BIOORGANIC CHEMISTRY 2, 179–190 (1973)

REVIEW

Stereochemical Reaction Mechanism Formulations for Enzyme-Catalyzed Pyrophosphate Hydrolysis, ATP Hydrolysis, and ATP Synthesis

EPHRAIM F. KORMAN[1] AND JEROME MCLICK[2]

Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin 53706

Received August 3, 1972

Fundamental concepts pertaining to the stereochemistry paths of polar addition–elimination (nucleophilic substitution) reactions at phosphate phosphorus centers are reviewed and employed to analyze ^{18}O exchange reactions catalyzed by inorganic pyrophosphatase and mitochondrial ATP synthetase. The analysis suggests reasonable choices for the stereochemistry path of the ^{18}O exchanges. This, in turn, permits reasonable choices for the stereochemistry paths of hydrolysis of pyrophosphate catalyzed by pyrophosphatase and of hydrolysis and synthesis of ATP catalyzed by ATP synthetase.

Over the past dozen years there has been a significant advance in our knowledge of stereochemistry and reaction mechanisms at second-row element centers such as silicon and phosphorus (*1–3*). It is widely recognized that such fundamental knowledge will be important in understanding biological reactions involving second-row element centers. Of such reactions, those involving phosphate phosphorus centers are among the most important. In this paper we review some fundamental concepts of phosphorus stereochemistry and polar reaction mechanisms and show how these concepts may be applied to certain biological reactions involving phosphates. The biological phosphate reactions under consideration have been observed to be accompanied by exchanges of ^{18}O from $H_2{}^{18}O$ of the medium into the pertinent phosphate phosphorus reaction centers. The ^{18}O exchange data are recognized as the expression of underlying chemical reaction mechanisms catalyzed by the enzymes. We utilize ^{18}O exchange data in combination with fundamental concepts of phosphorus stereochemistry and reaction mechanisms to propose reasonable reaction mechanisms for the enzyme-catalyzed reactions.

FUNDAMENTAL PHOSPHORUS STEREOCHEMISTRY IN POLAR ADDITION–ELIMINATION REACTIONS (NUCLEOPHILIC SUBSTITUTION)

Stable pentacoordinate phosphorus compounds such as PF_5 have a trigonal bipyramidal structure (*4*). A classical trigonal bipyramidal structure is depicted in Fig. 1

---

[1] Present address: Department of Chemistry, Eastern Illinois University, Charleston, IL 61920.

[2] Present address: P.O. Box 466, Madison, WI 53701.

and is described in the accompanying legend, including such features as apical and equatorial bonds. Structural (*4*, *5*) and theoretical (*6*, *7*) studies on stable pentacoordinate phosphorus compounds lead to the conclusion that apical bonds in such trigonal bipyramidal phosphorus structures are longer and weaker than the equatorial bonds. This is highly reasonable in terms of the probable character of the bonding orbitals of the central phosphorus atom, namely, more *s* character in the three equatorial bonding orbitals (probably sp^2) and more *p* character in the two apical bonding orbitals (probably *p* mixed with *d*).[3]

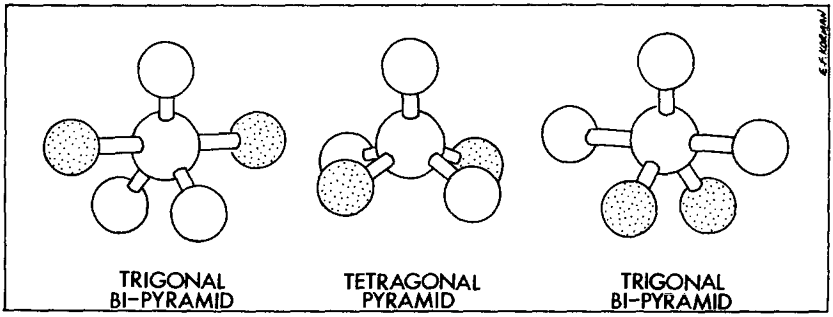


FIG. 1. Classical trigonal bipyramidal geometry (see the structure at the extreme left). The central atom has five atoms (or groups) bonded to it. Two of the atoms (stippled) are each bonded by a long, relatively weak apical bond and together are linear with the central atom. Three of the atoms (unshaded) are each bonded by a short, relatively strong equatorial bond and they form, together with the central atom, the equatorial plane. The apical bonds are perpendicular to the equatorial plane.

Pseudorotation of a trigonal bipyramidal structure. In the structure at the extreme left, the pair of stippled atoms each start out apically bonded. By a process of bond bending the stippled atoms become, in the structure at the extreme right, equatorially bonded. At the same time, in the original structure at the extreme left, the lower pair of equatorially bonded atoms (unshaded) become by the bond-bending process each apically bonded in the structure at the extreme right. The structure in the center is a tetragonal (square base) pyramid and is the transition state halfway between the two trigonal bipyramidal structures. In structures having pairs of atoms (or groups) indistinguishable from each other (as pairs), the trigonal bipyramidal structure seemingly undergoes a 90° rotation, i.e., pseudorotates.

