Chemistry 5.07SC Biological Chemistry I

Fall Semester, 2013

Lecture 10. Biochemical Transformations II. Phosphoryl transfer and the kinetics and thermodynamics of energy currency in the cell: ATP and GTP.

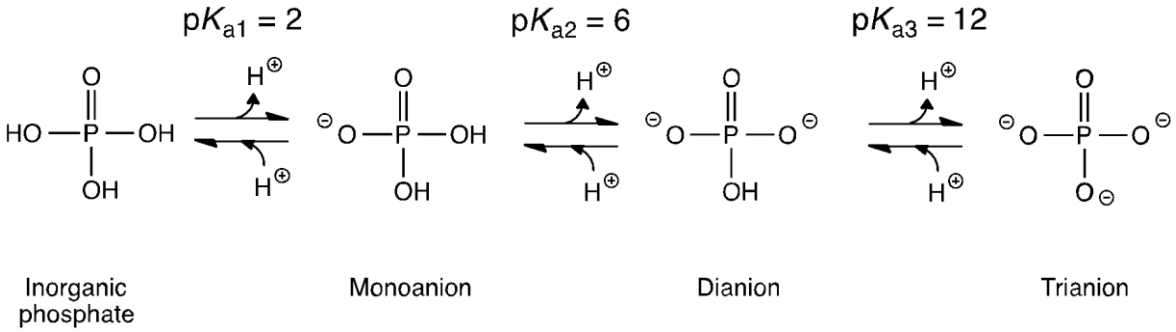
Outline:

I. Phosphorylation *in vivo*
II. Phosphate chemistry
III. ATP
IV. Mechanism of phosphoryl transfer with ATP
V. Use of ATP in vivo
VI. ATP as the energy currency of the cell

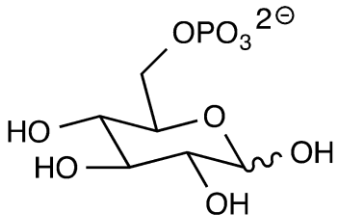
I. Roles of phosphorylated species in biology. P is an element that many of you have not previously thought about, but it is a constituent of almost all the cofactors in the cell (NAD, FAD, CoA, TPP, Vitamin B_{12} etc), of the energy currency of the cell (ATP and GTP), and of the molecules involved in information transfer in the cell (DNA, RNA). In addition, in both the glycolysis and gluconeogenesis pathways, all the sugars are phosphate monoesters. Phosphorylation is also a major regulatory mechanism in signal transduction pathways (fight or flight; fed state or starved state). S, T, Y are commonly phosphorylated in mammalian systems in a post-translational modification process. The phosphate then acts by an allosteric mechanism to control enzymatic activity or by altering interactions with other proteins or by targeting the enzyme for degradation. Phosphorylation of H, D, E and C have also been reported but are much less prevalent. His phosphorylation in bacteria provides the major signaling pathways.

II. Basic chemistry of phosphates. Phosphate is one of the major buffers inside the cell. There are three dissociable protons associated with phosphoric acid and their pKas are indicated below. [Recall that pKa = pH where the group of interest is 50% ionized.] There is an electrostatic barrier in going from the monoanionic to the dianionic state. The pK_{a2} is the one that is relevant

to biological systems. Phosphate is a common buffer used in biochemical studies in vitro as well as in the cell.



The pKa of phosphate monoesters, $ROPO_3H_2$, is very similar to inorganic phosphate. At pH 7 inside the cell, glucose-6-P (a phosphate monoester) is 90% in the dianionic state. All the sugar intermediates (metabolites) in glycolysis/gluconeogenesis/PPP are phosphorylated.



Glucose-6-phosphate
(G-6-P)

