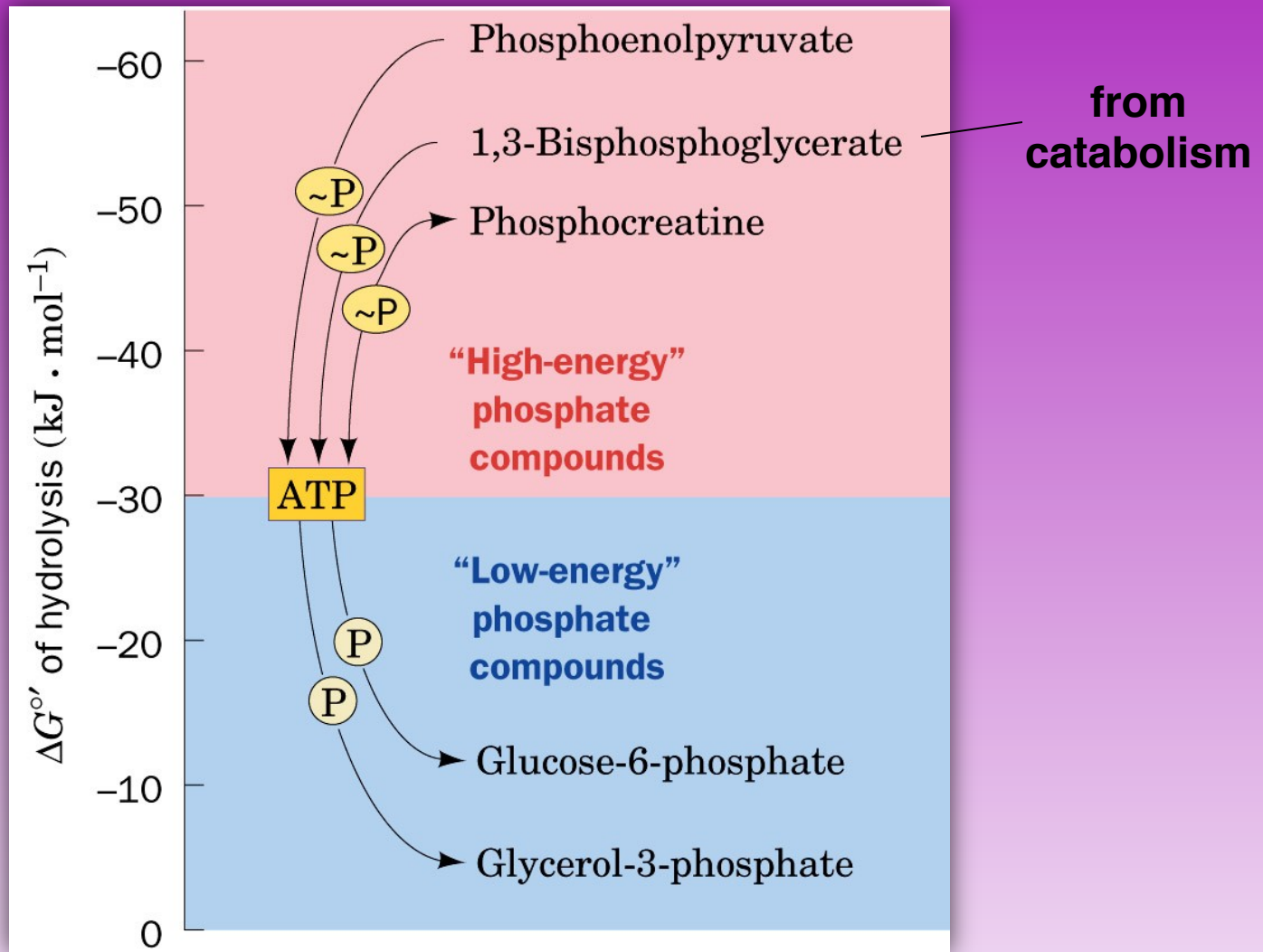
Oxidation States of Carbon

The carbon atoms in biological molecules can assume different oxidation states depending on the atom to which they are bonded. For example, a carbon atom bonded to less electronegative hydrogen atoms is more reduced than a carbon atom bonded to highly electronegative oxygen atoms.

The simplest way to determine the oxidation number (and hence the oxidation state) of a particular carbon atom is to examine each of its bonds and assign the electrons to the more electronegative atom. In a C—O bond, both electrons “belong” to O; in a C—H bond, both electrons “belong” to C; and in a C—C bond, each carbon “owns” one electron. An atom’s oxidation number is the number of valence electrons on the free atom (4 for carbon) minus the number of its lone pair and assigned electrons. For example, the oxidation number of carbon in CO₂ is 4 - (0 + 0) = +4, and the oxidation number of carbon in CH₄ is 4 - (0 + 8) = -4. Keep in mind, however, that oxidation numbers are only accounting devices; actual atomic charges are much closer to neutrality.

The following compounds are listed according to the oxidation state of the highlighted carbon atom. In general, the more oxidized compounds have fewer electrons per C atom and are richer in oxygen, and the more reduced compounds have more electrons per C atom and are richer in hydrogen. But note that not all reduction events (gain of electrons) or oxidation events (loss of electrons) are associated with bonding to oxygen. For example, when an alkane is converted to an alkene, the formation of a carbon-carbon double bond involves the loss of electrons and therefore is an oxidation reaction although no oxygen is involved. Knowing the oxidation number of a carbon atom is seldom required. However, it is useful to be able to determine whether the oxidation state of a given atom increases or decreases during a chemical reaction.

Compound	Formula	Oxidation Number
Carbon dioxide	O=C=O	4 (most oxidized)
Acetic acid	$\text{H}_3\text{C}-\overset{\text{O}}{\parallel}{\text{C}}-\text{OH}$	3
Carbon monoxide	:C≡O:	2
Formic acid	$\text{H}-\overset{\text{O}}{\parallel}{\text{C}}-\text{OH}$	2
Acetone	$\text{H}_3\text{C}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3$	2
Acetaldehyde	$\text{H}_3\text{C}-\overset{\text{O}}{\parallel}{\text{C}}-\text{H}$	1
Formaldehyde	$\text{H}-\overset{\text{O}}{\parallel}{\text{C}}-\text{H}$	0
Acetylene	HC≡CH	-1
Ethanol	$\text{H}_3\text{C}-\overset{\text{H}}{\underset{\text{H}}{\text{C}}}-\text{OH}$	-1
Ethene	$\text{H}_2\text{C}=\overset{\text{H}}{\underset{\text{H}}{\text{C}}}$	-2
Ethane	$\text{H}_3\text{C}-\overset{\text{H}}{\underset{\text{H}}{\text{C}}}-\text{H}$	-3
Methane	$\text{H}-\overset{\text{H}}{\underset{\text{H}}{\text{C}}}-\text{H}$	-4 (least oxidized)



The flow of phosphoryl groups from "high-energy" phosphate donors, via the ATP-ADP system, to "low-energy" phosphate acceptors (note the central role of ATP as energy currency).