According to current theory a very important property of pentacoordinate phosphorus compounds is their structural nonrigidity, i.e., their capacity for pseudorotation (*2*, *9*).[4] Pseudorotation of a trigonal bipyramidal structure is depicted in Fig. 1 and described in the accompanying legend. The concept of pseudorotation was first advanced by Berry (*10*) to account for the nuclear magnetic resonance properties of the stable pentacoordinate phosphorus species PF_5 and PCl_5. Since that time, the concept

---

[3] It should be understood that the amount of *d* orbital participation in trigonal bipyramidal phosphorus apical bonding could range from no *d* orbital participation to full *d* orbital participation, as is discussed by Van Der Voorn and Drago (*7*) and by Sommer *et al.* (*8*).

[4] By pseudorotation we mean the Berry process, as carefully defined by Muetterties (*9*). Of various conceivable polytopal isomerizations of trigonal bipyramids, the Berry process is the one involving minimal change in shape parameters and is the most symmetrical. Although pseudorotation is a general term, we equate it here with the Berry process.

has been successfully applied to a number of other stable pentacoordinate phosphorus compounds.

There is excellent evidence that pseudorotation is governed by electronic stability factors which express themselves in the following ways: (1) Electron-withdrawing ligands prefer apical bonding at the central phosphorus atom, and (2) electron-releasing ligands prefer equatorial bonding at the central phosphorus atom (*11*). This behavior relates to the nature of the phosphorus orbitals involved in the trigonal bipyramidal bonding, namely, equatorial sp^2 orbitals which bond best with their electrons being close to the phosphorus center, and apical *pd* orbitals which bond best with their electrons further removed from the phosphorus center (*2*).

The stability of a given trigonal bipyramidal phosphorus structure with respect to pseudorotation will depend upon the relative polarities of the five ligands bonded to the phosphorus atom and their orientations. The most favored path for pseudorotation will be to accord apical bonding to the most highly electron-withdrawing groups.

Westheimer and co-workers (*2*, *12*) have applied these fundamental concepts to reactions of phosphate esters. In their chemical reaction mechanism formulations they propose pentacoordination at a phosphorus reaction center in unstable reaction intermediates having trigonal bipyramidal geometry. Furthermore, they propose that these intermediates are sufficiently long-lived to undergo pseudorotation.

From the conclusion that in trigonal bipyramidal structures the apical bonds are longer and weaker than the equatorial bonds, it follows that for polar reactions at phosphorus involving relatively electronegative ligands, apical bonds comprise relatively unstable, reactive bonds, while equatorial bonds comprise much more stable bonds. Consistent with this, Westheimer and co-workers (*2*, *12*) formulate their phosphate ester reaction mechanisms as polar addition–elimination reactions (i.e., nucleophilic substitutions) with the addition of a ligand (nucleophile) to a tetrahedral phosphate phosphorus reaction center to form an apical bond in the resulting pentacoordinate trigonal bipyramidal reaction intermediate. They formulate the subsequent elimination of a ligand (leaving group) as the cleavage of an apical bond to give tetrahedral geometry at the phosphorus center in the product.

In the formulations for phosphate reactions proposed by Westheimer (*2*), two different reaction mechanisms in terms of stereochemistry paths are proposed for apical entry and apical departure, depicted in Fig. 2 and described in the accompanying legend. In the first reaction mechanism depicted in Fig. 2, which is the simpler, the entering ligand and the departing ligand are colinear with the phosphorus reaction center. Such a reaction mechanism would lead to stereochemical inversion of configuration at the phosphorus center, i.e., would comprise an *inversion stereochemistry path.* In the second reaction mechanism depicted in Fig. 2, the entering and departing ligands are not colinear but instead have an angular relationship close to perpendicular. Such a reaction mechanism involving a pseudorotation would lead to stereochemical retention of configuration at the phosphorus reaction center, i.e., would comprise a *retention stereochemistry path.*[5]

---

[5] For a useful pictorial introduction to reaction stereochemistry at tetrahedral substrates, using silicon as an example, see Ref. *1*, Chap. 11. It should be noted that designation of reaction stereochemistry (retention or inversion of configuration) is most commonly made in cases where the reaction centers under study are asymmetric and the relative configurations of those centers in the starting and

There are several critical aspects of these two possible stereochemistry paths as they relate to phosphate reaction mechanisms. The first of these has to do with initial formation of a trigonal bipyramidal reaction intermediate. The ligand coapical with the ligand which enters must be sufficiently electron-withdrawing to favorably occupy an apical position. A negatively charged oxygen ($-O^-$) would be less favorable for apical orientation than an oxygen whose negative charge has been attenuated, as for example in an $-OH$ or in an $-OR$ (alkoxy) group. Better still for apical orientation would be an oxonium ($-OH_2^+$) group which amounts to a water ligand apically bound at the reaction center. The second critical matter has to do with whether or not pseudorotation can occur. In order for a pseudorotation to occur, the initial pair of apical ligands must not be so electron withdrawing that they cannot go equatorial while at the same time a pair of initial equatorial ligands must not be so electron releasing that they cannot go apical. Whether a ligand is apically or equatorially favored is a relative matter, i.e., relative to the electron-withdrawing and electron-releasing capacities of all the other ligands. Westheimer and co-workers (*2*, *12*) have utilized various protonation states of oxygen ligands in pentacoordinate reaction intermediates in formulating their reaction mechanisms in phosphate ester reactions. The relative polarity preference rules implicitly utilized by these workers are summarized below:

