Phosphoryl Group Chemistry and Importance of ATP

32

Reactions involving transfer of phosphoryl groups are central to the metabolism of all living beings. This chapter brings out the unique features that make phosphate and its derivatives ideal candidates for driving metabolism. Chemistry at the phosphorus atom is almost always catalyzed by a suitable enzyme. Reaction mechanisms pertaining to these enzymes are presented. Aspects of high-energy compounds like ATP and their role in group transfer reactions are highlighted.

32.1 Why Nature Chose Phosphates

Phosphoric acid esters and anhydrides are cardinal players in metabolism and enzyme chemistry Westheimer 1987). They are found uniformly in the pathways of all biomolecules – nucleic acids, proteins, carbohydrates, and lipids. A few important phosphate compounds representing a range of linkages are listed in Table 32.1.

Phosphoric acid, its esters, and anhydrides are particularly selected by biological systems on the following counts. First, even as its diester (read genetic material like DNA!), phosphate retains one negative charge and is thus noticeably stable to nucleophilic attack. Consider this with 55.5 M of water as a reasonable nucleophile around. Second, the permanent negative charge serves to retain phosphate compounds inside – as they cannot cross the phospholipid bilayer without assistance. Third, negative charges on phosphates are excellent specificity/recognition entities of phosphorylated substrates in binding to enzymes. Lastly, phosphate (and at times pyrophosphate) is usually a good leaving group in many nucleophilic displacement reactions.

Phosphoric acid is a tribasic acid. Its successive ionization constants differ by factors of greater than 10^5. The three pKas of phosphoric acid are well spaced – $pKa_1 = 2.12$, $pKa_2 = 7.21$, and $pKa_3 = 12.32$. Not many other poly-anionic compounds are endowed with this feature. Therefore, phosphoric anhydrides exhibit many favorable properties. They are protected by these negative charges from rapid

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Table 32.1 Phosphate derivatives in metabolism

Nature of linkage to phosphoric acid	Examples
Monoester	Glucose-6-phosphate, dihydroxyacetone phosphate
Diester	DNA, RNA, and phospholipids
Enolic ester	Phosphoenolpyruvate
Amide	Phosphocreatine
Anhydride	ATP, pyrophosphate, and acetyl phosphate
Pyrophosphate ester	Isopentenyl pyrophosphate, 5-phosphoribosyl-1-pyrophosphate

attack of water and other nucleophiles. Thus, even though thermodynamically unstable, they are kinetically quite stable in aqueous environment. This remarkable combination of thermodynamic instability and kinetic stability makes them stand out as potentially *energy-rich* compounds (like ATP, see below). They can drive uphill chemical reactions in the presence of a suitable catalyst. Nature prefers phosphate esters because they are stable yet they can be attacked and cleaved by enzymatic hydrolysis.

32.2 Chemical Mechanisms at the Phosphoryl Group

Phosphoryl group ($-PO_3^-$) transfers are ubiquitous in intermediary metabolism. And they are invariably enzyme-catalyzed reactions (Knowles 1980, Cleland and Hengge 2006). Every thermodynamically uphill step of metabolism involves a displacement at the phosphorous atom of a phosphoric monoester or anhydride. Mechanistically phosphoryl group transfer reactions can be studied at three levels: (a) whether a phospho-enzyme is formed during the reaction, (b) which is the rate-limiting step and the nature of the transition state, and (c) whether the displacement at phosphorus atom is associative or dissociative in nature.

Bond Cleavage at Phosphorus Atom In all phosphoryl and pyrophosphoryl group transfers, the phosphorus atom reacts as an electrophilic center. It is therefore handed over from one nucleophile to the other. *All enzymatic phosphoryl transfers proceed with the cleavage of the phosphorus–oxygen bond*, and a nucleophile forms a bond to phosphorus. During phosphoryl ester hydrolysis, for instance, the oxygen of water appears in phosphate – implying a nucleophilic attack by "O" of water on P. This is easily demonstrated by performing the hydrolysis in ^{18}O-labeled water. Whenever phosphorylation serves to activate a group, the situation is different. The acyl group transfer and glycosyl group transfer reactions are illustrative. In both these cases, the carbon–oxygen bond is cleaved and a nucleophile forms a bond to carbon. We will discuss about *activation of groups* and the concept of a *good leaving group* later on.

