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Phosphoryl Transfer Enzymes and Hypervalent Phosphorus Chemistry

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ABSTRACT

The coordination tendencies of phosphorus to form a hexacoordinated state from a pentacoordinated state, which might assist in describing the mechanistic action of phosphoryl transfer enzymes, are delineated. The factors discussed include substrate and transition or intermediate state anionicity, hydrogen bonding, packing effects, that is, van der Waals forces, the ease of formation of hexacoordinate phosphorus from lower coordinate states, and the pseudorotation problem common to nonrigid pentacoordinate phosphorus. In view of the work reported in this Account and recent work on enzyme promiscuity and moonlighting activities, it is suggested that donor action should play a role in determining active site interactions in phosphoryl transfer enzyme mechanisms.

Introduction

Enzyme active sites of phosphoryl transfer enzymes and cAMP invariably are portrayed with nearby residues involved in hydrogen bonding with the substrate but not with donor interactions at the phosphorus atom.[1−3] In general, it has been assumed that a tetracoordinate phosphate substrate will proceed to an activated complex in a nucleophilic displacement reaction where the activated complex or transition state is pentacoordinate by virtue of the incoming nucleophile. The pentacoordinate phosphorus species is formulated as a trigonal bipyramid with the entering and departing group doing so at axial sites, that is, an inline process.[1,2,4−12]

The coordination tendencies of phosphorus that might assist in describing the mechanistic action of phosphoryl transfer enzymes and cAMP has not been used up to the present. In this regard, biochemists have persisted in referring to knowledge of hypervalent phosphorus chemistry, as it existed over a quarter of a century ago. However, the area has experienced major advances, particularly the ease of formation of hexacoordinated phosphorus and its relation to pentacoordinated phosphorus.[1,6,13] It is the purpose of this Account to delineate the dominant features of hypervalent phosphorus chemistry that may have applicability in formulating active site mechanisms of phosphoryl transfer enzymes and cAMP. It is our contention that residues at active sites of phosphoryl transfer enzymes are capable of entering into donor interaction at the phosphorus atom and as a consequence assist in nucleophilic attack.[1]

In what is presented here, we outline developments in higher valent phosphorus chemistry that reasonably may be used by biochemists in refining their current proposals concerning mechanistic models of nucleophilic displacements at active sites of phosphoryl transfer enzymes.

A principal tenet that will guide our discussion is the likelihood that active sites of phosphoryl transfer enzymes and cAMP will incorporate donor coordination at the phosphorus center in phosphate substrates and active site complexes. As a corollary, such coordination will distort the phosphate substrate from tetracoordinate toward pentacoordinate and alter the proposed mechanistic activated complex from pentacoordinate toward hexacoordinate. Accompanying these geometrical changes induced by coordination of donor atoms that are prevalent at active sites, consideration will be given to the effects brought about by associated changes in energy and concomitant activation energies for nucleophilic displacement reactions. What will not be treated is a digression about transition states or activated intermediates and whether "bond" making or "bond" breaking is a guiding feature of displacement reactions.[14]

As pointed out in my ACS Monograph,[2] although the term 'transition state' refers to a crest in a potential energy surface and 'intermediate' refers to a trough, many authors use these terms somewhat interchangeably, even though in most cases, the intermediate is not isolated. In a recent X-ray study Dunaway-Mariano et al.[15] obtained the first structural evidence of a pentacoordinated phosphorane *intermediate* stabilized by an enzyme-catalyzed phosphoryl transfer reaction, the isomerization of β-glucose 1-phosphate to β-glucose 6-phosphate catalyzed by β-phosphoglucomutase from *Lactococcus lactis*. A trigonal bipyramidal oxyphosphorane intermediate formed during the phosphoryl group transfer from the C(l) oxygen atom of glucose 1,6-(bis)phosphate to the nucleophilic oxygen atom of Asp8 carboxylate. A schematic of the active site geometry is reproduced in Figure 1.