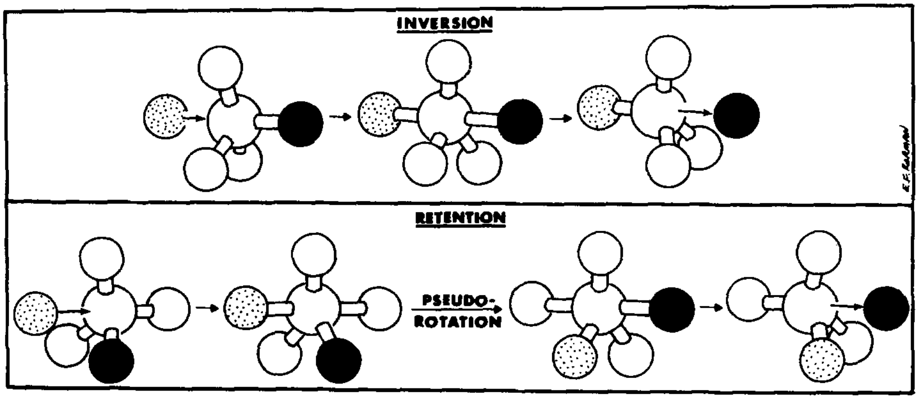


FIG. 2. Inversion and retention stereochemistry paths. In the inversion path the entering group (represented as a stippled atom) adds to the reaction center to become coapical with the group which eventually leaves (represented as a dark atom). The leaving group is eliminated apically from the reaction center. In the retention path the entering group adds to the reaction center to become apical while the group which eventually leaves is equatorial. Pseudorotation places the entering group equatorial and the leaving group apical. The leaving group is eliminated apically from the reaction center.

(1) $-OH_2^+$ (oxonium) ligands prefer apical bonding,
(2) $-O^-$ groups prefer equatorial bonding,

product molecules can be deduced by physical methods (e.g., optical rotation measurements). Nevertheless, for purposes of mechanistic description and arguments the notation of reaction stereochemistry is just as useful and appropriate for symmetric reaction centers as it is for asymmetric centers. It merely must be understood that in the case of symmetric reaction centers the reaction stereochemistry cannot be experimentally determined by physical methods dependent on molecular asymmetry.

(3) –OH groups, which are neutral, will have relatively no bonding orientation preference.

The fundamental stereochemistry and reaction mechanism paths outlined above in this section will be used to discuss certain enzyme-catalyzed reactions involving phosphate groups. Of course, reaction mechanisms other than those of the polar addition–elimination type, such as dissociative (prior elimination) reaction mechanisms and free radical reaction mechanisms, could conceivably be formulated for these enzyme-catalyzed reactions, but such formulations will not be considered in this paper. Consistent with our treatment of polar addition–elimination reaction mechanisms is our use of various protonation states of the oxygen ligands about phosphate phosphorus reaction centers. Such protonation states are reasonable not only for polar addition–elimination reactions in free solution but also, we believe, for such reactions when catalyzed by enzymes.

POSSIBLE STEREOCHEMISTRY PATH OF PYROPHOSPHATE HYDROLYSIS AND P_i–$H_2{}^{18}O$ OXYGEN EXCHANGE CATALYZED BY PYROPHOSPHATASE

Among the enzymes which catalyze reactions involving phosphorus centers of phosphates and also catalyze exchanges of ^{18}O from water of the medium into such centers is inorganic pyrophosphatase (*13*, *14*). Pyrophosphatase not only catalyzes the hydrolysis of pyrophosphate, but also an exchange of ^{18}O from $H_2{}^{18}O$ into inorganic phosphate (P_i) even in the absence of pyrophosphate (*13*). Although ^{18}O from $H_2{}^{18}O$ is rapidly exchanged into P_i, Cohn (*13*) observed no ^{18}O exchange into pyrophosphate.

Since it is reasonable that the same enzyme active site catalyzes both the hydrolysis of pyrophosphate and the P_i–$H_2{}^{18}O$ oxygen exchange reaction, the chemical reaction mechanism of the oxygen exchange into P_i might be germane to our understanding of the chemical reaction mechanism of the hydrolysis of pyrophosphate. In light of the discussion of fundamental phosphorus stereochemistry and reaction mechanisms given above, two different addition–elimination stereochemistry paths suggest themselves for the P_i–$H_2{}^{18}O$ oxygen exchange. The first would be an inversion stereochemistry path in which the $H_2{}^{18}O$ ligand which enters and the oxygen ligand (unlabeled) which eventually leaves as H_2O from the phosphorus center are coapical in a trigonal bipyramidal reaction intermediate (or transition state). The second possibility would be a retention stereochemistry path in which the $H_2{}^{18}O$ ligand which enters is apical while the oxygen ligand (unlabeled) which eventually leaves as H_2O is equatorial. The reaction intermediate would have to pseudorotate placing the ^{18}O-labeled oxygen ligand equatorial, while placing unlabeled oxygen ligand apical, from which orientation the latter could leave as H_2O.

The two stereochemical reaction paths possible for the rapid enzyme-catalyzed P_i–$H_2{}^{18}O$ oxygen exchange outlined here for pyrophosphatase have been deduced as possible stereochemical reaction paths for isotopic exchanges at tetrahedral second-row element centers in free solution. For example, Sommer and co-workers (*15*) have found that exchange of $^{36}Cl^-$ into the asymmetric tetrahedral silicon center of an optically active chlorosilane can occur by way of an inversion path as well as by a retention path.

Westheimer (*2*) has proposed a retention path for ^{18}O exchange from $H_2{}^{18}O$ into unhydrolyzed hydrogen ethylene cyclic phosphate ester in free solution.