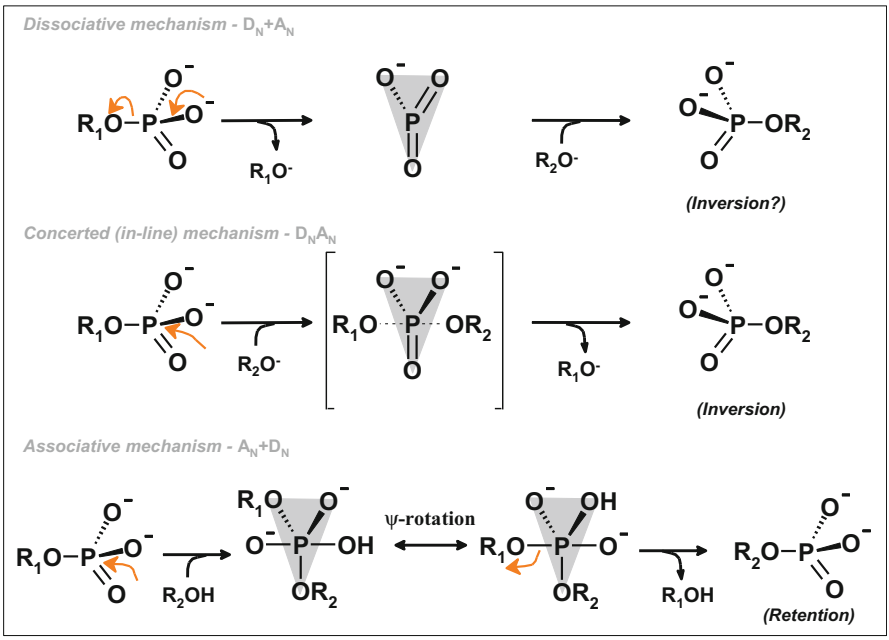


Fig. 32.1 Mechanisms of phosphoryl group transfer. The trigonal plane defining the phosphoryl group is shown as gray triangle. (1). Dissociative pathway goes through an unstable metaphosphate intermediate. While a racemic product is expected in solution, the spatial arrangements of substrates at the enzyme active site govern the stereochemical outcome. (2). The concerted (in-line) reaction between the two substrates leads to inversion. (3). The associative (adjacent) mechanism always leads to retention of configuration. The pathway goes through pseudo(ψ)-rotation at the penta-coordinate intermediate stage. Subsequently, the leaving group (R_1OH) always leaves from an apical position of the trigonal bipyramid

Phosphoryl group transfer reactions broadly fall into three types (Fig. 32.1). These differ in the mechanism of bond cleavage/formation at the phosphorus atom.

1. In the *dissociative mechanism*, a meta-phosphate (PO_3^-) intermediate is formed prior to attack by the incoming nucleophile (Fig. 32.1). The outgoing nucleophile dissociates in the first step (D_N), while the incoming nucleophile attacks the meta-phosphate in the second (A_N). In the IUPAC nomenclature, it is denoted as $D_N + A_N$ mechanism and is analogous to the S_N1 reaction mechanism in carbon chemistry (see Chap. 29). Evidence (through kinetic isotope effects; Chap. 27) for a meta-phosphate-like transition state (loose *TS*) was obtained for the bovine protein tyrosine phosphatase. Similarly, stabilized meta-phosphate entity has been experimentally observed with fructose-1,6-bisphosphatase and *Lactococcus lactis* β-phosphoglucomutase.
2. The *concerted mechanism* proceeds via a penta-coordinate transition state. In this case (which is S_N2 like and is also known as D_NA_N mechanism), the attacking nucleophile enters opposite the leaving group. In this *in-line mechanism*, no

reaction intermediate is involved. Adenylate kinase is an example of this type. Its mechanism involves an inversion at the P atom; this can be tested using a chiral phosphate ester and examining the stereochemical outcome of the overall reaction (see below).

3. The third possibility is of an *associative mechanism*. Here the incoming nucleophile attacks first to give a penta-coordinated phosphorane intermediate (Fig. 32.1). It is therefore shown as $A_N + D_N$ mechanism. The penta-covalent intermediate is of trigonal bipyramid geometry; the five substituents therefore are found either at *equatorial* or at *apical* position. In this *adjacent mechanism*, the nucleophile enters on the same side as the leaving group. Since groups can enter or leave only from the apical position, the trigonal bipyramid formed has to rearrange. This movement – termed *pseudorotation* – brings the originally equatorial leaving group to apical position for expulsion. A well-characterized example of associative mechanism is bovine pancreatic ribonuclease A. The 2′-OH of ribose sugar attacks the phospho-diester via an associative mechanism to form a divalent transition state stabilized by Lys-41.

We know that tetravalent carbon compounds are stable. But phosphorus can form stable trivalent (planar), tetravalent (tetrahedral), and pentavalent (a trigonal bipyramid with three equatorial and two axial bonds to P) compounds. This has a bearing on the relevant bond orders to P atom, possible during reaction. Concerted mechanism of phosphoryl transfer reactions straddles the two outer limits of an associative (five bonds to P) to a fully dissociative (three bonds to P) transition state. The character of the transition state thus ranges from (a) being associative with the sum of the axial bonds between one and two, (b) to S_N2 with the sum of the axial bonds equal to one, (c) to dissociative with the sum of the axial bonds less than one. As a general rule, at least with nonenzymatic phosphoryl transfer reactions, the trend is loose transition states for monoesters, a synchronous reaction for diesters, and a tight transition state for triesters. As the phosphate is more esterified (like in phosphodiesters and phosphotriesters), these stabilized phosphoesters require additional bond order from the nucleophile to achieve transition state. In reality, progressively more associative mechanisms occur on a continuum. This also depends on whether a good leaving group is found on the P atom (such as a concerted mechanism) or not (such as in a fully associative mechanism).

Stereochemistry of Phosphoryl Transfer Stereochemical course of a reaction provides excellent clues to the reaction mechanism. This is true as well with the chemical mechanisms at P atom. Phosphates contain three apparently identical oxygen substituents. And it is convenient that three isotopes of oxygen are available – ^{16}O, ^{17}O, and ^{18}O. Chiral phosphate esters can be prepared to act as enzyme substrates. Upon reaction, chiral phosphate ester products may be generated (Fig. 32.1). Absolute configuration of such products can be analyzed, albeit with some chemical (technical) skill and spectroscopy. In the final outcome, we can