It is little trouble for structural enzymologists to ascertain whether donor coordination takes place as most X-ray analyses these days are performed with sufficient resolution to pinpoint active site distances. In the present case, one of the authors was kind enough to supply these distances. The distances show that the potential donor atoms from nearby residues are outside the range for effective coordination with the phosphorus atom. However, structural analysis by solid state X-ray is indicative

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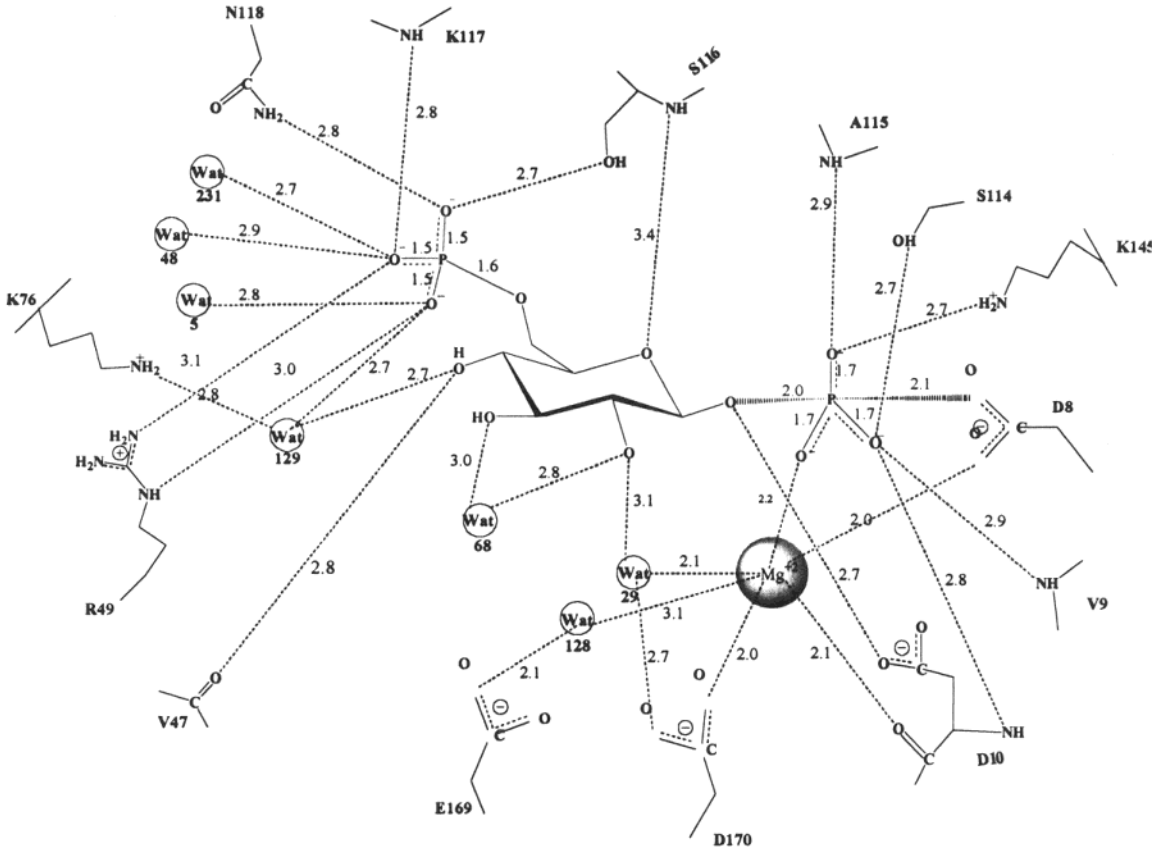


FIGURE 1. Schematic of the active site geometry of β-phosphoglucomutase from *Lactococcus lactis*. Reprinted from ref 15 with permission. Copyright 2003 AAAS.

but suffers from the definition of what actually transpires in an actual enzyme reaction. In the more flexible enzymatic reaction, the phosphoryl substrate may readily experience labile interaction with nearby donor groups leading to some degree of hexacoordination. As X-ray structural analysis of active sites show, water molecules are present that also could serve in this capacity in solution.