Standard free energies of hydrolysis for specific types of bonds common in biochemistry

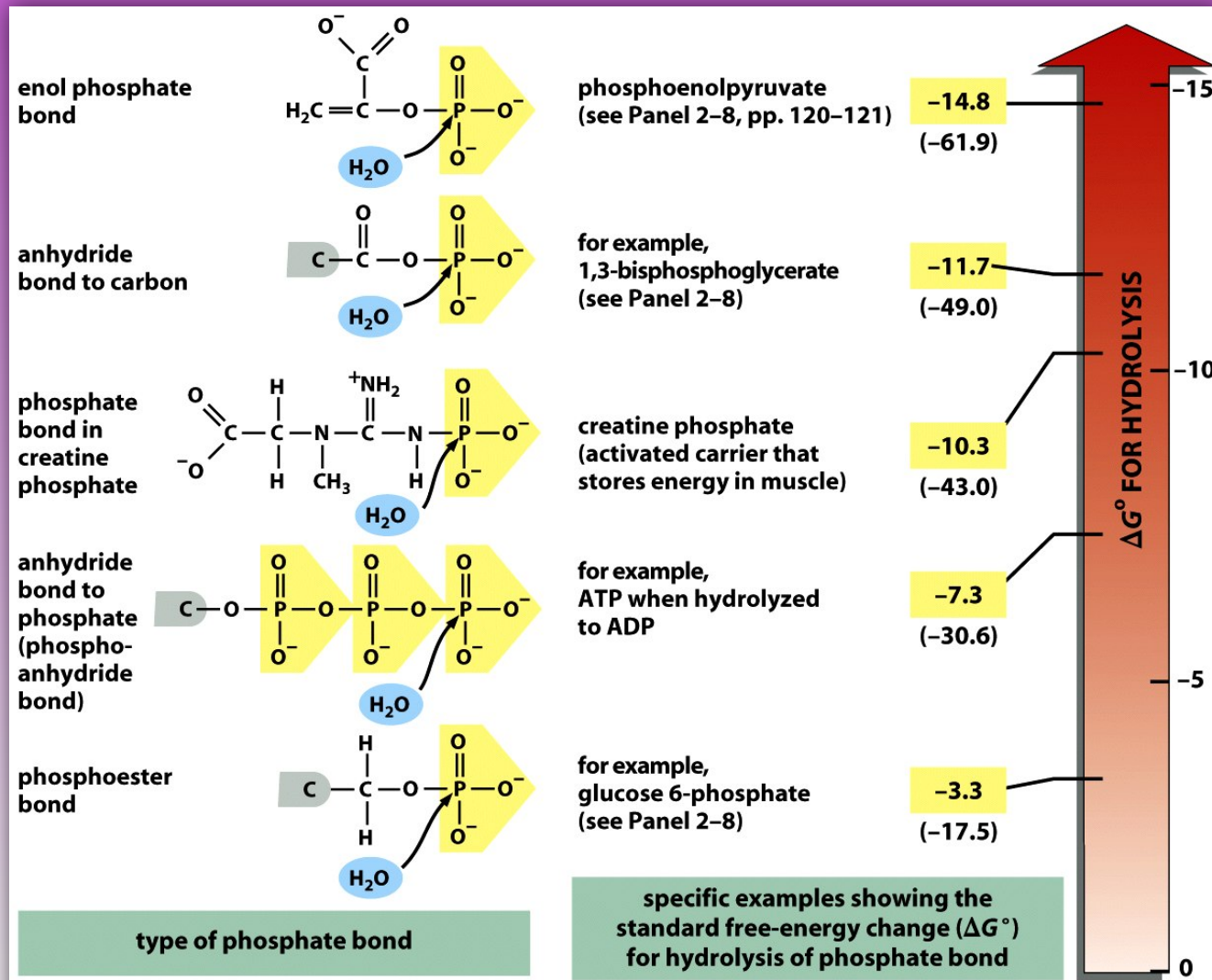


Figure 2-74 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Standard free energies of phosphate hydrolysis of some compounds of biological interest: Two modes of hydrolysis of ATP

Compound	ΔG (kJ · mol ⁻¹)
Phosphoenolpyruvate	-61.9
1,3-Bisphosphoglycerate	-49.4
Acetyl phosphate	-43.1
Phosphocreatine	-43.1
PP _i	-33.5
ATP (→ AMP + PP_i)	-32.2
ATP (→ ADP + P_i)	-30.5
Glucose-1-phosphate	-20.9
Fructose-6-phosphate	-13.8
Glucose-6-phosphate	-13.8
Glycerol-3-phosphate	-9.2

Source: Jencks, W.P., in Fasman, G.D. (Ed.), Handbook of Biochemistry and Molecular Biology (3rd ed.), Physical and Chemical Data, Vol. I, pp. 296-304, CRC Press (1976).

The structure of ATP indicating its relationship to ADP, AMP, and adenosine

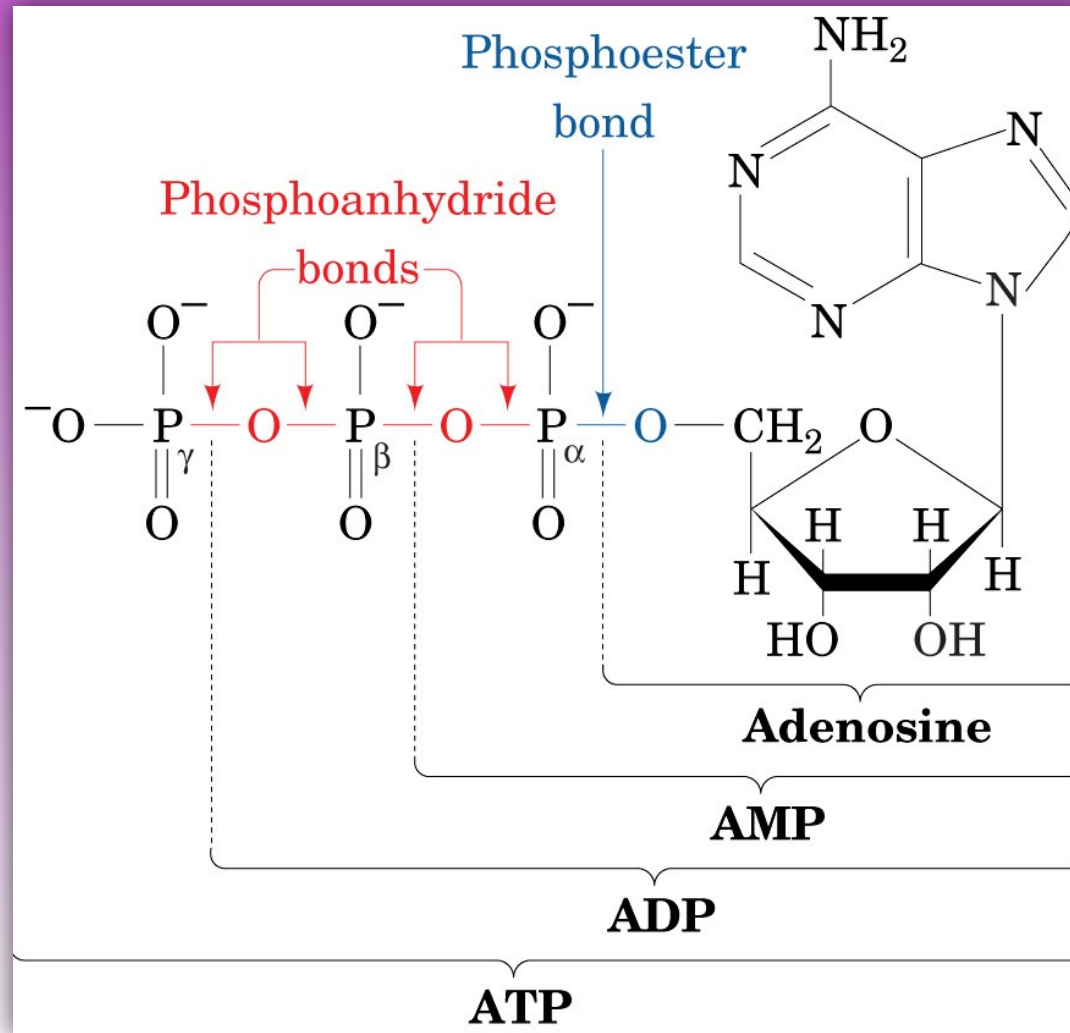


TABLE 13–5 Adenine Nucleotide, Inorganic Phosphate, and Phosphocreatine Concentrations in Some Cells

	Concentration (mM)*				
	<i>ATP</i>	<i>ADP</i> [†]	<i>AMP</i>	<i>P_i</i>	<i>PCr</i>
Rat hepatocyte	3.38	1.32	0.29	4.8	0
Rat myocyte	8.05	0.93	0.04	8.05	28
Rat neuron	2.59	0.73	0.06	2.72	4.7
Human erythrocyte	2.25	0.25	0.02	1.65	0
<i>E. coli</i> cell	7.90	1.04	0.82	7.9	0

*For erythrocytes the concentrations are those of the cytosol (human erythrocytes lack a nucleus and mitochondria). In the other types of cells the data are for the entire cell contents, although the cytosol and the mitochondria have very different concentrations of ADP. PCr is phosphocreatine, discussed on p. 505.