It is possible and, we feel, likely that the reaction mechanism of the P_i–$H_2{}^{18}O$ oxygen exchange catalyzed by pyrophosphatase is analogous to the reaction mechanism of pyrophosphate hydrolysis. This possibility was listed among other possibilities cited by Cohn (*13*). In P_i–$H_2{}^{18}O$ oxygen exchange as well as in hydrolysis of pyrophosphate, the entering ligand is the nucleophile H_2O ($H_2{}^{18}O$). In the oxygen exchange into P_i the leaving ligand is H_2O, while in the hydrolysis of pyrophosphate the leaving ligand is a phosphate group. If the stereochemistry path of the P_i–$H_2{}^{18}O$ oxygen exchange were known, this might shed light on the stereochemistry path of the hydrolysis of pyrophosphate, and vice versa. Although the enzyme may not be absolutely specific as to the oxygen ligands about the phosphorus center of species it accepts and binds as substrates, the enzyme is very likely to be specific with regard to the type of reaction mechanism it catalyzes and the stereochemistry path of that mechanism.

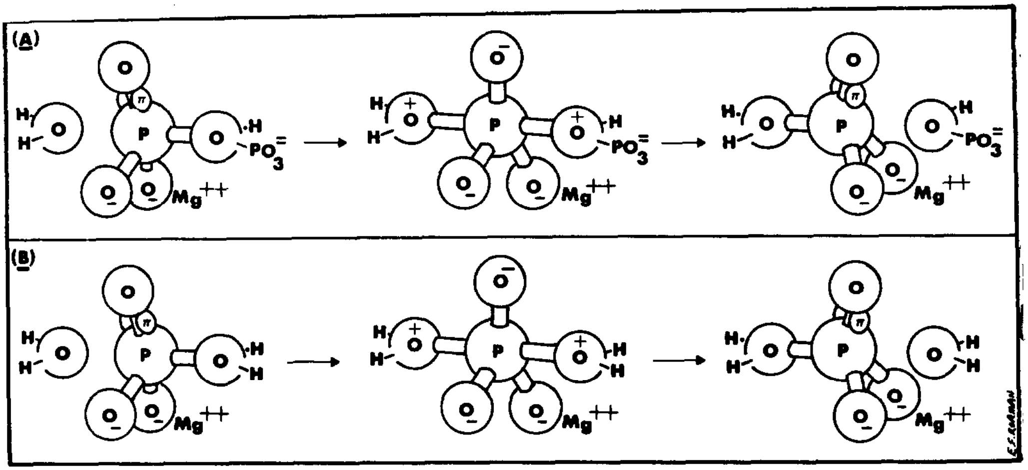
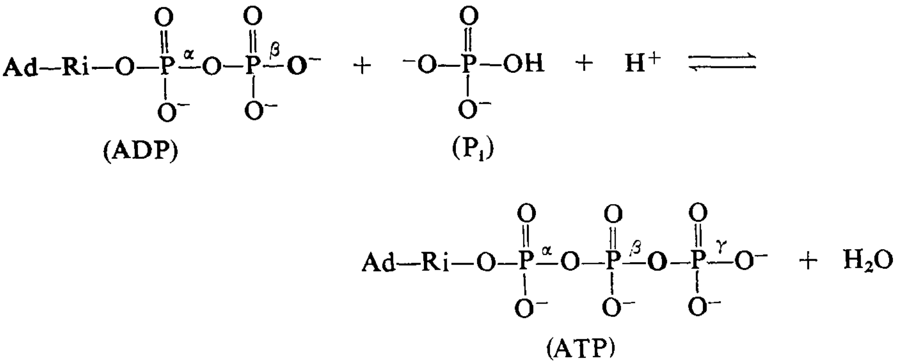


FIG. 3. Proposed inversion stereochemistry path for hydrolysis of pyrophosphate and P_i–H_2O oxygen exchange catalyzed by pyrophosphatase. In both A (hydrolysis of pyrophosphate) and B (P_i–H_2O oxygen exchange), H_2O ($H_2{}^{18}O$) adds to the phosphorus reaction center to become an apically bonded oxonium group. At the same time a proton of the enzyme associates with the oxygen atoms of the respective leaving groups, giving them oxonium character. The respective leaving groups are each eliminated apically. Concomitant with the departure of the respective leaving groups, a proton from the entering H_2O transfers to the enzyme.

Until the reaction mechanism and stereochemistry path catalyzed by pyrophosphatase is experimentally determined, we can merely point out that an addition–elimination mechanism having an inversion stereochemistry path seems particularly well suited for pyrophosphate hydrolysis. In an inversion path for hydrolysis, when H_2O apically adds to the phosphorus reaction center of pyrophosphate a phosphate moiety would be coapical with H_2O. The phosphate moiety, as an electron-withdrawing group, not only would have a high preference for apical orientation thus facilitating pentacoordination at the reaction center, but also would be thermodynamically favored relative to H_2O as a leaving group. Thus, the phosphate moiety could directly leave apically, giving the observed hydrolysis. If the pyrophosphate hydrolysis reaction occurs by way of the inversion stereochemistry path, then it is likely that the P_i–$H_2{}^{18}O$ oxygen exchange also occurs by way of a classical inversion path. In Fig. 3 we depict proposed reaction mechanisms for hydrolysis of pyrophosphate and for P_i–$H_2{}^{18}O$ oxygen exchange by way of a common inversion stereochemistry path.