Anionic Phosphorus and Hydrogen Bonding

Our work with oxygen donor atoms has modeled active sites of phosphoryl transfer enzymes by the inclusion of two important features, anionic phosphorus and hydrogen bonding.[16,17] In one such study,[16] the synthesis of the pentacoordinate phosphorane shown here was rendered hexacoordinate by virtue of P−O donor interaction revealed in an X-ray structure with P−O distance of 2.984(2) Å. The anionic counterpart formed by treatment with triethylamine showed a P−O distance shortened by over an angstrom to 1.898(1) Å.[17] Both the anionic form and the precursor acid existed in the presence of hydrogen bonding interactions.

Additional studies were conducted on a series of phosphorus compounds **I**−**III** containing carboxyl groups[16] and their anionic counterparts **IV**−**VI**[17] obtained by treatment of the precursor acid forms with amines that also served to introduce hydrogen bonding interactions. The structures, a hexacoordinated anionic phosphoranate **IVA** and **IVB**, a trigonal bipyramidal anionic phosphine **V**, and a trigonal bipyramidal phosphine oxide **VI**, revealed the presence of P−O donor coordination, which was stronger for all members than that which existed in the precursor acid forms **I** to **III** (Chart 1). The extent of the P−O bond

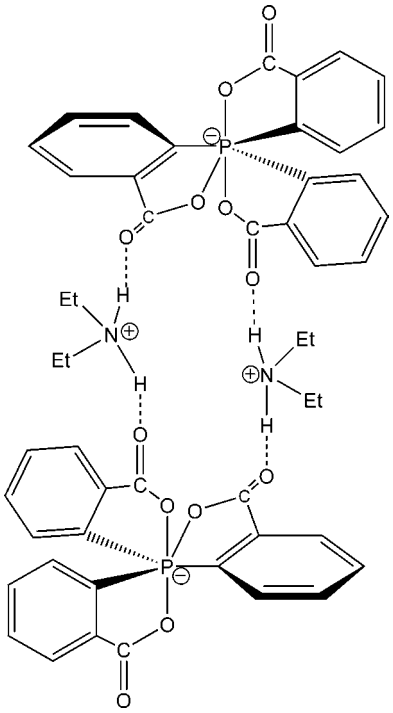
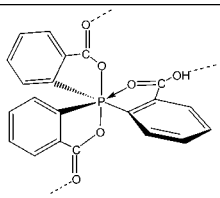
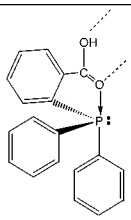
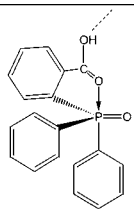
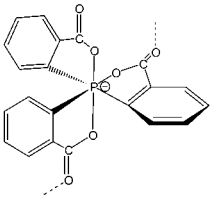
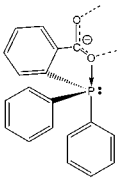
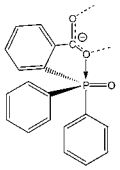


Chart 1

				
^{31}P, ppm	-56.9		-4.8	38.3
P-O, Å	2.984(2) Ave.		2.806(1)	3.075(2)
	I		**II**	**III**
				
	$Et_2NH_2^+$	Et_3NH^+	$Et_2NH_2^+$	$Et_2NH_2^+$
^{31}P, ppm	-135.5	-124.9	-5.7	30.3
P-O, Å	1.901(1)	1.898(1)	2.696(2)	2.863(2)
	IVA	**IVB**	**V**	**VI**
Δ, Å	1.083	1.086	0.110	0.212

distance shortening is expressed by the Δ values listed at the bottom of the chart.

Energies of Donor Coordination vs Energies of Hydrogen Bonding

It is possible to compare P−O donor coordination and hydrogen bonding in a more quantitative fashion. The hydrogen bond interactions are shorter for **V** and **VI** and are confined to a narrow range, 2.66−2.73 Å. Examination of the literature on N−H···O hydrogen bonding with regard to the relation between enthalpy and P−O distance[18] suggests that a value of about 4.5 kcal/mol applies here. The phosphoranates **IVA** and **IVB** that have achieved hexacoordination by forming a covalent P−O bond have longer hydrogen bond distances, presumably because of the reduced electron density at the carbonyl oxygen atom.