[†]This value reflects total concentration; the true value for free ADP may be much lower (see Box 13–1).

WORKED EXAMPLE 13–2 Calculation of ΔG_p

Calculate the actual free energy of hydrolysis of ATP, ΔG_p , in human erythrocytes. The standard free energy of hydrolysis of ATP is -30.5 kJ/mol, and the concentrations of ATP, ADP, and P_i in erythrocytes are as shown in Table 13–5. Assume that the pH is 7.0 and the temperature is 37°C (body temperature). What does this reveal about the amount of energy required to *synthesize* ATP under the same cellular conditions?

Solution: The concentrations of ATP, ADP, and P_i in human erythrocytes are 2.25, 0.25, and 1.65 mM, respectively. The actual free energy of hydrolysis of ATP under these conditions is given by the relationship (see Eqn 13–4)

$$\Delta G_p = \Delta G'^{\circ} + RT \ln \frac{[\text{ADP}][P_i]}{[\text{ATP}]}$$

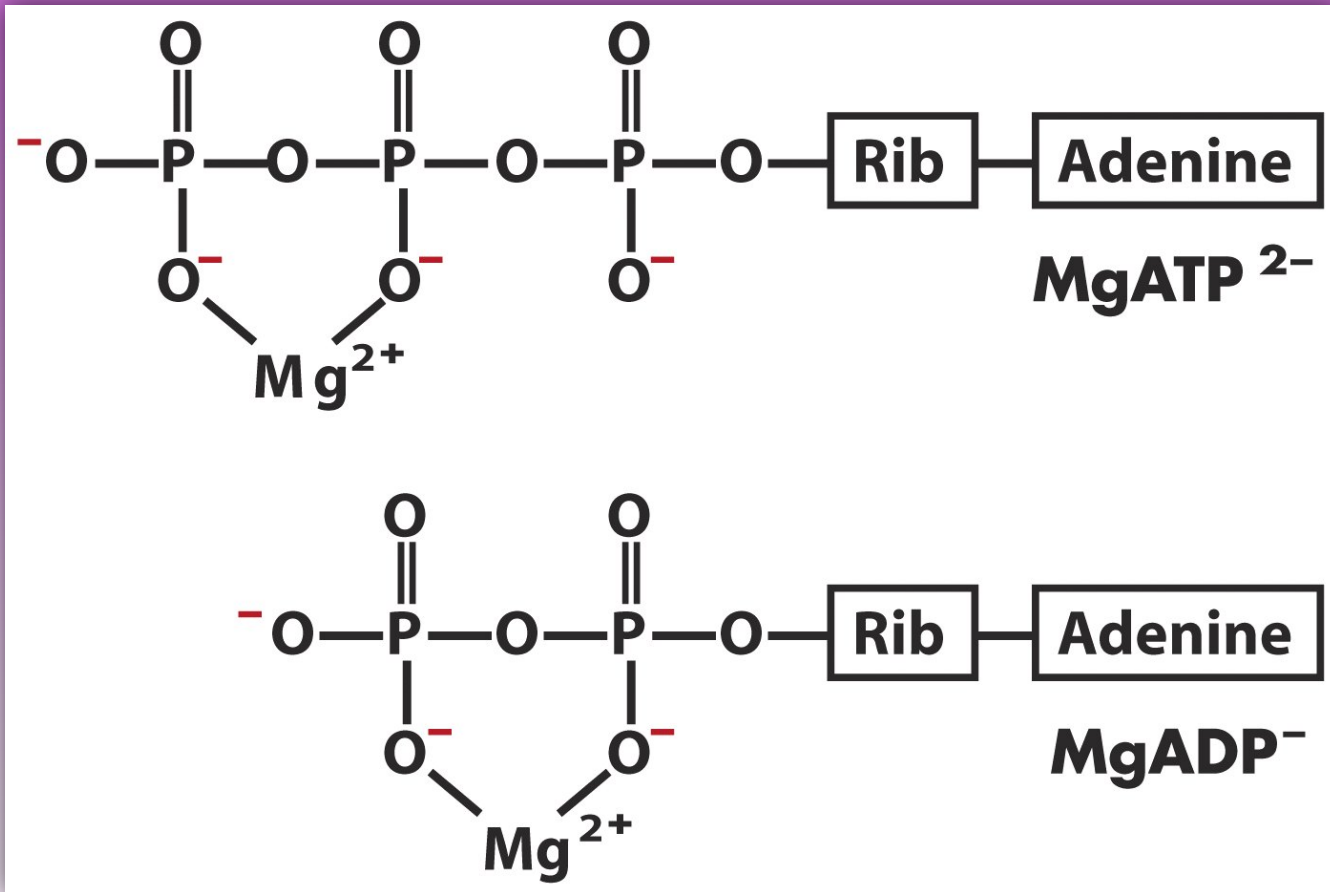
Substituting the appropriate values we get

$$\begin{aligned}\Delta G_p &= -30.5 \text{ kJ/mol} + \left[(8.315 \text{ J/mol} \cdot \text{K})(310 \text{ K}) \ln \frac{(0.25 \times 10^{-3})(1.65 \times 10^{-3})}{(2.25 \times 10^{-3})} \right] \\ &= -30.5 \text{ kJ/mol} + (2.58 \text{ kJ/mol}) \ln 1.8 \times 10^{-4} \\ &= -30.5 \text{ kJ/mol} + (2.58 \text{ kJ/mol})(-8.6) \\ &= -30.5 \text{ kJ/mol} - 22 \text{ kJ/mol} \\ &= -52 \text{ kJ/mol}\end{aligned}$$

(Note that the final answer has been rounded to the correct number of significant figures (52.5 rounded to 52), following rules for rounding 5 down to the nearest even number to avoid the bias inherent in rounding “up.”) Thus ΔG_p , the actual free-energy change for ATP hydrolysis in the intact erythrocyte (-52 kJ/mol), is much larger than the standard free-energy change (-30.5 kJ/mol). By the same token, the free energy required to *synthesize* ATP from ADP and P_i under the conditions prevailing in the erythrocyte would be 52 kJ/mol.

$\Delta G'^{\circ}$ and ΔG :
ATP hydrolysis
in vivo

The metallated, biologically active forms of ADP and ATP



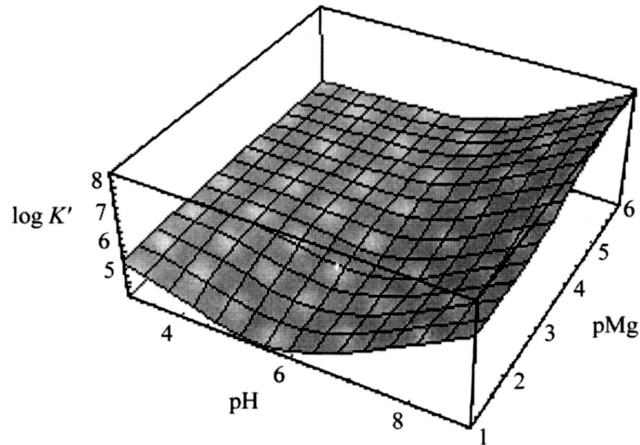


Figure 1.9 Plot of the base 10 logarithm of the apparent equilibrium constant for the hydrolysis of ATP to ADP and P_i at 298.15 K and 0.25 M ionic strength