POSSIBLE STEREOCHEMISTRY PATH OF ^{18}O EXCHANGES ASSOCIATED WITH MITOCHONDRIAL ATP SYNTHESIS AND HYDROLYSIS

Another enzyme which catalyzes a reaction involving phosphorus centers of phosphate groups and also catalyzes exchange of ^{18}O from water of the medium into such centers is the mitochondrial ATP synthetase. ATP synthetase not only catalyzes the synthesis and hydrolysis of ATP (Eq. 1) but also catalyzes an exchange of ^{18}O from $H_2{}^{18}O$ into the γ-phosphate phosphorus center of ATP and also into P_i (*16–19*). The ATP–$H_2{}^{18}O$ and P_i–$H_2{}^{18}O$ oxygen exchanges are rapid relative to ATP synthesis. Since ATP synthesis, ATP hydrolysis, ATP–$H_2{}^{18}O$ oxygen exchange, and P_i–$H_2{}^{18}O$ oxygen exchange are all inhibited by the antibiotic oligomycin (*19*, *20*), they are recognized as being intimately related probably at the level of reaction mechanisms. Since the sensitivity to oligomycin suggests that all of the reactions occur at the same enzyme active site, the chemical reaction mechanisms of the ATP–$H_2{}^{18}O$ and P_i–$H_2{}^{18}O$ oxygen exchanges might be germane to an understanding of the chemical reaction mechanism of ATP synthesis and hydrolysis.



$$\mathrm{Ad{-}Ri{-}O{-}}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}\overset{\alpha}{-}\mathrm{O}{-}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}\overset{\beta}{-}\mathrm{O^-} + {}^-\mathrm{O}{-}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}{-}\mathrm{OH} + \mathrm{H^+} \rightleftharpoons$$

(ADP) (P_i)

$$\mathrm{Ad{-}Ri{-}O{-}}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}\overset{\alpha}{-}\mathrm{O}{-}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}\overset{\beta}{-}\mathrm{O}{-}\overset{\overset{O}{\|}}{\underset{\underset{O^-}{|}}{P}}\overset{\gamma}{-}\mathrm{O^-} + \mathrm{H_2O} \quad (1)$$

(ATP)

Unlike the ^{18}O exchange data in the case of pyrophosphatase where there is no observed ^{18}O exchange from $H_2{}^{18}O$ into reisolated pyrophosphate, the mitochondrial ATP synthetase catalyzes an exchange of ^{18}O from $H_2{}^{18}O$ into reisolated ATP (*17*, *18*). The rate of the ATP–$H_2{}^{18}O$ oxygen exchange has been found (*21*) to be more than 10 times faster than the rate of overall reversal of the reaction catalyzed by the enzyme (Eq. 1), where overall reversal is measured by the rate of incorporation of $^{32}P_i$ from the medium into ATP initially unlabeled with ^{32}P (ATP–$^{32}P_i$ exchange). Mitchell, Hill, and Boyer (*21*) have given a kinetic analysis which outlines how the relatively rapid rate of

ATP–$H_2{}^{18}O$ oxygen exchange might be arising in terms of overall dynamic reversal of Eq. 1. This would involve disparities in the rates at which the various substrates come on and off the enzyme as well as lack of absolute spatial selectivity in the binding of oxygen atoms of tetrahedral phosphate to enzyme.

Alternatively, Boyer (*19*) has listed among the possibilities to explain the rapid ATP–$H_2{}^{18}O$ oxygen exchange one in which the exchange may not involve overall dynamic reversal of Eq. 1, but instead may occur if the catalytic site lacks absolute spatial selectivity for the oxygens of a pentavalent phosphorus intermediate involving the γ-phosphate group of ATP. This possibility requires analysis in stereochemical reaction mechanism terms. Either inversion or retention are conceivable for the oxygen exchange. In a retention reaction mechanism for the exchange, the entering $H_2{}^{18}O$ ligand, which would add to the γ-phosphate phosphorus center of ATP, would be apical while the oxygen ligand (unlabeled) which eventually leaves as H_2O would be equatorial. The pentacoordinate reaction intermediate then would have to pseudorotate placing the entering ^{18}O ligand equatorial while placing the unlabeled oxygen ligand apical, from which orientation the latter ligand could leave as H_2O. In forming the pentacoordinate reaction intermediate, the entering $H_2{}^{18}O$ ligand could either enter coapical with the β,γ bridge oxygen to ADP (which we will call the ADPO group) or enter coapical with one of the other oxygens of the γ-phosphate group. It should be noted that, in the case where $H_2{}^{18}O$ would enter coapical with ADPO, a pseudorotation would be unexpected on the basis of polarity preference rules. Instead, direct apical elimination of the *good leaving group ADPO* (as $ADPO^-$) would be expected. In the case where $H_2{}^{18}O$ would add at 90° to the ADPO group, pseudorotation would be expected to place the initially equatorial ADPO group apical together with an unlabeled oxygen ligand. However, apical elimination of the unlabeled oxygen ligand to result in ^{18}O exchange into the γ-phosphate group of ATP would be unexpected. Instead, apical elimination of $ADPO^-$ would be expected. Thus, both of the possibilities for a retention stereochemistry path for ^{18}O exchange into the γ-phosphate group of ATP seem to us unlikely.