To establish a relationship between P−O bond distance and interaction energy, we employed an exponential function governing these quantities. The P−O single bond energy is estimated as 80 kcal/mol and the sum of the covalent radii of phosphorus and oxygen is 1.83 Å.[19] With the use of the sum of the van der Waals radii of 3.35 Å for these two atoms and setting this distance to correspond to zero P−O bond energy, one may define the exponential relation to connect these limits.

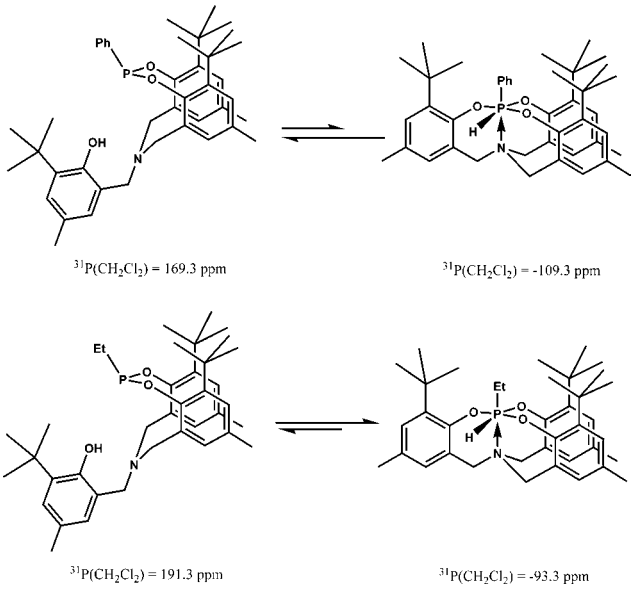
This procedure allows an estimation of the P−O donor energies for **IV**−**VI**. These are (in kcal/mol) **V** (16) and **VI** (6.5). The close approach of the P−O bond distance to the covalent value for the phosphoranates **IVA** and **IVB** suggests that their energy is near 80 kcal/mol. This same procedure for the acid forms gave P−O values (in kcal/mol) **II** (10), **III** (3.5), and **I** (5.0).[16] For these latter substances, the P−O donor energies were estimated to range from less than to more than the O−H···O hydrogen bond energy, which was assigned a value of 5.5 kcal/mol.[18] In the present series, the anionic forms, **IV**−**VI**, have greater P−O donor energies than that which exists in the acid forms as reflected in the shorter P−O distances, and as a consequence, these values all exceed the estimated hydrogen bond energies.

The present anionic series **IV**−**VI** and their precursor acid forms **I**−**III** all possess three aryl bonds to phosphorus and as such are not the most electrophilic. In our previous studies where we employed more electrophilic compounds, (phosphates, phosphites, and pentaoxyphosphoranes, containing flexible eight-membered ring systems), it was found that donor action for these neutral entities lacking hydrogen bonding increased, respectively, in the latter order.[6,20−38] For example, the P−O donor distances for the pentaoxyphosphoranes spanned the range from 1.94 to 2.65 Å.[22,23] This corresponds to a displacement from a TBP to an octahedron from 28 to 82% with the upper part of the range approaching that observed in the phosphoranate anions **IVA** and **IVB**. On this basis, it seems reasonable to assume that the initial TBP transition state formed by inline attack by a nucleophile in a phosphoryl transfer reaction may undergo rate enhancement from a nearby residue at the active site capable of donor interaction at phosphorus leading to a hexacoordinated state. We have reported an outline of this type of mechanism.[24]

Equilibria Between Tricoordinate and Hexacoordinate Phosphorus

The ease with which hexacoordinated phosphorus forms from lower coordinate phosphorus was typified in our report of the first example of the conversion of three coordinate to six coordinate phosphorus on-going from the solid to the solution state, and the existence of these two disparate geometries in equilibrium with one another in solution, as shown below.[39a] The X-ray structures showed that the unreacted hydroxyl group in the tricoordinated phosphonite oxidatively added to phosphorus in the hexacoordinated phosphorane-phosphatrane, leading to the formation of three additional bonds, a P−O, a P−H, and a P−N linkage in the case of the ethyl derivative. In solution, ^{31}P measurements assisted by solid-state ^{31}P measurements revealed that each of the compounds existed in both structural forms. VT ^{31}P established equilibria where the solid-state structures predominated in each case, thus demonstrating the modest energy difference accompanying the conversion between the lower and higher coordinate states.[39a,39b]