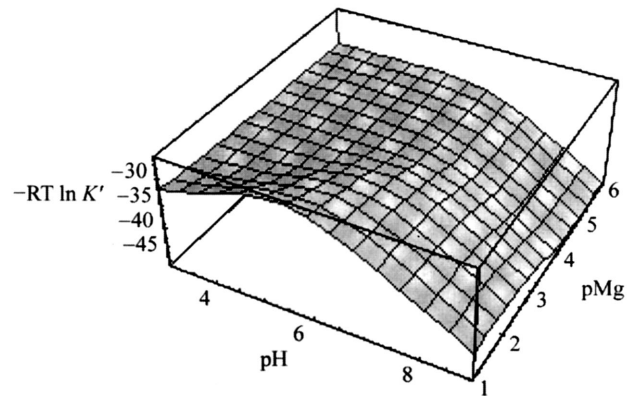
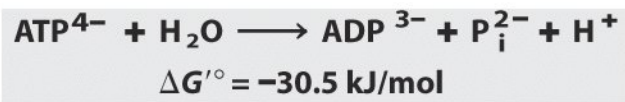
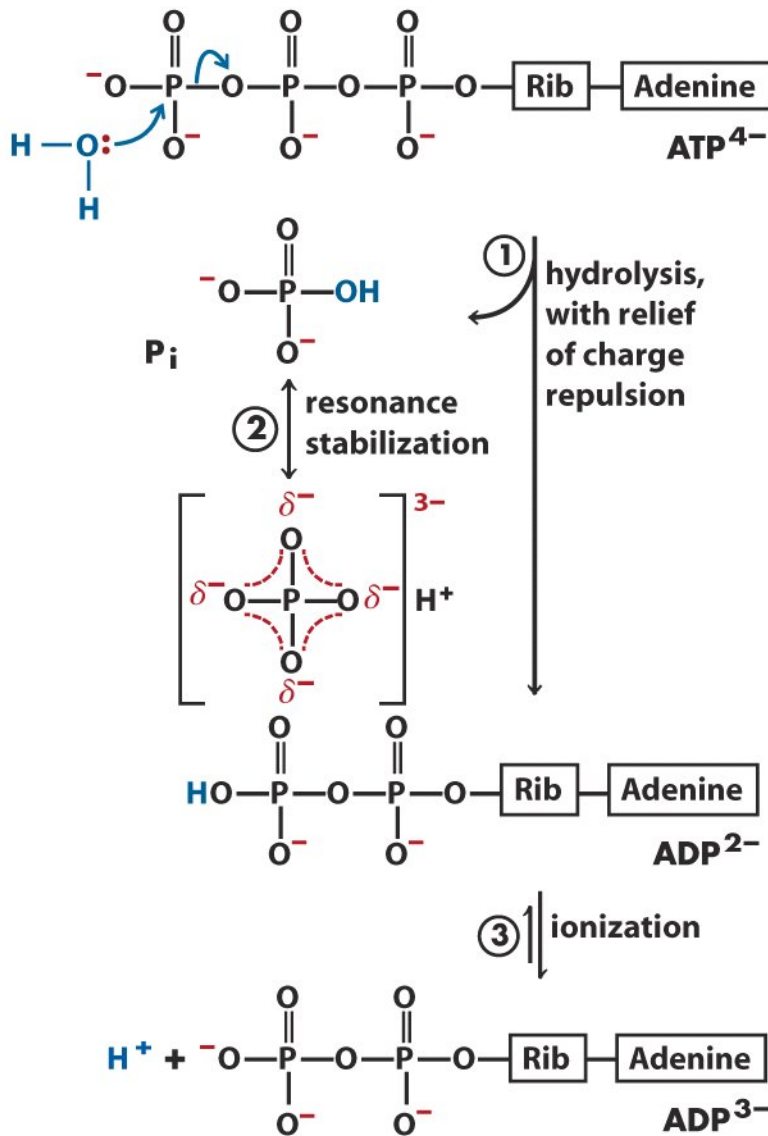
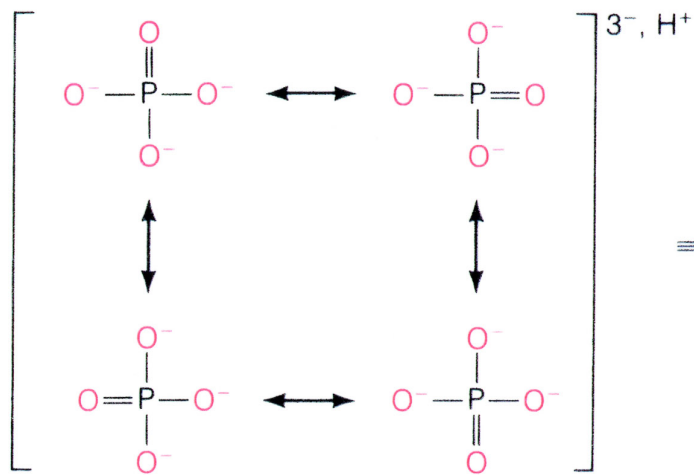


Figure 1.10 Plot of $-RT \ln K'$ in kJ mol^{-1} versus pH at 298.15 K and 0.25 M ionic strength

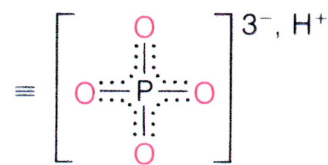
Effect of pH and pMg on K' of ATP hydrolysis and on the free energy of ATP hydrolysis



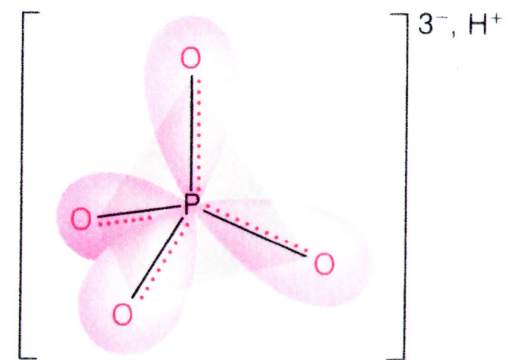
Summary of factors responsible for the negative standard free energy of hydrolysis of ATP



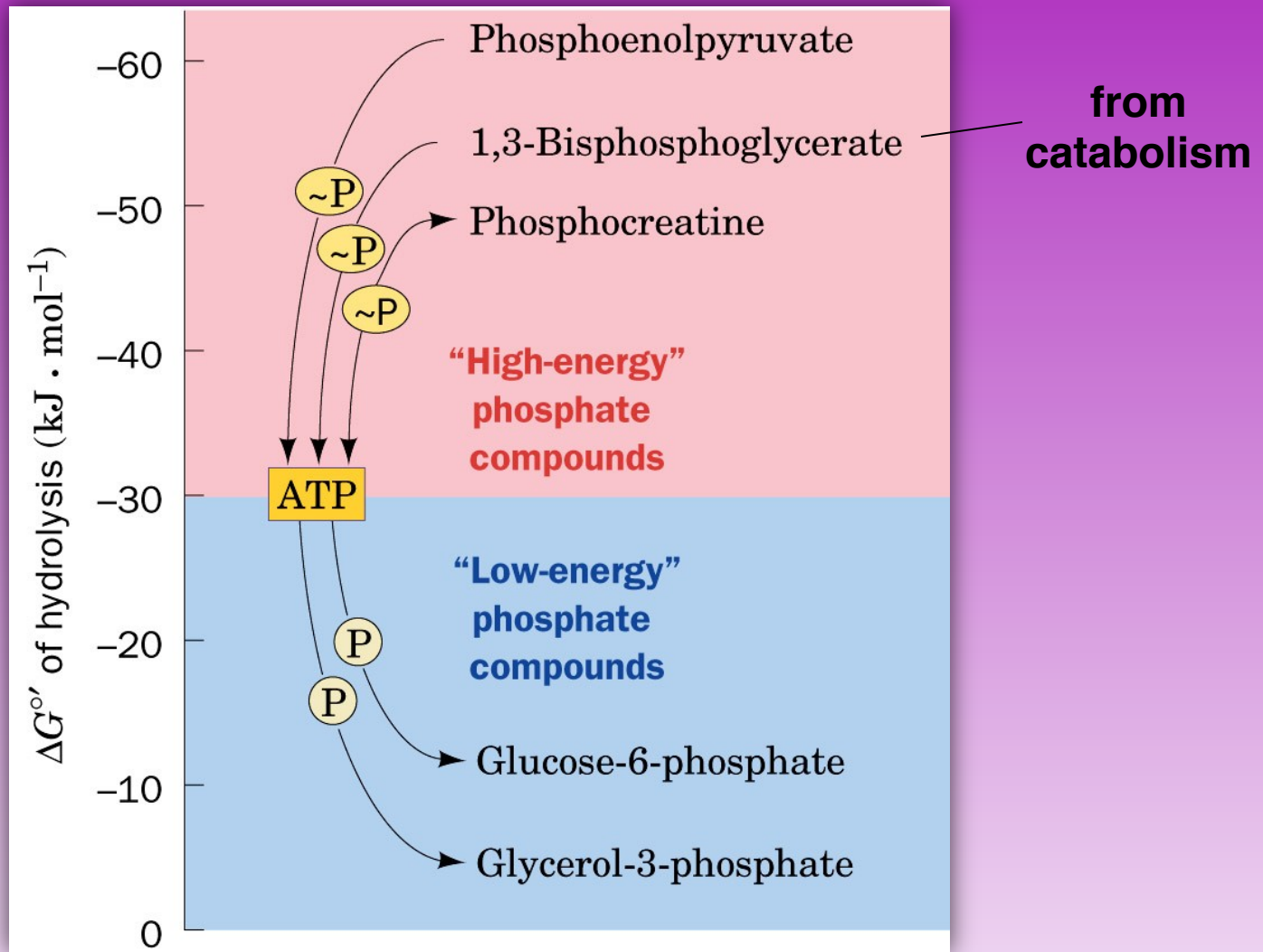
(a) Structures of phosphate ion contributing to resonance stabilization