In an inversion reaction mechanism path for ^{18}O exchange into ATP, the $H_2{}^{18}O$ ligand which would enter, and the oxygen ligand (unlabeled) which would eventually leave as H_2O, would be coapical in the pentacoordinate reaction intermediate. Direct apical elimination of H_2O would result in incorporation of ^{18}O label into the γ-phosphate group of ATP. Such a direct invertive ^{18}O exchange into ATP catalyzed by ATP synthetase would be stereochemically analogous to the invertive reaction mechanism which we proposed in the previous section as reasonable for the P_i–$H_2{}^{18}O$ oxygen exchange catalyzed by pyrophosphatase. Figure 4 depicts a direct invertive ATP–$H_2{}^{18}O$ oxygen exchange.

As was mentioned above, a rapid P_i–$H_2{}^{18}O$ oxygen exchange is catalyzed by the ATP synthetase as well as a rapid ATP–$H_2{}^{18}O$ oxygen exchange. The inversion stereochemistry path which we find reasonable for that ATP–$H_2{}^{18}O$ exchange would be equally reasonable for the P_i–$H_2{}^{18}O$ exchange. In such a context P_i would be acting as an analog of the γ-phosphate group of ATP. Thus, a capacity for an inversion reaction mechanism path for the ATP–$H_2{}^{18}O$ oxygen exchange catalyzed by ATP synthetase may find expression in a rapid P_i–$H_2{}^{18}O$ oxygen exchange having an analogous inversion reaction mechanism path. This would mean that both the ATP–$H_2{}^{18}O$ oxygen exchange and the P_i–$H_2{}^{18}O$ oxygen exchange of ATP synthetase may be stereochemically and mechanistically analogous with the P_i–$H_2{}^{18}O$ oxygen exchange of pyrophosphatase. Such an analogy would be consistent with the earlier suggestion of Cohn (*13*) that a mechanistic analogy may exist between the respective P_i–$H_2{}^{18}O$ oxygen exchanges.

Our purpose in considering the ATP–$H_2{}^{18}O$ and P_i–$H_2{}^{18}O$ oxygen exchanges of the mitochondrial ATP synthetase is the same as our purpose in considering the P_i–$H_2{}^{18}O$ oxygen exchange of pyrophosphatase. In the case of pyrophosphatase, the stereochemistry and reaction mechanism of the P_i–$H_2{}^{18}O$ oxygen exchange have been examined because they may shed light on the stereochemistry and reaction mechanism of the hydrolysis of pyrophosphate. In the case of ATP synthetase, the stereochemistry and reaction mechanism of the ATP–$H_2{}^{18}O$ oxygen exchange (and of the P_i–$H_2{}^{18}O$ oxygen exchange which may be analogous) have been examined because they may shed light on the stereochemistry and reaction mechanism of the synthesis and hydrolysis of ATP. It is convenient to first consider the relationship of ATP–$H_2{}^{18}O$ oxygen exchange to the hydrolysis of ATP. In Fig. 4 depicting the ATP–$H_2{}^{18}O$ oxygen exchange, the pentacoordinate reaction intermediate has the ADPO group equatorial. In order for ADPO to leave the phosphorus reaction center to give ATP hydrolysis, ADPO would first have to become apical. ADPO could become apical if the reaction intermediate were to pseudorotate, as depicted in Fig. 5. Pseudorotation would result in another pentacoordinate reaction intermediate from which ADPO could apically leave to give $ADPO^-$ and P_i, also depicted in Fig. 5. Thus, the hydrolysis of ATP would be possible in the context of ATP–$H_2{}^{18}O$ oxygen exchange since that hydrolysis has as one of its reaction intermediates the very same reaction intermediate involved in ATP–$H_2{}^{18}O$ oxygen exchange. Of course, ATP hydrolysis may be the microscopic reverse of ATP synthesis. Therefore, Fig. 5 may depict not only ATP–$H_2{}^{18}O$ oxygen exchange and ATP hydrolysis, but also ATP synthesis as well.

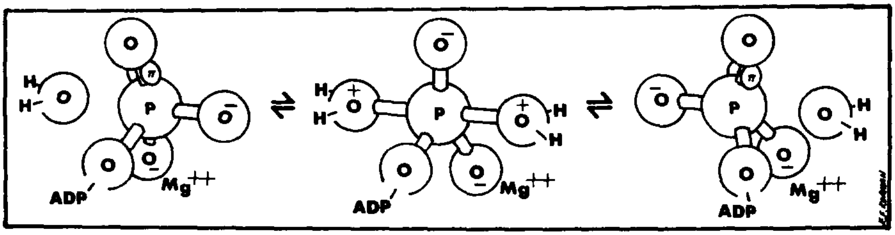


FIG. 4. Proposed inversion stereochemistry path for ATP–H_2O oxygen exchange. H_2O ($H_2{}^{18}O$) adds to the γ-phosphate phosphorus center of ATP to become an apically bonded oxonium group. At the same time the oxygen atom which eventually leaves as H_2O and which is coapical with the entering H_2O group develops bonds with a pair of protons from the enzyme, thus acquiring oxonium character. The leaving group is eliminated apically as H_2O group, a pair of protons from the entering H_2O ($H_2{}^{18}O$) transfer to the enzyme.