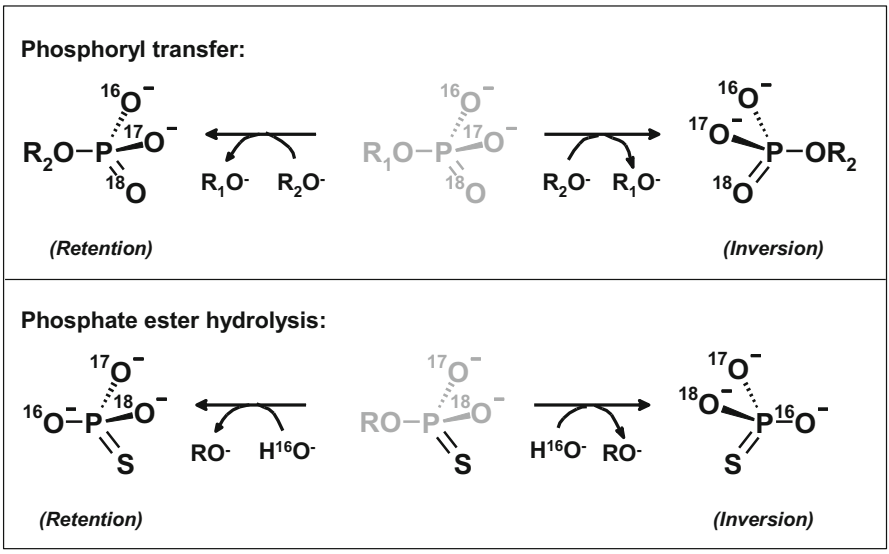


Fig. 32.2 Stereochemistry at the phosphorus atom. Phosphoryl group has the same tetrahedral geometry as the saturated carbon compound. The three oxygen isotopes (^{16}O, ^{17}O, and ^{18}O) along with the unique R group on the fourth oxygen define the stereochemistry of phosphoryl transfer. For phosphate ester hydrolysis, however, S atom of thiophosphate serves as the fourth substituent to define the stereochemistry of phosphate release

clarify whether the enzymatic reaction proceeds with retention or inversion of configuration at the P atom.

Defining the stereochemical course for phosphate (when compared to its ester) is a bit tricky. The phosphate has four equivalent oxygen atoms, while only three isotopes of oxygen are available to mark them. This limitation can be overcome by using sulfur as the fourth substituent (Fig. 32.2). Fortunately, many enzymes do accept substrates with corresponding thiophosphate groups. Analyzing the configuration of [^{16}O,^{17}O,^{18}O]-thiophosphate product formed is all that is needed.

Stereochemical evidence is the most diagnostic of mechanistic pathway of phosphoryl group transfer. This is particularly true in cases where a phosphorylated enzyme intermediate cannot be isolated and characterized. A single displacement at phosphorus generally results in inversion of stereochemistry. Reactions with no phosphoryl enzyme intermediate (a direct *in-line* attack leading to phosphoryl transfer) usually go with inversion. Phosphokinases proceed with inversion at P suggesting a direct transfer between the two substrates. Phosphomutases – viewed as internal kinases – proceed with retention. This results from two inversions because of a double displacement involving a phosphoryl enzyme intermediate. In enzymes such as alkaline phosphatase – where a phosphoryl enzyme form exists – the reaction ends up with retention. Presence of a phospho-enzyme intermediate (for *E. coli* alkaline phosphatase) was demonstrated by incorporation of ^{32}P from the labeled

substrate on to the enzyme Ser residue. The associative (adjacent) mechanism always leads to retention as the configuration is retained in each step.

Phosphoryl Transfer Mechanism: Single or Double Displacement?

The transfer of a phosphoryl group (from the donor substrate to the acceptor substrate) in principle can occur in two different ways. (1) The phosphorylium group may be directly transferred from the donor substrate to the acceptor substrate – a *single displacement* route. (2) The phosphoryl group is first transferred to a suitable group on the enzyme (phosphoenzyme covalent intermediate is formed) and is then transferred from the enzyme to the acceptor substrate in the second step – a *double displacement* route.

Examples to illustrate the two different modes of phosphoryl transfer are adenylate kinase and nucleoside bisphosphate kinase. The chemical reactions catalyzed by the two enzymes are apparently similar.

Adenylate Kinase

$$\mathbf{AMP + Mg\text{-}ATP \rightleftarrows ADP + Mg\text{-}ADP}$$

Nucleoside Bisphosphate Kinase

$$\mathbf{Mg\text{-}GDP + Mg\text{-}ATP \rightleftarrows Mg\text{-}GTP + Mg\text{-}ADP}$$

However adenylate kinase follows a single displacement mechanism, while nucleoside bisphosphate kinase operates through a double displacement mechanism. A kinetically competent phospho-enzyme (see Table 31.4) has been demonstrated for the latter enzyme. The types of experimental evidence to support and contrast these two mechanisms are tabulated below.

Single displacement (adenylate kinase)	Double displacement (nucleoside bisphosphate kinase)
Phospho-enzyme intermediate not formed	Phospho-enzyme intermediate is formed
Stereochemical inversion at P center	Stereochemical retention at P center
Sequential kinetic mechanism	Ping-pong kinetic mechanism
No partial exchange reactions occur	Partial exchange reactions are observed