(b) Resonance hybrid



(c) Molecular orbitals of tetrahedral phosphate ion



The flow of phosphoryl groups from "high-energy" phosphate donors, via the ATP-ADP system, to "low-energy" phosphate acceptors (note the central role of ATP as energy currency).

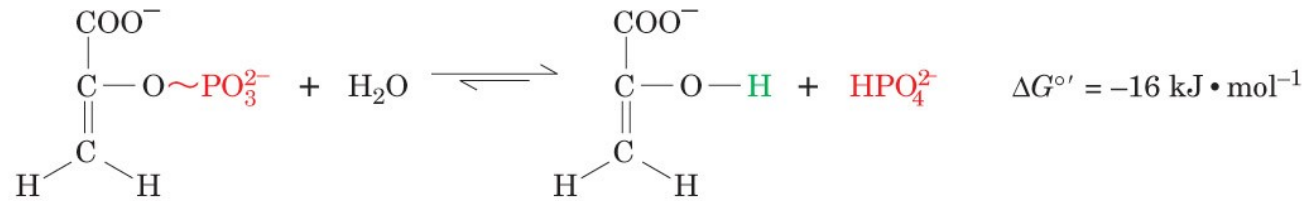
Standard free energies of phosphate hydrolysis of some compounds of biological interest: Phosphoenolpyruvate (PEP)

Compound	ΔG (kJ · mol ⁻¹)
Phosphoenolpyruvate	-61.9
1,3-Bisphosphoglycerate	-49.4
Acetyl phosphate	-43.1
Phosphocreatine	-43.1
PP _i	-33.5
ATP (→ AMP + PP_i)	-32.2
ATP (→ ADP + P_i)	-30.5
Glucose-1-phosphate	-20.9
Fructose-6-phosphate	-13.8
Glucose-6-phosphate	-13.8
Glycerol-3-phosphate	-9.2

Source: Jencks, W.P., in Fasman, G.D. (Ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), *Physical and Chemical Data*, Vol. I, pp. 296–304, CRC Press (1976).

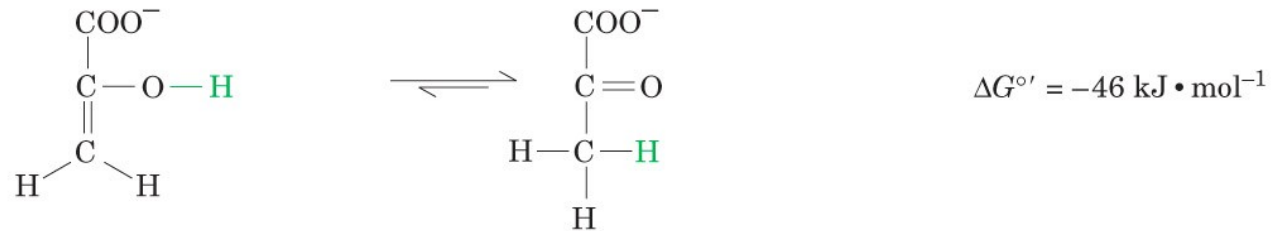
Hydrolysis of phosphoenolpyruvate (PEP)

Hydrolysis



**Phosphoenol-
pyruvate**

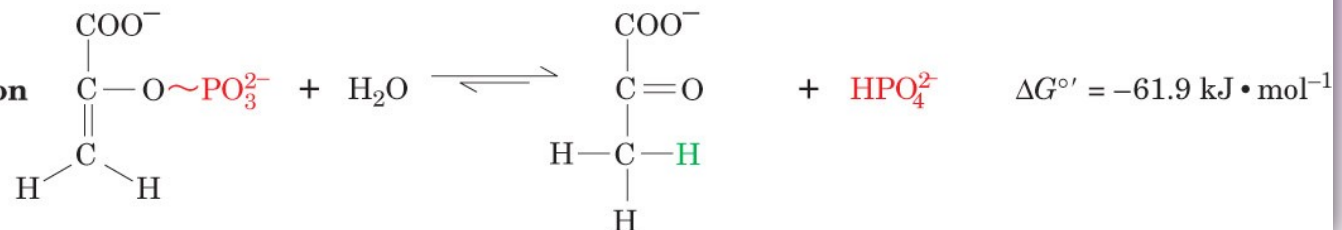
Tautomerization



**Pyruvate
(enol form)**

**Pyruvate
(keto form)**

Overall reaction



Standard free energies of phosphate hydrolysis of some compounds of biological interest: Glucose 6P

Compound	ΔG (kJ · mol ⁻¹)
Phosphoenolpyruvate	-61.9
1,3-Bisphosphoglycerate	-49.4
Acetyl phosphate	-43.1
Phosphocreatine	-43.1
PP _i	-33.5
ATP (→ AMP + PP_i)	-32.2
ATP (→ ADP + P_i)	-30.5
Glucose-1-phosphate	-20.9
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Glucose-6-phosphate	-13.8
Glycerol-3-phosphate	-9.2

Source: Jencks, W.P., in Fasman, G.D. (Ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), *Physical and Chemical Data*, Vol. I, pp. 296–304, CRC Press (1976).