Scheme 1



The solution equilibrium also points out the fact that nitrogen donor action at the phosphorus atom takes place in the more highly coordinated form as a consequence of the greater electrophilicity associated with hypervalent phosphorus, and supports the premise that donor coordination caused by an amino acid residue at an enzyme active site would increase in a pentacoordinated phosphorus activated state compared to that at the phosphate substrate. Accordingly, if such action occurred, this should be one of the factors accounting for the large reaction rates associated with phosphoryl transfer enzymes. The energy associated with these coordination changes is low, of the order of a few kcals.[22,39a,39c]

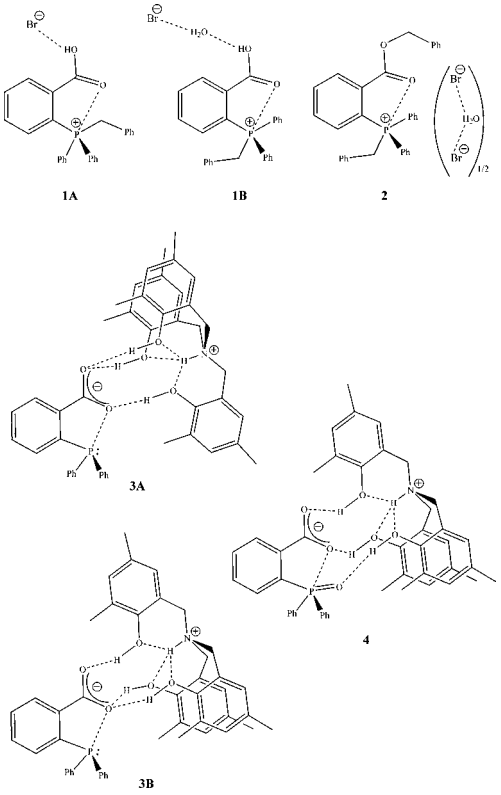
Reactivity of Pentaoxyphosphoranes

Reactivity of pentaoxyphosphoranes may be tested by conducting studies where the strength of the donor interaction is varied by altering substituent composition. For example, the X-ray structures of the following series[22,28] show an increase in displacement toward an octahedron from a pentacoordinate form in the order from left to right displayed below.

A displacement reaction conducted with catechol for this series of oxyphosphoranes shows an increase in reaction rate that parallels the increase in displacement from a trigonal bipyramid toward an octahedron. Second-order kinetics are followed implying an associative reaction.[22,40]

One may conclude that there will be a tendency toward hexacoordinate formation for a proposed trigonal bipyramid engaged at an enzyme active site in donor action with a nearby residue.

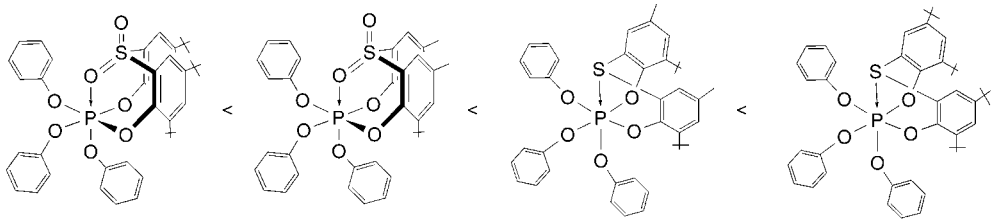
Chart 2. The Phosphonium Salts 1A, 1B, and 2, the Anionic Phosphines 3A and 3B, and the Anionic Phosphine Oxide 4



Packing Effects (van der Waals Forces)

An additional factor that deserves attention is the influence of so-called packing effects, that is, van der Waals forces in determining active site geometry and its consequences. To address this feature, we synthesized a series composed of the phosphonium salts **1A**, **1B**, and **2**, the anionic phosphines **3A** and **3B**, and the anionic, phosphine oxide **4**.[41] These are shown schematically in Chart 2 based on their X-ray structures. Extensive hydrogen bonding is seen.