32.3 Adenosine Triphosphate: Structure Relates to Function

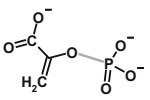
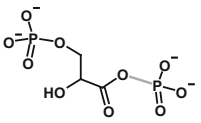
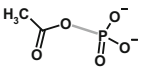
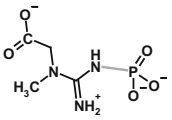
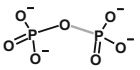
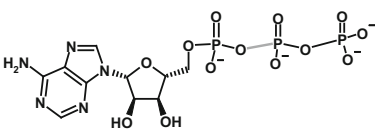
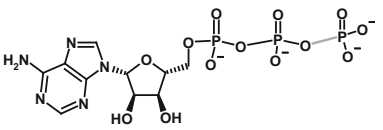
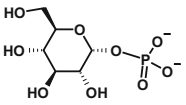
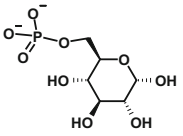
The most common phosphoryl group donor in metabolism is adenosine triphosphate – commonly abbreviated as ATP (Ramasarma 1998). It was identified as a derivative of adenosine with three phosphates by Fiske and SubbaRow in 1929. ATP is a derivative of adenosine-5′-phosphate with two more phosphates attached to its 5′-phosphate via anhydride linkages. Todd et al. confirmed this by chemical synthesis in 1949. As noted above, and in contrast to carboxylic anhydrides, phosphoric anhydride groups in ATP are protected by their negative charges from rapid attack by water (and other nucleophiles). Fritz Lipmann's observation was prescient in ascribing the kinetic stability of ATP to the negative charges in ATP. This makes hydrolysis of ATP thermodynamically favorable (large $-\Delta G^{\circ}$) but kinetically unfavorable (large $\Delta G^{\neq}$) – a virtue exploited by nature to use ATP as an ideal free energy currency.

ATP Is a High-Energy Compound Combination of kinetic stability and thermodynamic instability imparts ATP its *energy-rich* nature. Hydrolysis of ATP can therefore be coupled to drive uphill chemical reactions in the presence of a suitable enzyme catalyst. *ATP provides energy by group transfer and not by simple hydrolysis*. The term *energy rich* implies that (a) it has a high phosphate group transfer potential and (b) on hydrolysis of its phosphoric anhydride bonds, sufficient ΔG (free energy) is available for the formation of other bonds. The ΔG° for ATP hydrolysis (ATP ⇄ ADP + Ⓟ) is negative and very large (around 7.0 kcal/mol, at 25 °C and pH 7.0). This corresponds to an equilibrium constant for hydrolysis of about 140,000 M!

What makes ATP a *high-energy compound* and confers it *high phosphate group transfer potential*? Several factors that contribute include (a) electrostatic repulsions between neighboring negative charges of ATP, (b) relative bond energies of the reactants and products, and (c) better solvation and relative resonance stabilization of the products (ADP + Ⓟ) of hydrolysis. Other compounds (listed above ATP in Table 32.2) may also be recognized as high-energy compounds on similar grounds. Since negative charges on ATP are a function of phosphate group ionization, pH and ionic strength have a significant effect on ΔG° for ATP hydrolysis. ATP invariably occurs as a complex with divalent metal ions – Mg^{2+} in particular (see below). For these reasons, the likely ΔG° for ATP hydrolysis in vivo may be as high as 12.0 kcal/mol (and not 7.3 kcal/mol).

Like ATP, several phosphorylated compounds can transfer their phosphoryl groups to water. The associated free energy changes (ΔG° of hydrolysis) are listed in Table 32.2. The larger the negative ΔG° for a phospho-compound, the greater is its phosphoryl group transfer potential. This sets up a nice hierarchy of energy transfer in coupled reactions. Under standard conditions, for instance, compounds above ATP (in Table 32.2) can transfer phosphoryl group to ADP. And those below cannot. Phosphoenolpyruvate has the highest negative ΔG°; this is because the enol formed quickly converts to the keto form making the reaction further exergonic.

Table 32.2 ΔG° **of hydrolysis for important phospho-compounds**

Phospho-compound	Structure[a]	$-\Delta G^{\circ}$ (kcal/mol) (25°C, pH 7.0)
Phosphoenolpyruvate		14.8
1,3-Bisphosphoglycerate		11.8
Acetyl phosphate		10.3
Phosphocreatine		10.3
Inorganic pyrophosphate (Ⓟ-Ⓟ)		8.0
ATP (→AMP + Ⓟ-Ⓟ)		7.7
ATP (→ADP + Ⓟ)		**7.3**
Glucose-1-phosphate		4.8
Glucose-6-phosphate		3.3

[a]The ΔG° of hydrolysis for *high-energy* bond (shown in gray) is listed in the last column (1.0 kcal = 4.184 kJ). Note that glucose-1-phosphate and glucose-6-phosphate are phosphate esters that do not contain a *high-energy* bond. Whenever abbreviated, the phosphoryl group is shown as "Ⓟ" throughout this book

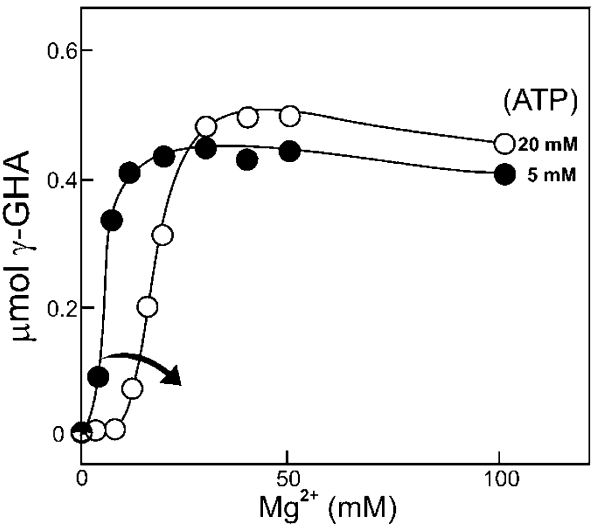


Fig. 32.3 Mg^{2+} saturation profile of glutamine synthetase. Mg-ATP is the true substrate of this enzyme (see Table 32.3 later, for the reaction). A small fraction of total ATP is present as Mg-ATP at lower [Mg^{2+}]. The higher the initial [ATP] used, the curve is shifted further to the right (arrow). This apparent sigmoidicity is a manifestation of equilibrium between free [ATP], free [Mg^{2+}], and their various complexes and does not reflect cooperative enzyme kinetics

Phosphoenolpyruvate has a higher phosphoryl transfer potential than ATP; it can therefore phosphorylate ADP to ATP.