Coupled reactions involving ATP: The phosphorylation of glucose to form glucose-6-phosphate and ADP

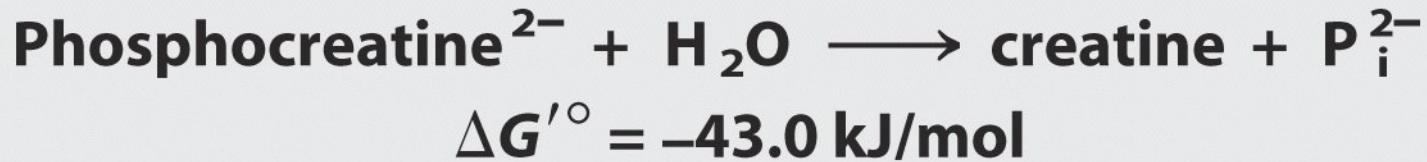
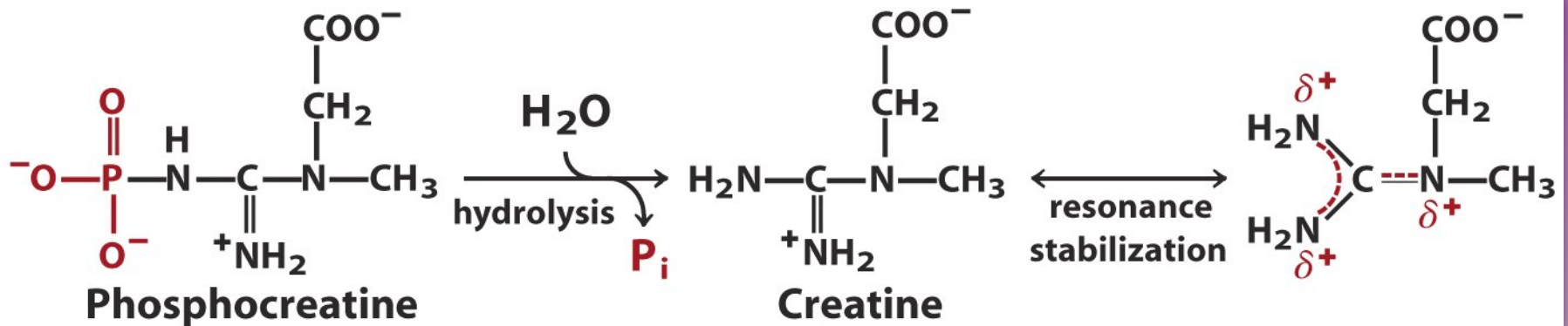
				<u>$\Delta G^{\circ'}$ (kJ \cdot mol⁻¹)</u>
Endergonic half-reaction 1	$P_i + \text{glucose}$	\rightleftharpoons	$\text{glucose-6-P} + \text{H}_2\text{O}$	+13.8
Exergonic half-reaction 2	$\text{ATP} + \text{H}_2\text{O}$	\rightleftharpoons	$\text{ADP} + P_i$	-30.5
Overall coupled reaction	$\text{ATP} + \text{glucose}$	\rightleftharpoons	$\text{ADP} + \text{glucose-6-P}$	-16.7

Standard free energies of phosphate hydrolysis of some compounds of biological interest: Phosphocreatine

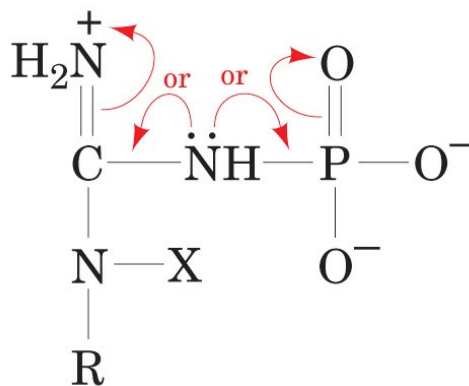
Compound	ΔG (kJ · mol ⁻¹)
Phosphoenolpyruvate	-61.9
1,3-Bisphosphoglycerate	-49.4
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PP _i	-33.5
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Glucose-1-phosphate	-20.9
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Glucose-6-phosphate	-13.8
Glycerol-3-phosphate	-9.2

Source: Jencks, W.P., in Fasman, G.D. (Ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), *Physical and Chemical Data*, Vol. I, pp. 296–304, CRC Press (1976).

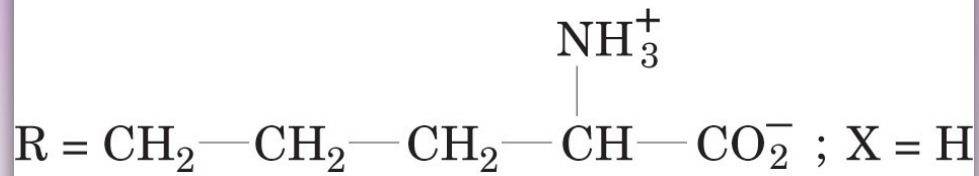
Phosphocreatine: a high-energy phosphate found largely in muscle



Analogous competing resonance structures in phosphoguanidines



Phosphocreatine

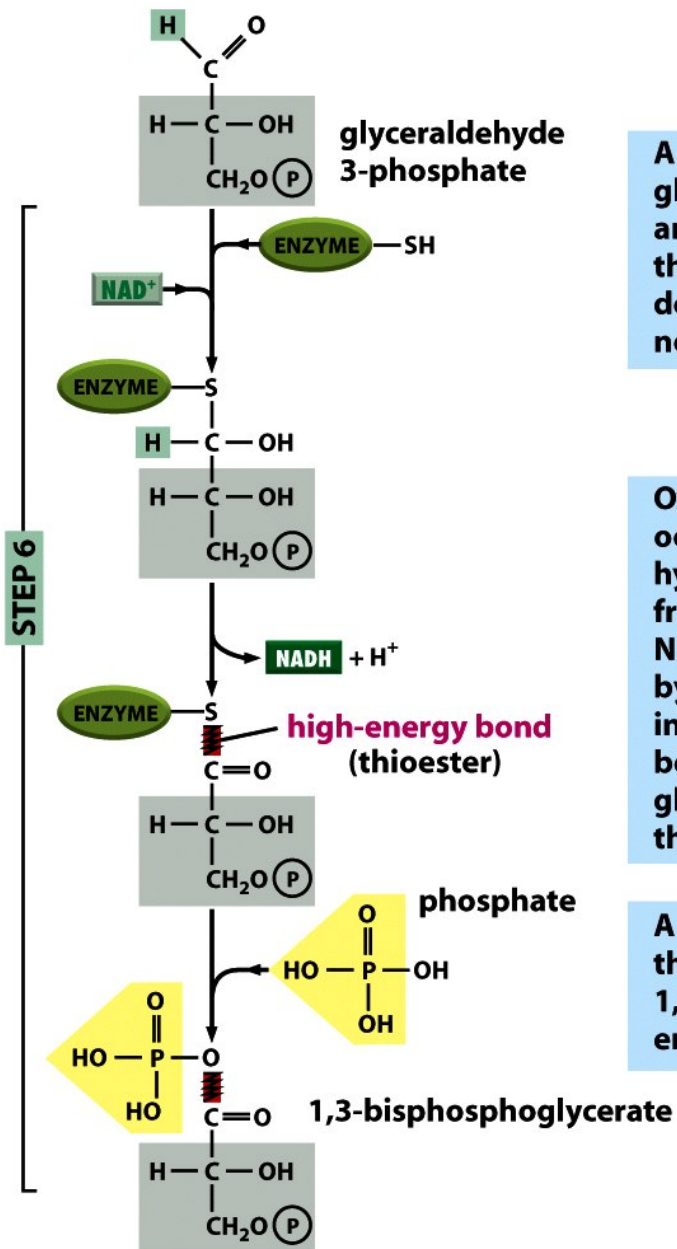


Phosphoarginine

Standard free energies of phosphate hydrolysis of some compounds of biological interest: 1,3-bisphospho-D-glycerate (1,3 BPG)

Compound	ΔG (kJ · mol ⁻¹)
Phosphoenolpyruvate	-61.9
1,3-Bisphosphoglycerate	-49.4
Acetyl phosphate	-43.1
Phosphocreatine	-43.1
PP _i	-33.5
ATP (→ AMP + PP_i)	-32.2
ATP (→ ADP + P_i)	-30.5
Glucose-1-phosphate	-20.9
Fructose-6-phosphate	-13.8
Glucose-6-phosphate	-13.8
Glycerol-3-phosphate	-9.2

Source: Jencks, W.P., in Fasman, G.D. (Ed.), Handbook of Biochemistry and Molecular Biology (3rd ed.), Physical and Chemical Data, Vol. I, pp. 296-304, CRC Press (1976).



A covalent bond is formed between glyceraldehyde 3-phosphate (the substrate) and the -SH group of a cysteine side chain of the enzyme glyceraldehyde 3-phosphate dehydrogenase, which also binds noncovalently to NAD⁺.