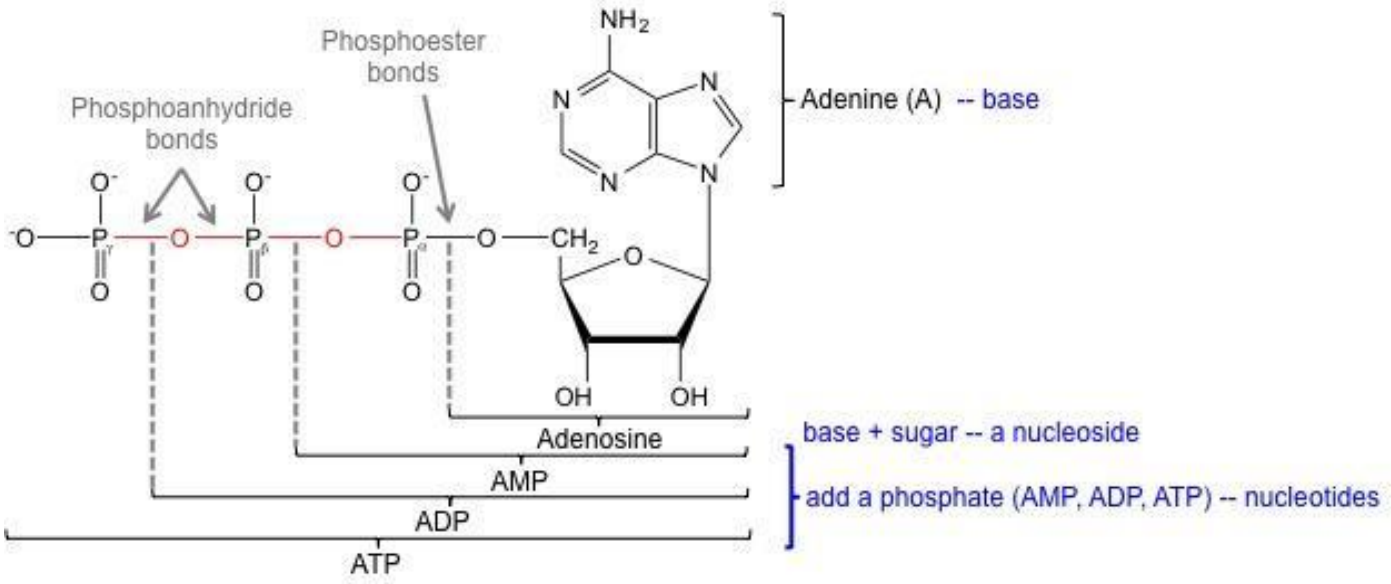
Glucose (G) itself is neutral and phosphorylation converts it to a charged state where it cannot diffuse through the membrane, out of the cell. Thus Nature in general has used this strategy to trap sugars and other small molecules, inside the cell. As G-6-P can be mono anionic or dianionic and the enzyme can use this charge as a handle to bind substrates of different ionization states.

III. ATP is the universal phosphorylating agent inside cell. Enzymes that use ATP to phosphorylate small molecules or proteins are called kinases. The structure of ATP is shown below and should become part of your basic vocabulary. The nucleoside adenosine contains the

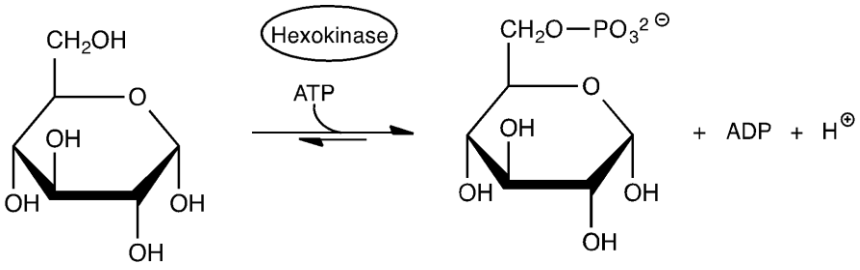
base adenine and the sugar (ribose). Adenine is the purine base alone. A nucleotide is the nucleoside that is phosphorylated (AMP, ADP or ATP).

IV. Generalizations about the mechanism of phosphoryl transfer with ATP.

1. The tripolyphosphate of ATP is composed of phosphoanhydride linkages and a phosphomonoester linkage. These linkages are the business end of the molecule where in general, chemistry occurs at either the γ or the α P. In the former case the substrate is phosphorylated and in the latter case the substrate is adenylated (AMP attached):



An example of phosphorylation is shown below. Hexokinase catalyzes the first step in the glycolysis pathway (glucose to glucose-6-phosphate).