Complexes of ATP with Metal Ions Metabolically relevant ATP occurs as a divalent metal ion complex. Most often this metal ion is Mg^{2+}. The shielding of ATP-negative charges (Chap. 6) is important for access to nucleophiles. Mg-ATP^{2-} is the true substrate of most enzymes that are generally described as *ATP-dependent*. In reality, there is nothing like a pure solution of Mg-ATP^{2-}. An equimolar mixture of ATP and $MgCl_2$ at pH 7.0 contains the following species at different concentrations (in their decreasing order!): Mg-ATP^{2-}, ATP^{4-}, $HATP^{3-}$, Mg^{2+}, Cl^-, Mg-$HATP^-$, Mg_2-ATP, and $MgCl^+$. Moreover their proportions vary with (a) the total [ATP] and [$MgCl_2$], (b) the pH and buffering species present, and (c) the ionic strength. For these reasons studying interactions of Mg-ATP^{2-} with an enzyme obviously requires much care. Otherwise phenomena like spurious cooperativity (Fig. 32.3) may occur (Punekar et al. 1985). To avoid such kinetic artifacts, it is important to accurately evaluate the true concentration of Mg-ATP^{2-} at a given total [ATP] and [$MgCl_2$]. The stability constants for many of the metal complexes of biochemical interest have been measured. It is thus possible to calculate the concentration of any complex given the concentrations of free components. There are simple computer programs to do such iterative calculations.

Three common experimental designs are found in literature – while deciding [ATP] and [$MgCl_2$] to fix Mg-ATP^{2-} concentration. It does not help to either (a) vary [ATP] and [$MgCl_2$] at equimolar ratios or (b) fix [$MgCl_2$] at a very high value (say 10 mM) while [ATP] is varied. The third and the best option is to keep the total [$MgCl_2$] in constant excess over the total [ATP]. One final factor to consider is the variation in ionic strength as the component ($MgCl_2$ and ATP) concentrations are

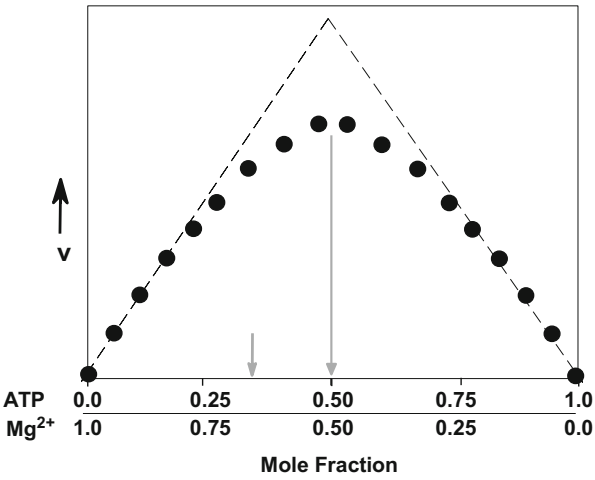


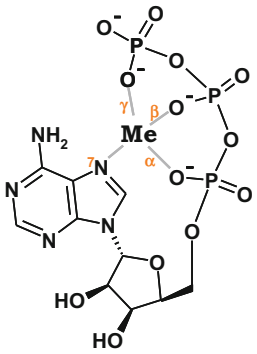
Fig. 32.4 Job plot for an enzyme requiring Mg-ATP as substrate. The maximal enzyme activity is at the mole fraction of 0.5 (1:1 ratio of ATP:Mg^{2+}; the long vertical gray arrow). The peak activity would move to the left for an optimal mole fraction of 0.33 (1:2 ratio of ATP:Mg^{2+}; short gray arrow)

increased. It is desirable to maintain the ionic strength constant. A value of about 0.15 M may be appropriate since it approximates the ionic strength in vivo.

A method of continuous variation may also be used to arrive at the best ratio of [ATP] and [$MgCl_2$]. This protocol – called the *Job plot* – provides useful information in optimizing the assay under a standard set of conditions. Mole fractions of [ATP] and [$MgCl_2$] are varied such that the total molarity remains constant. If the enzyme prefers a 1:1 complex, then the enzyme activity will be maximal at mole fraction of 0.5 (as shown in Fig. 32.4). However, if the active species is a 1:2 complex (of ATP:Mg^{2+}), then the enzyme will be most active at mole fraction of 0.33.

Free ATP in solution (when not in a complex with divalent metal ion) takes a linear extended conformation. It is a flexible molecule because P-O single bonds enjoy many degrees of freedom. As a metal complex, however, ATP conformation is frozen. ATP assumes specific folded forms when so complexed. Different divalent metal ions interact differently with ATP; variations include interactions with α-, β-, and γ-phosphates and N7 of adenine (Fig. 32.5). This has a bearing on which ATP-metal ion complex can serve as substrate for a given enzyme.