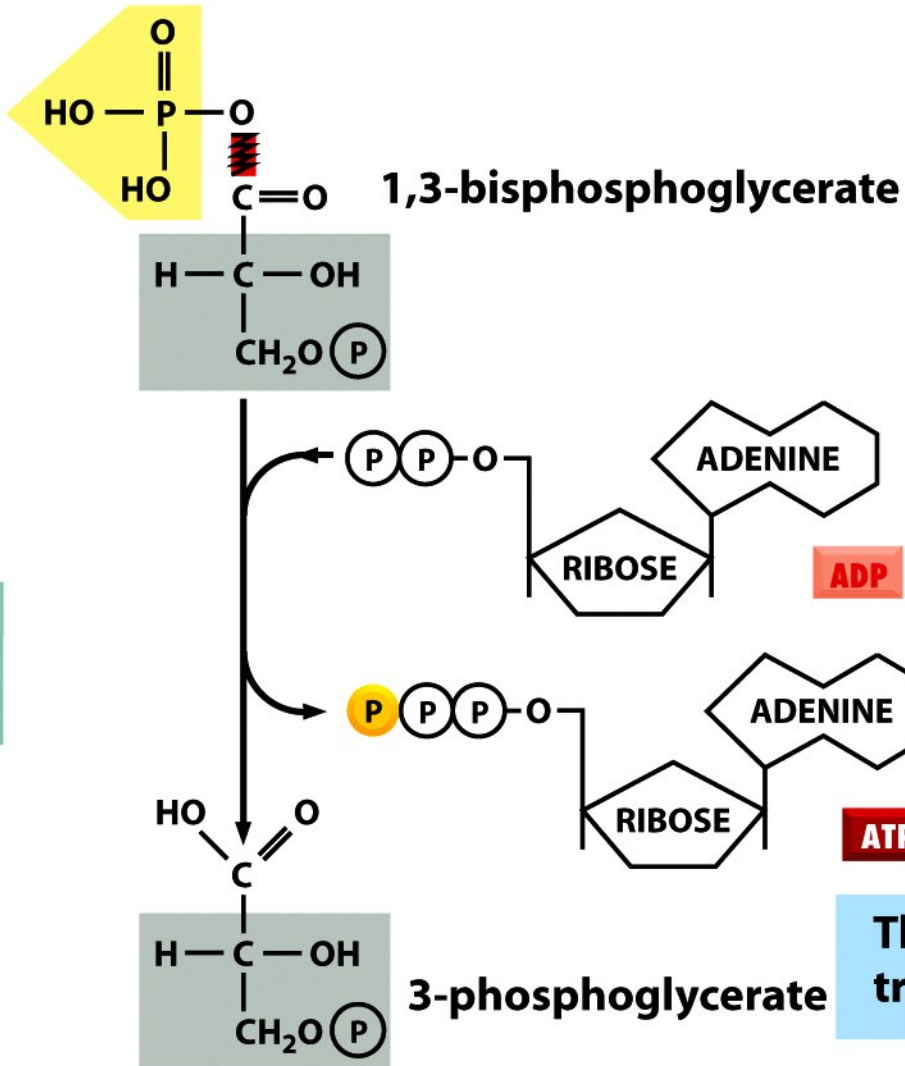
Oxidation of glyceraldehyde 3-phosphate occurs, as two electrons plus a proton (a hydride ion, see Figure 2-60) are transferred from glyceraldehyde 3-phosphate to the bound NAD⁺, forming NADH. Part of the energy released by the oxidation of the aldehyde is thus stored in NADH, and part goes into converting the bond between the enzyme and its substrate glyceraldehyde 3-phosphate into a high-energy thioester bond.

A molecule of inorganic phosphate displaces the high-energy bond to the enzyme to create 1,3-bisphosphoglycerate which contains a high-energy acyl-anhydride bond.

Figure 2-72a part 1 of 2 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Generation of a high-energy phosphate via glycolysis

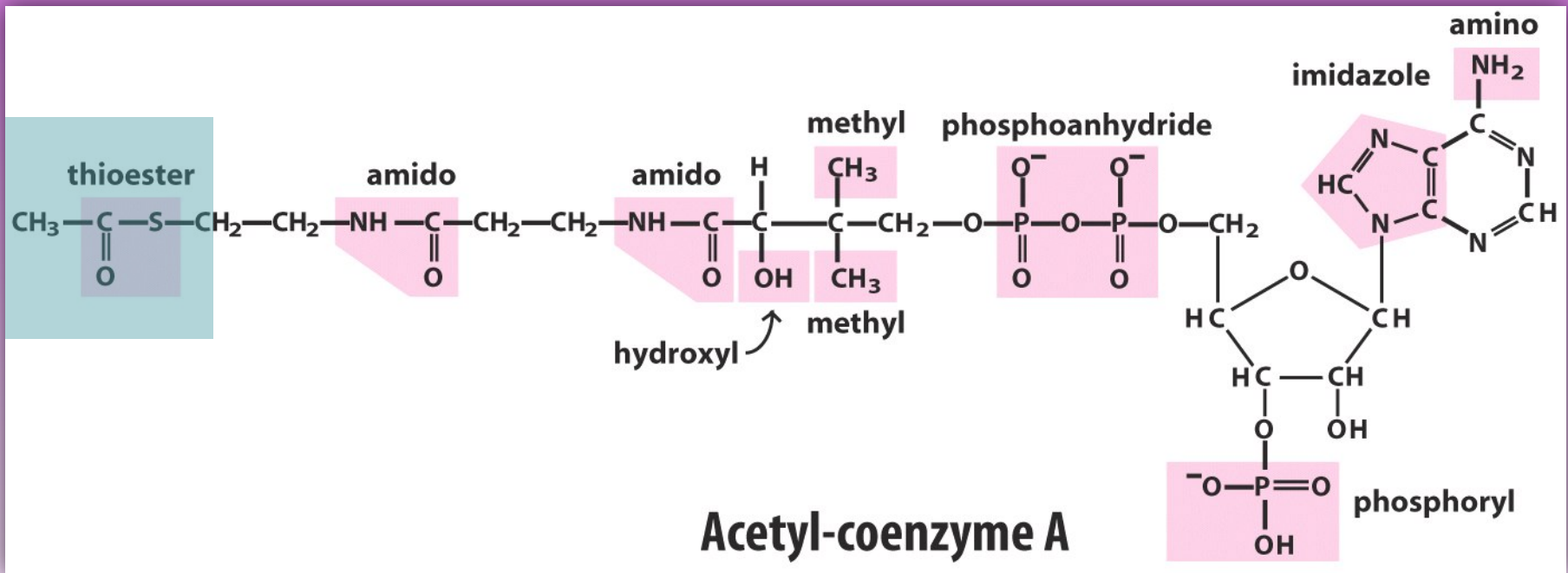
STEP 7



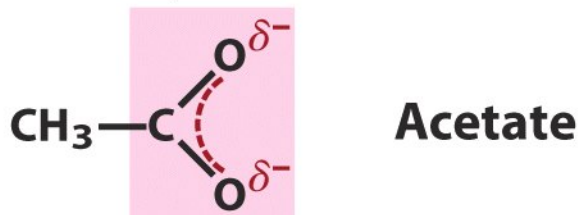
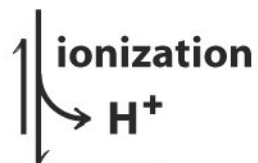
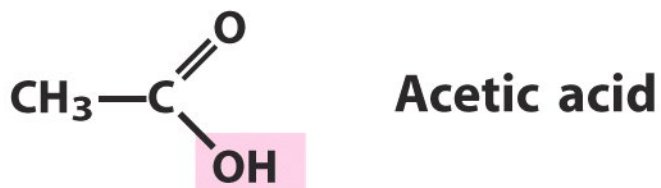
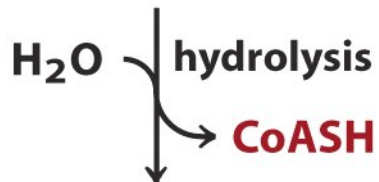
Substrate-level phosphorylation (ATP generation coupled with catabolism)

The high-energy bond to phosphate is transferred to ADP to form ATP.