The ATP Synthesis Reaction Mechanism

The stereochemical reaction mechanism possibility for ATP synthesis which emerges here deserves careful scrutiny. In the formulation of the reaction mechanism in the direction of ATP synthesis, ADPO$^-$ would apically add to the phosphorus center of P_i, where P_i would be bound to the enzyme by way of hydrogen bonding and Mg^{2+} coordination. The oxygen ligand coapical with ADPO would be coordinated with Mg^{2+}. This coordination would among other things attenuate negative charge, permitting apical orientation of that oxygen ligand. In the trigonal bipyramidal reaction intermediate, two of the equatorial oxygen ligands would have oxonium character. Protons would bridge between the enzyme and those oxonium oxygens. These four bridging protons participating in the reaction intermediate emerged as necessary from the need for protons in two water molecules involved in the ATP–$H_2{}^{18}O$ oxygen exchange. These protons would comprise not only an important part of tight and stereospecific binding of substrates and reaction intermediates to enzyme, but also their participation would comprise a salient feature of the reaction mechanism. The importance of protons in such a phosphate reaction mechanism is reasonable, as has been recognized for reactions of phosphate esters in free solution (*12*). This is because

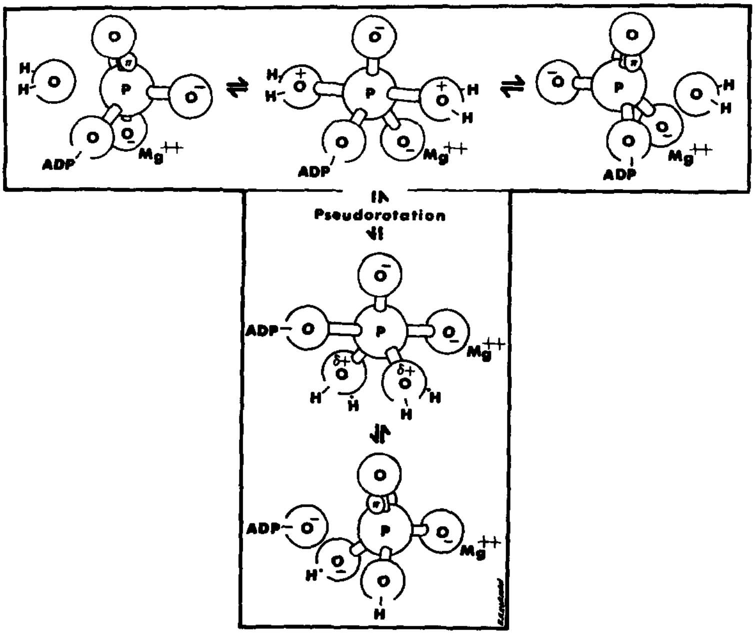


FIG. 5. Proposed retention stereochemistry path for ATP hydrolysis and synthesis in the context of inversion ATP–H_2O oxygen exchange. The horizontal portion of the diagram depicts invertive ATP–H_2O exchange as proposed in Fig. 4, including the pentacoordinate double-oxonium reaction intermediate with the ADPO group equatorial. Pseudorotation would place the two oxonium groups equatorial concomitant with attenuation of oxonium character on each oxonium group by transfer of bridging protons to the enzyme. ADPO would go from equatorial to apical bonding. Apical elimination of ADPO would give ADPO$^-$ and P_i, both enzyme bound. This sequence would constitute hydrolysis of ATP. The sequence is depicted as reversible and thus would describe synthesis of ATP as well as hydrolysis of ATP in the context of ATP–H_2O oxygen exchange. Both ATP hydrolysis and synthesis would follow a retention stereochemistry path in the context of an inversion stereochemistry path for ATP–H_2O oxygen exchange.

the protonation state of a given oxygen ligand determines its relative polarity, and relative polarities of ligands underlie pseudorotational events. Each of the two equatorial oxygen ligands having oxonium character probably would have a tendency to become apical. Acting together as a pair, they would probably have a preference for apical bonding near equal to the preference of ADPO paired with O^-Mg^{2+} for apical bonding. This would permit pseudorotation to give the reaction intermediate in the ATP–$H_2{}^{18}O$ oxygen exchange. The pseudorotation would transform the bonding of ADPO at the reaction center from apical to equatorial, which would constitute an equatorial capture of ADPO at that center. In the reaction intermediate in which ADPO is equatorially captured, the two apical oxygen ligands would have essentially full oxonium character and may be thought of as H_2O ligands. This reaction intermediate can be described as a "double-oxonium" or "double-water" reaction intermediate.[6] Apical elimination of either one or the other of the H_2O ligands would give enzyme-bound H_2O and thus would achieve ATP.

In such a formulation, ATP synthesis (and hydrolysis) would be occurring by way of a *retention* stereochemistry path in which ADPO would have a near 90° geometric relationship with the H_2O involved in the reaction. This retention stereochemistry path for ATP synthesis (and hydrolysis) would be, of course, in the context of an *inversion* stereochemistry path for ATP–$H_2{}^{18}O$ oxygen exchange. Any single ATP–$H_2{}^{18}O$ exchange event would go through a reaction intermediate identical with the reaction intermediate that any one ATP synthesis (or hydrolysis) event would go through, namely, the double-oxonium reaction intermediate. Thus, there would be a unity among all these reactions.