Both tri-dentate and bi-dentate (Mg^{2+} bonded to O atoms of β- and γ-phosphate) complexes of Mg-ATP are formed in solution. Furthermore, β,γ-bi-dentate Mg-ATP exists as a rapidly equilibrating mixture of **Λ** and **Δ** screw-sense isomers (Fig. 32.6). These two isomers have opposite CD spectra. It is tricky to determine which of the three Mg-ATP forms the true substrate for a given enzyme. This can be attempted in the following ways:



Metal ion (Me)	Bonds to
Mg^{2+}	$^{\beta}O$, $^{\gamma}O$ (and ^{7}N)
Mn^{2+}	$^{\alpha}O$, $^{\beta}O$, $^{\gamma}O$ and ^{7}N
Co^{2+}	$^{\beta}O$, $^{\gamma}O$ and ^{7}N
Zn^{2+}	$^{\beta}O$, $^{\gamma}O$ and ^{7}N
Ca^{2+}	$^{\beta}O$ and $^{\gamma}O$

Fig. 32.5 Each divalent metal ion interacts differently with ATP. Different combinations of phosphate oxygens ($^{\alpha}O$, $^{\beta}O$, and $^{\gamma}O$) and/or ^{7}N of adenine coordinate to the metal ion are shown in the accompanying table

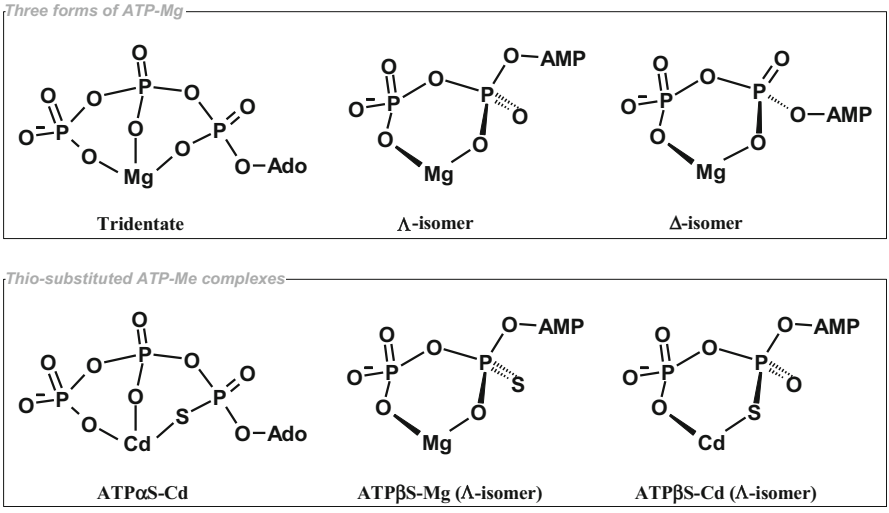


Fig. 32.6 Various forms of Me-ATP complexes in solution. The screw-sense isomers (**Λ** and **Δ** forms) of Mg-ATP are in rapid equilibrium. The α-phosphate of ATPαS is coordinated to Cd through the S atom. The β-phosphate of ATPβS becomes chiral as one O is replaced by S atom. The screw-sense isomers formed with Mg and Cd are reversed when the other stereoisomer of ATPβS is used. The adenosine moiety is abbreviated as Ado

1. ATP coordination complexes of ATP with Cr^{3+}, Co^{3+}, and Rh^{3+} are inert in water – their stable screw-sense isomers can be separated as pure Λ and Δ forms and tested as possible substrates. For instance, hexokinase uses the Λ isomer of β,γ-bi-dentate Cr-ATP, while adenylate kinase prefers the Δ isomer.

2. Chiral sulfur substituted ATP complexes differently with Mg^{2+} and Cd^{2+}. Mg^{2+} prefers to coordinate to O over S by a factor of 31,000, whereas Cd^{2+} prefers S over O by a factor of 60. And when sulfur replaces one of the two non-bridge oxygens of β-phosphate, the resulting ATPβS becomes chiral (Fig. 32.6). Therefore with ATPβS the two metal ions form distinct (predominant) screw-sense complexes. Specific preference of an enzyme for Mg^{2+} or Cd^{2+} complex of ATPβS is thus indicative of its screw-sense isomer specificity. The two Λ screw-sense complexes shown in Fig. 32.6 are substrates for yeast hexokinase, for example.
3. ATPαS is useful in deciding whether or not the α-phosphate is coordinated to the metal ion during reaction. If α-phosphate coordination is significant for enzyme catalysis, then a reversal in the α-S isomer specificity is expected when Mg^{2+} is replaced by Cd^{2+}. The Mg^{2+} to Cd^{2+} switch affects α-S isomer specificity of creatine kinase but not of hexokinase. Thus, β,γ-bi-dentate Mg-ATP is the substrate for hexokinase, whereas it is the tri-dentate Mg-ATP for creatine kinase.

ATP Binding to Enzymes Negative charges of ATP tend to protect it from the attack by incoming nucleophiles. Then how is it handled at the enzyme active site? ATP exists in vivo largely as a complex with Mg^{2+}. Typically Mg^{2+} neutralizes two of the negative charges. Ion pair interactions with active site Arg (guanidinium group) and Lys (ammonium group) residues contribute to binding (also hydrogen bonds) and further negative charge neutralization. Together such interactions generally define the productive binding of ATP to the enzyme active site. While many subtle variations on this theme are possible, there is a degree of active site conservation to accommodate ATP. The sequence popularly known as *Walker motif*-A (also called P loop) is thought to be the nucleotide binding site in many proteins. It consists of the sequence A/GX_4GKT/S (in one-letter code) and is flanked by a β-strand and an α-helix. Walker motif loops around the triphosphate moiety of ATP. The Walker motif-B, on the other hand, consists of X_4D (where X is almost exclusively a hydrophobic residue), occurs at the end of a β-strand, and interacts with the Mg^{2+} ion coordinated to the triphosphate moiety of ATP.

Different Modes of ATP Cleavage ATP is a versatile molecule serving as a free energy source as well as a donor of its constituent groups, namely, phosphate (Ⓟ), pyrophosphate (Ⓟ-Ⓟ), and AMP. Accordingly, ATP hydrolysis may occur at its γ-, β-, or α-phosphate group (Fig. 32.7).