Acetyl CoA: a biological thioester (high-energy bond)

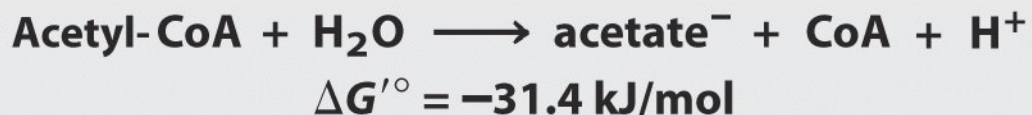


Coenzyme A = CoASH

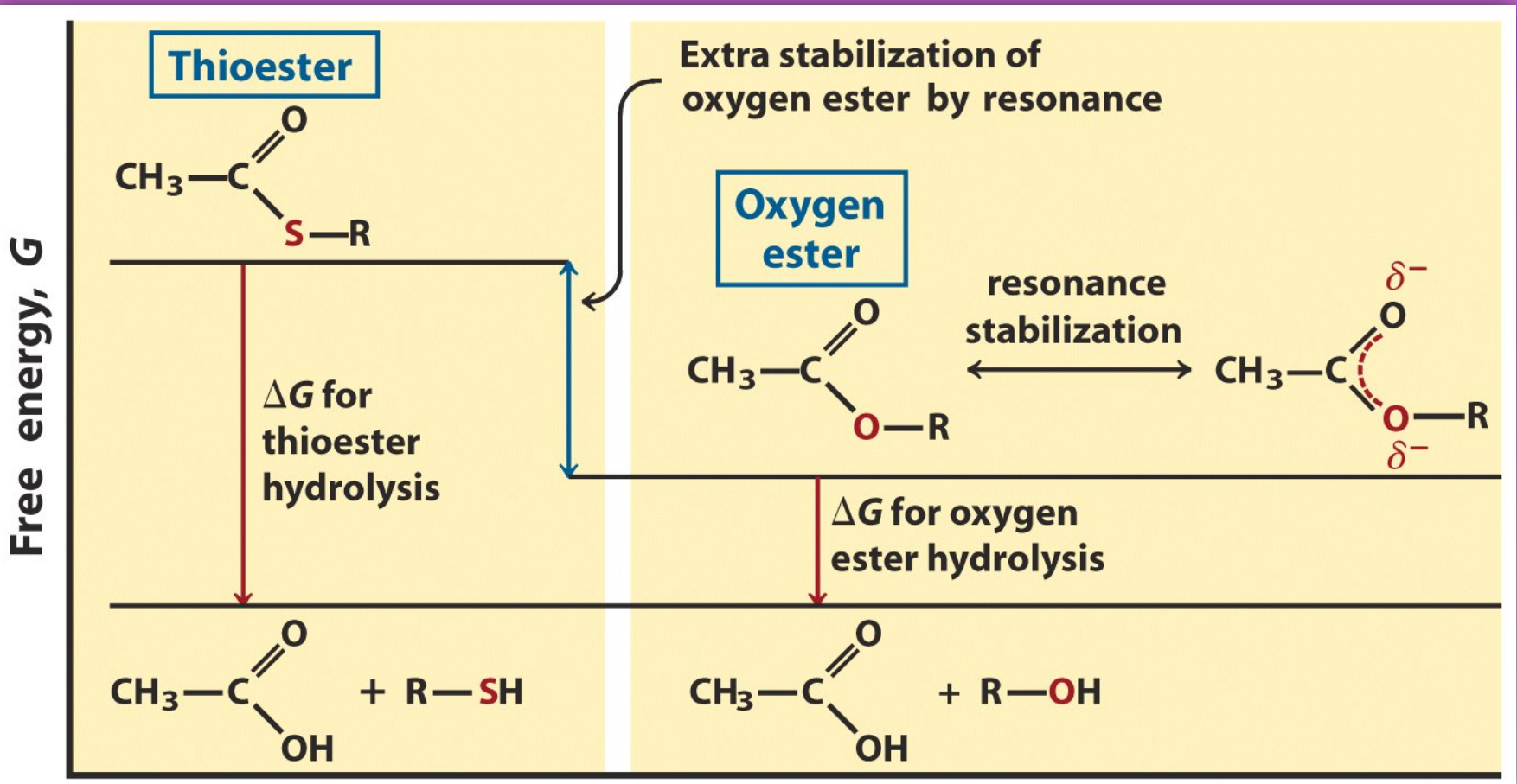


resonance
stabilization

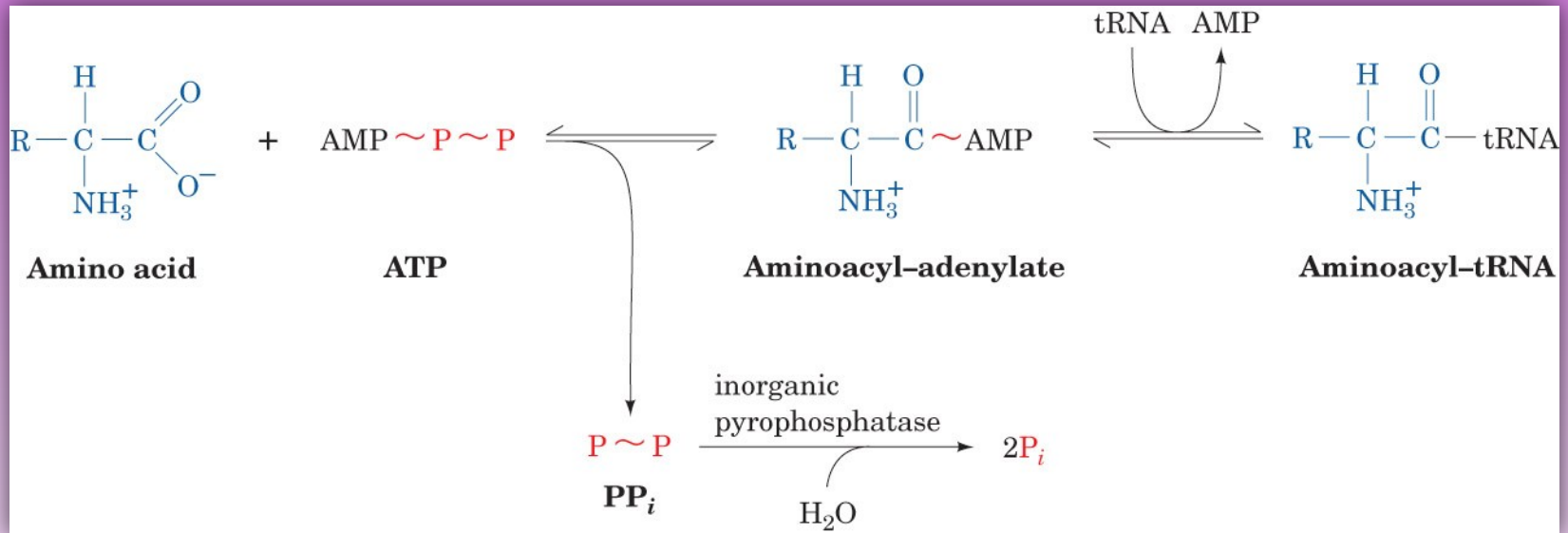
Why is the standard free energy of hydrolysis of acetyl CoA so negative?



Further explanation of the energetics of acetyl CoA hydrolysis



Biological activation of an α -amino acid: Pyrophosphate and acyl phosphate cleavage in the synthesis of an aminoacyl-tRNA (charged tRNA)



Calculating the ΔG associated with a concentration gradient across a biological membrane (for non-ionic solutes)