Glucose Glucose-6-P

An example of adenylation involves acetylCoA synthesis catalyzed by acetylCoA synthetase

$$CH_3CO_2^- + ATP \leftrightarrows CH_3COAMP + PPi$$

$$CH_3COAMP + \text{-}SCoA \rightarrow AMP + CH_3COSCoA$$

adenylated acetate

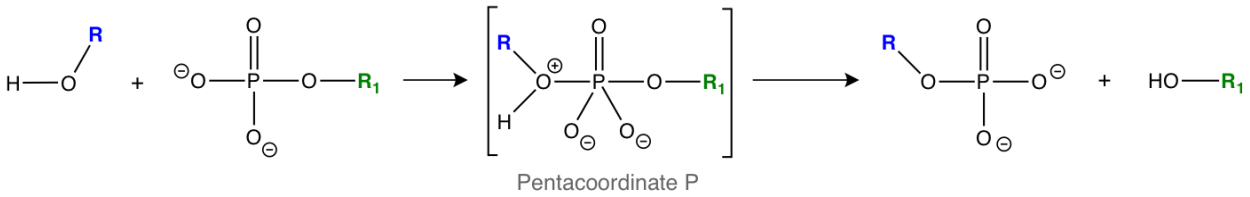
2. In general, the tripolyphosphate is always coordinated to at least one Mg^{2+}. The K_d for this interaction is sufficiently tight that all nucleoside triphosphates are 100% coordinated to Mg^{2+} inside the cell. This coordination reduces the negative charges on the molecule. This charge neutralization is essential for a nucleophile to attack on the γ or α P. In addition Ks, Rs in the active site of ATP requiring enzymes are also often involved in charge neutralization. In the active site of the kinase, the Mg^{2+} can be coordinated to the α,β Ps, the β, γ Ps or to the α, β, γ Ps and can isomerizes between coordination states during the kinase catalyzed conversion of substrate to product. Also conserved in the ATP binding domain are glycines that provide the flexibility essential for Mg^{2+} to reorganize during turnover.

3. There are two mechanisms for phosphorylation: an associative mechanism similar to the SN2 reaction you learned about with carbon chemistry and a dissociative mechanism (Figure 1) similar to an SN1 mechanism.

4. ATP is kinetically stable because of all the negative charges hinder nucleophilic attack.

5. ATP is thermodynamically labile. ATP, as described in detail below, is often used in a coupling reaction in metabolism to drive a reaction that is unfavorable to the right.

A.



B.

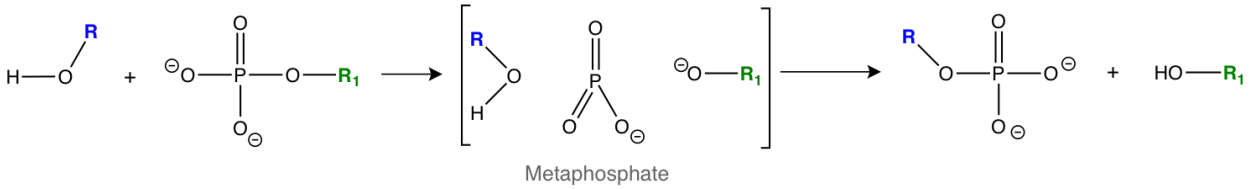


Figure 1. Mechanisms of phosphate transfer. A. Associative mechanism. B. Dissociative mechanism.

Digression - Brief review of spontaneity.

How does one predict spontaneity of a reaction? The state function to predict spontaneity is the Gibbs Free Energy: G. $\Delta G < 0$, the reaction is spontaneous, exergonic; $\Delta G > 0$, the reaction is non-spontaneous, endergonic; $\Delta G = 0$, the reaction is at equilibrium.

For a given reaction:

$$aA + bB \leftrightarrows cC + dD$$

you have learned in Freshman Chemistry (or Chemistry 5.60) that

$$\Delta G = \Delta G^\circ + RT\ln [C]^c[D]^d/[A]^a[B]^b$$

ΔG° is the free energy change for all reactants and products in their standard state; it is a reference state where intrinsic free energy changes under equivalent conditions can be compared. R is the gas constant, T is the absolute temperature, and the reactants are present at 1M. **Note for all A, B etc one looks at the total concentration, ignoring ionization states**. The standard state for biochemists differs from that used by chemists as most biological reactions are run at pH 7 in dilute aqueous solution. When a H^+ is a reactant, its activity has the value of 1, corresponding to a pH of 7. The activity of water is also taken as 1. Instead of using ΔG°, biochemists thus use $\Delta G^{\circ\prime}$. As we discussed in the lecture on kinetics, kilocalories (kcal) or kilojoules (kj) are units of energy where 1 kcal = 4.184 kj

At equilibrium $\Delta G = 0$ and $\Delta G^{\circ\prime} = - RT\ln [C_{eq}]^c[D_{eq}]^d/[A_{eq}]^a[B_{eq}]^b$