The ATP α-bond is cleaved in nucleotidyltransferase reactions, the γ-bond is cleaved in kinase (phosphotransferase) reactions and both the α and γ bonds are mobilized in S-adenosylmethionine synthesis (Frey and Magnusson 2003, Struck et al. 2012). Cleavage at the β-bond is rare. Both α- and β-bonds are thought to proceed through an associative transition state – as it is crowded at the P atom. With its two negative charges and because it is a good leaving group, a dissociative

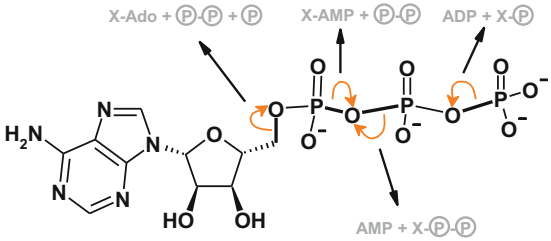


Fig. 32.7 Different modes of ATP cleavage. The electron movements (gray arrows) indicate the bond cleaved when a nucleophile (X:) attacks one of the P or C atoms of ATP. Some representative enzyme examples of these may be found in Table 32.3

Table 32.3 Different modes of ATP cleavage in metabolism

ATP cleavage pattern	Enzyme and reaction	Comment[a]
ATP → ADP + Ⓟ	*Glutamine synthetase*	Bond between β-γ-phosphates is split
	Glutamate +NH_3 + ATP → Glutamine +ADP + Ⓟ	
ATP + X → ADP + X-Ⓟ	*Hexokinase*	The γ-phosphate is transferred to acceptor
	Glucose+ATP → Glucose 6-Ⓟ + ADP	
ATP → AMP + Ⓟ-Ⓟ	*Aminoacyl-tRNA synthetase*	Bond between α-β-phosphates is split
	Amino acid +ATP + tRNA → Aminoacyl-tRNA+AMP + Ⓟ-Ⓟ	
ATP + X → AMP + X-Ⓟ-Ⓟ	*5-Phosphoribosyl-1-pyrophosphate synthetase*	Bond between α-β-phosphates split with pyrophosphate transfer to acceptor
	Ribose-5-Ⓟ + ATP → 5-Phosphoribosyl-1-pyrophosphate +AMP	
ATP + X → X-AMP + Ⓟ-Ⓟ	*FAD synthetase*	Bond between α-β-phosphates split with AMP transfer to acceptor
	FMN + ATP → FAD+Ⓟ-Ⓟ	
ATP + X → X-Adenosine + Ⓟ-Ⓟ + Ⓟ	*S-Adenosylmethionine synthetase*	Bonds at α- and γ-phosphates split with adenosine transfer to acceptor
	Methionine + ATP → S-Adenosylmethionine + Ⓟ-Ⓟ + Ⓟ	

[a]Refer to Fig. 32.7 to visualize which bond(s) of ATP are mobilized

transition state is expected when the γ-bond of ATP is cleaved. Representative examples of all these modes of ATP cleavage are listed in Table 32.3.

Table 32.4 Compounds with high group transfer potential

Group transferred	High-energy compound	Examples
Acetyl (acyl) group	Oxygen-ester	Aminoacyl tRNA, acylcarnitine
	Thioester	Acyl CoA, acyl carrier protein
	Mixed anhydride	Acetyl phosphate
Phosphoryl group[a]	Acid anhydride	ATP, Ⓟ-Ⓟ
	Phosphoramidate (P-N bond)	Phosphocreatine

[a]More examples of high phosphoryl group transfer potential may be found in Table 32.2

32.4 Investing Group Transfer Potential to Create Good Leaving Groups

The *group transfer potential* of a compound is defined as the negative of free energy of its hydrolysis. The transfer of a group (such as γ-phosphate from ATP) to water (hydrolysis) provides a standard for use in comparing such reactions. The more positive the group transfer potential, the greater is the tendency to transfer the group to an acceptor. (This concept is similar to how we define standard reduction potential and rank reducing agents.) Thus a comparison of group transfer potentials provides the means for establishing the direction of group transfer among various donor-acceptor molecules. Recall that since phosphoenolpyruvate has higher phosphoryl transfer potential, it can phosphorylate ADP to ATP (Table 32.2). The concept of group transfer potential is not just limited to phosphoryl transfers but may be extended to others like acyl group transfers. Some relevant examples of compounds with high group transfer potential are listed in Table 32.4.

Leaving groups in a reaction are quite often nucleophiles (see Chap. 31). And a *good leaving group* is one that is stable in solution. Generally, the lower the base strength, the greater the ease of expulsion. In keeping with this, the leaving group order is acetate (pKa, 4.76) > phosphate (pKa, 7.00) > hydroxyl (pKa, 15.8). Alkoxides (RO^-; pKa, 16.0) and amide ions (RNH^-; pKa, 30) are difficult to expel because they are strongly basic. They need to be protonated to leave from a reaction center – enzymes provide suitable acid groups to donate protons and help them leave. More importantly, a poor leaving group has to be activated for expulsion. This is done through energy-rich compounds that exhibit high group transfer potential (Table 32.4). A major objective of investing phosphate transfer potential of ATP into different recipient substrates is thus to achieve better leaving group chemistry for the reaction. Tagging poor leaving groups with different parts of ATP serves to activate them. For instance, methyl transfer from methionine is difficult because basic thiolate anion has to be expelled. But when methionine is activated as S-adenosylmethionine (see Table 32.3), a facile leaving group is created. This is S-adenosyl-homocysteine – a neutral, nonbasic sulfur compound. In this sense, *high group transfer potential is used to craft a good leaving group*. We will elaborate this concept below by describing acyl group activation, frequently encountered example in metabolism.