Relationship Between ATP–$H_2{}^{18}O$ Exchange and ATP Synthesis

In the unitary formulation given above, the relationship between the ATP–$H_2{}^{18}O$ oxygen exchange and ATP synthesis would not be a trivial one. The relationship can best be appreciated by asking why ATP synthesis may be going by way of a double-oxonium reaction intermediate. It should be recognized that compared with an H_2O ligand, ADPO is a good leaving group from a pentacoordinate phosphate phosphorus center. ADPO is, therefore, correspondingly a group with a high preference for apical bonding at such a center, and has relatively little tendency to become equatorial. For ADPO to go from apical to equatorial (i.e., to be equatorially captured) the apical preference of ADPO must be at least nearly matched by the apical preference of *two* oxonium ligands formed by protonation. When two H_2O ligands express their combined preference for apical bonding and go from equatorial to apical, ADPO would be equatorially captured by the pseudorotation giving the double-oxonium reaction intermediate. The double-oxonium reaction intermediate, which would be an expression of the need for a pair of oxonium ligands to capture ADPO equatorially in a pseudorotation, also may express itself in a capacity of the enzyme to catalyze rapid ATP–$H_2{}^{18}O$ oxygen exchange. In the context of the synthesis of ATP by way of a double-oxonium reaction intermediate, ATP–$H_2{}^{18}O$ oxygen exchange would not be trivial but indeed would be at the heart of the synthesis mechanism.

---

[6] It is interesting to note that this double-water intermediate can be viewed as a doubly hydrated metaphosphate ester.

CONCLUSION

For enzyme-catalyzed reactions involving phosphate phosphorus centers, ^{18}O exchange reactions at such centers may shed light on the reaction mechanism and stereochemistry path. Using fundamental phosphorus stereochemistry in polar addition–elimination reaction mechanism formulations, we have attempted to outline how such ^{18}O exchanges may be analyzed to such a purpose. While it is not possible through such an analysis to provide definitive answers, it is nevertheless possible to offer reasonable choices. We have offered such choices for the hydrolysis of pyrophosphate catalyzed by pyrophosphatase and for the synthesis of ATP catalyzed by mitochondrial ATP synthetase.

ACKNOWLEDGMENTS

This work was supported in part by Program Project Grant GM-12,847 from the Institute of General Medical Sciences, U.S. Public Health Service. We thank Mr. Jan Galligan for expert photographic services.

REFERENCES

1. L. H. Sommer, "Stereochemistry, Mechanism and Silicon." McGraw–Hill, New York, 1965.
2. F. H. Westheimer, *Acc. Chem. Res.* **1**, 70 (1968).
3. M. J. Gallagher and I. D. Jenkins, "Topics in Stereochemistry" (E. L. Eliel and N. L. Allinger, Eds.), Vol. 3, p. 1. Interscience, New York, 1968.
4. L. S. Bartell and K. W. Hansen, *Inorg. Chem.* **4**, 1777 (1965).
5. W. C. Hamilton, S. J. La Placa, F. Ramirez, and C. P. Smith, *J. Amer. Chem. Soc.* **89**, 2268 (1967).
6. (a) F. A. Cotton, *J. Chem. Phys.* **35**, 228 (1961); (b) R. J. Gillespie, *Inorg. Chem.* **5**, 1634 (1966); (c) L. S. Bartell, *Inorg. Chem.* **5**, 1635 (1966).
7. P. C. Van Der Voorn and R. S. Drago, *J. Amer. Chem. Soc.* **88**, 3255 (1966).
8. L. H. Sommer, G. A. Parker, N. C. Lloyd, C. L. Frye, and K. W. Michael, *J. Amer. Chem. Soc.* **89**, 857 (1967).
9. (a) E. L. Muetterties, *J. Amer. Chem. Soc.* **91**, 4115 (1969); (b) E. L. Muetterties, *Acc. Chem. Res.* **3**, 266 (1970).
10. R. S. Berry, *J. Chem. Phys.* **32**, 933 (1960).
11. E. L. Muetterties, W. Mahler, and R. Schmutzler, *Inorg. Chem.* **2**, 613 (1963).
12. (a) R. Kluger, F. Covitz, E. Dennis, L. D. Williams, and F. H. Westheimer, *J. Amer. Chem. Soc.* **91**, 6066 (1969); (b) R. Kluger and F. W. Westheimer, *J. Amer. Chem. Soc.* **91**, 4143 (1969).
13. M. Cohn, *J. Biol. Chem.* **230**, 369 (1958).
14. (a) J. Josse and S. C. K. Wong, "The Enzymes" (P. D. Boyer, Ed.), Vol. IV, p. 499. Academic Press, New York, 1971; (b) L. G. Butler, "The Enzymes" (P. D. Boyer, Ed.), p. 529.
15. L. H. Sommer, G. D. Homer, A. W. Messing, J. L. Kutschinski, F. O. Stark, and K. W. Michael, *J. Amer. Chem. Soc.* **93**, 2093 (1971).
16. M. Cohn, *J. Biol. Chem.* **201**, 735 (1953).
17. M. Cohn and G. R. Drysdale, *J. Biol. Chem.* **216**, 831 (1955).
18. P. C. Chan, A. L. Lehninger, and T. Enns, *J. Biol. Chem.* **235**, 1790 (1960).
19. P. D. Boyer, "Current Topics in Bioenergetics" (D. R. Sanadi, Ed.), Vol. 2, p. 99. Academic Press, New York, 1967.
20. P. C. Hinkle, H. S. Penefsky, and E. Racker, *J. Biol. Chem.* **242**, 1788 (1967).
21. R. A. Mitchell, R. D. Hill, and P. D. Boyer, *J. Biol. Chem.* **242**, 1793 (1967).