$= -RT\ln K'_{eq}$ When $R = 1.98 \times 10^3$ kcal/mol/ $^\circ$ and T = 298 K

Thus $K'_{eq} = 10^{-\Delta G^{o\prime}/1.38}$

Where have you seen this before? **THINK ABOUT the KINETICS lecture. A shift in the equilibrium constant by a factor of 10, requires a standard free energy change at 25°C of 1.38 kcal/mol** (Table 1)**.**

K_{eq}	10^{-6}	10^{-4}	10^{-2}	10^{-1}	10^{0}	10^{1}	10^{2}	10^{4}	10^{6}
$\Delta G^{o\prime}$ (kj/mol)	34.3	22.8	11.4	5.7	0	5.7	11.4	22.8	34.3

1.38 kcal/mol

Table 1. Standard free energy changes at varying equilibrium constants.

There exist two common ways in biochemical systems to influence the spontaneity of a reaction.

1. change the concentrations of the reactants/products
2. couple an unfavorable reaction with a favorable reaction

END DIGRESSION.

V. Use of ATP in vivo: ATP plays a major role in making an endergonic reaction work, due to its thermodynamic instability.

1. Concentrations affect spontaneity

$$ATP + H_2O \leftrightarrows ADP + Pi \qquad \Delta G^{\circ\prime} = -30.5 \text{ kj/mol}$$

Under physiological conditions the [ ]s of these species can vary (think about exercising versus resting muscle). For example [ATP] = 8mM; [ADP] = 1 mM and [Pi] = 8 mM

$$\Delta G' = \Delta G^{\circ\prime} + RT\ln [ADP][Pi]/[ATP]$$

Plugging in the [ ]s to the Equation given above, $\Delta G' = -49$ kj/mol

2. The additivity of free energy changes allows an endergonic reaction to be driven by an exergonic reaction (a coupling process)

1. A + B ⇆ C + D $\Delta G1$ $\Delta G1 > 0$ endergonic

2. D + E ⇆ F + G $\Delta G2$ $\Delta G2 < 0$ exergonic

1 + 2 A + B + E ⇆ C + F + G $\Delta G3 = \Delta G1 + \Delta G2 < 0$

Let us use a specific example from glycolysis: hexokinase or glucokinase overall reaction:

glucose + ATP ⇆ glucose-6-P + ADP $\Delta G^{o\prime} = -16.7$ kj/mol

glucose + Pi ⇆ glucose-6-P $\Delta G^{o\prime} = +13.8$ kj/mol

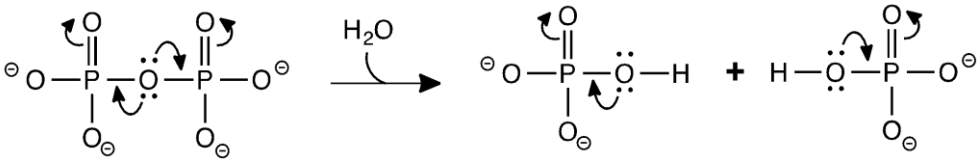
ATP + H_2O ⇆ ADP + Pi $\Delta G^{o\prime} = -30.5$ kj/mol

Conclusion: the thermodynamic lability of ATP is used frequently to drive reactions to the right.

VI. Why is ATP chosen as the energy currency of the cell? (Recall from the first lecture that ATP is continually being made and used) Why does ATP have such a large free energy of hydrolysis?

What is the driving force for **ATP's thermodynamic lability**?

a. Resonance stabilization differences between ATP, ADP, and Pi.



b. Destabilization of ATP relative to ADP or Pi by electrostatic delocalization

c. Solvation differences between ATP and ADP and Pi.

Appendix:

Thermodynamic units and constants that you may find useful.

Name	Value
Avagadro's Number (*N*)	6.0221×10^{-23} molecules/mol
Universal Gas Constant (*R*)	8.32 joule/mole•°K 1.69872 cal/°K•mol
Boltzmann's Constant (*k*)	1.38×10^{-23} joule/°K
Mechanical Equivalent of Heat (*J*)	4.19 joule/cal
1 Electronic Charge (*e*)	1.60×10^{-19} coulomb
Faraday (*F*)	96 485 joules/volt•mol 96 485 Cal/mol
Coulomb (C)	1.60×10^{-19} coulomb
1 Joule (J)	1 kg•m^2/s 1 N•m (newton meter)
1 Calorie (cal)	1 cal heats 1g of water from 14.5°C to 15.5°C 4.184 joules 1×10^{-3} Cal (large calories)

5.07SC Biological Chemistry I

Fall 2013