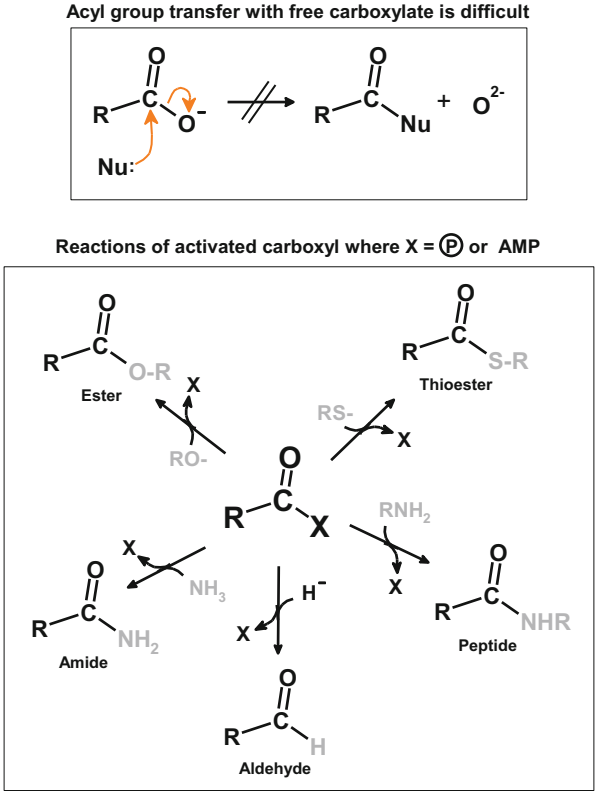


Fig. 32.8 Activating carboxylate for acyl group transfer. Since O^{2-} is a very poor leaving group, the carboxylate is first tagged with a better leaving group (shown as X) like Ⓟ or AMP. This can be easily displaced by the incoming nucleophile

Activating Carboxylate for Acyl Group Transfer Nucleophiles need to approach and attack the carboxylate carbon to form various products. Important examples include ester synthesis (R-OH as the incoming nucleophile), peptide/amide bond formation (R-NH_2 as the incoming nucleophile), and reduction of carboxylate to aldehyde (hydride as the incoming nucleophile). But the free carboxylate ($-COO^-$) group in water is not reactive toward a nucleophilic attack. This is because the incoming nucleophile has to displace O^{2-} from the carbonyl carbon (Fig. 32.8). The O^{2-} species is a very poor leaving group making the event thermodynamically unfavorable. Therefore, a modifying group must be built and tagged to the leaving carboxylate oxygen. Upon activation, this oxygen first becomes part of a good leaving group (Perler 1998). Subsequently, the incoming nucleophile displaces this group to establish a bond with the carbonyl carbon (Fig. 32.8).

Different representative examples of acyl activation in enzyme reactions are summarized in Table 32.5.

Table 32.5 Enzyme reaction mechanisms involving acyl activation

Activated acyl group	Nucleophile which accepts the acyl group	Enzyme example
Acyl phosphate – *phosphate as leaving group*		
γ-Glutamyl phosphate	NH_3	Glutamine synthetase
γ-Glutamyl phosphate	NH_2 of cysteine	γ-Glutamylcysteine synthetase (GSH biosynthesis)
γ-Glutamyl phosphate	Hydride (H^-)	γ-Glutamyl phosphate reductase
Aspartyl-β-phosphate	Hydride (H^-)	Aspartate β-semialdehyde dehydrogenase
1,3-Bisphosphoglycerate[a]	Enzyme-Cys-SH	Glyceraldehyde-3-phosphate dehydrogenase
Acyl adenylate – *AMP as leaving group*		
Fatty acyl-AMP	CoA-SH	Fatty acyl-CoA synthetase
Amino acyl-AMP[b]	NH_2 of amino acid	Non-ribosomal peptide synthetase
Amino acyl-AMP	tRNA-CCA-OH	Amino acyl-tRNA synthetase

[a]The acyl group is first transferred to enzyme-Cys-SH. This thioester is later reduced to aldehyde
[b]In the non-ribosomal peptide synthetase, the amino acyl group is first transferred to a thiol (Cys-SH and 4′-phosphopantetheine-SH) on the enzyme. This thioester is then attacked by the next incoming amino acid to form the peptide bond

32.5 Summing Up

Inorganic phosphate, phosphorylated metabolites, and high-energy phosphate compounds play pivotal yet universal roles in cellular metabolism. Phosphoric acid, a tribasic acid with unique ionization properties, is well exploited by nature.

Phosphorylation imparts charge and polarity to compounds. Such tags prevent them from escaping out of the cellular compartments.

Enzyme substrates are often derivatized with phosphate, adenosine, or AMP. Presence of an additional group like this provides molecular features for better recognition/discrimination at the enzyme active site. They in turn contribute to binding energy that can be exploited for catalysis.

The phosphate group has the same tetrahedral geometry as that of a saturated carbon center. Substitution by four different groups on a P atom generates a chiral center.

Phosphorus atom provides an electrophilic center in most reactions involving nucleophilic attack. Whenever group transfer occurs, phosphorylium (phosphoryl group, PO_3^-) is transferred and not the phosphate (PO_4^{3-}).

Group transfers are best achieved in a reaction with good leaving groups. High-energy compounds are used to modify the reactants so that a better leaving group is created. Phosphate, pyrophosphate, and AMP are examples of leaving groups derived from ATP.

In the final analysis, the ester/amide bond synthesis is an example of dehydration reaction. And ATP, being an effective anhydride, acts as a remarkable dehydrating agent. In this sense, high-energy compound nature of ATP is employed to drive uphill reactions. ATP provides chemical energy by group transfers and not by simple hydrolysis.

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