In **3B**, the donor oxygen atom has two hydrogen bonding interactions and is further from phosphorus (2.898(2) Å) than in **3A** (2.729(3) Å), which has only one hydrogen bonding interaction. This longer P−O distance in **3B** can be explained on the basis that both oxygen lone pairs are partly or fully involved in bonding with the OH hydrogen atoms, which leaves less bonding electron density for coordination of the donor oxygen atom with the phosphorus atom. The latter result confirms that there exists a competition between hydrogen bonding and P−O



interactions, which we have described earlier.[16,17] In addition, this also suggests that even a single hydrogen bond can help manipulate a nucleophile's proximity to phosphorus, which can either promote or block a reaction at a phosphorus center. That is, hydrogen bonding can act as an intermediary in the initial stages of formation or cleavage of P−O bonds. Though similar suggestions have been made earlier,[42] this is the first time concrete structural evidence supporting this postulate has been arrived at independently.[41]

Both **2** and **4** showed the influence of molecular packing effects in competition with hydrogen bonding interactions. For example, compound **2** crystallizes with three independent molecules in each asymmetric unit in the same crystal lattice and does so with considerable variation in P−O distances. This variation that extends from 2.807(4) Å to 2.970(4) Å must result from crystal lattice effects that have their basis in van der Waals terms that act to alter the packing arrangements of the three molecules. Compound **4** is similar in this regard. The study leads to the conclusion that van der Waals forces that are discussed in terms of conformational distortions in enzyme chemistry,[43] termed lattice or packing effects here, are important in controlling P−O donor interactions in compounds **2** and **4**. The variation in P−O donor distance for **2** extends over a range of 0.163Å and that for **4** extends over 0.127 Å. This is comparable to the effect of phosphorus anionicity in supporting donor action.

The Pseudorotation Problem

As an additional feature regarding hypervalency in enzyme reactions, the pseudorotation problem presents itself in certain situations.

If an enzymatic chiral reaction is involved, most reactions occur with inversion of configuration. If the chirality shows retention of configuration, then two sequential in-line displacement steps are suggested, again all by way of trigonal bipyramidal active states. Inline attack, that is, apical entry of the incoming nucleophile and apical departure of the leaving group, agrees with the definitive chemistry of pentacoordinate phosphorus, both structural[44] and in terms of reaction mechanisms[2] in the absence of an enzyme environment. In the more rare event of a retention process, biochemists are reluctant to propose adjacent attack at the phosphate substrate, that is, apical attack by the nucleophile and equatorial placement of the leaving group, as this would involve pseudorotation of the trigonal bipyramidal transition state at the enzyme active site in order to bring the leaving group for departure at a preferred apical site. A schematic representation of the retention process is shown in Figure 2.[45]

The latter reasoning seems valid if one views the rearrangement of the active site residues necessary to accommodate the repositioning of the ligands attached to phosphorus as a relatively high-energy process as was suggested earlier.[2,44]

However, it may be that in the presence of an enzyme, the necessary active site residues reposition themselves

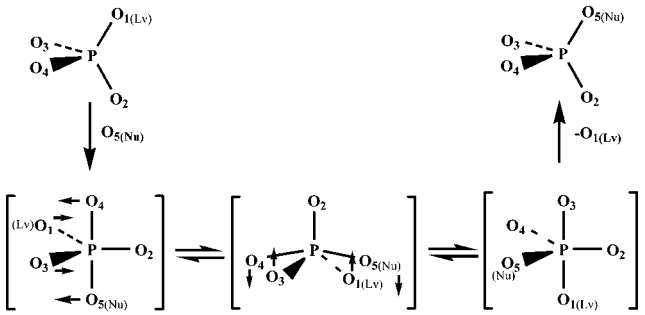


FIGURE 2. Pseudorotational process (simple Berry process) for intramolecular exchange leading to a retention mechanism in PO_4^- derivatives undergoing nucleophilic attack by OH^-. Bracketed isomers represent activated states.[45]

to accommodate the rearrangement of the phosphorus ligands with a reduction of energy associated with the pseudorotational process, and do so in a cooperative fashion. This could happen if the enzyme exhibits some degree of flexibility in causing a modest readjustment of residues at the active site in combination with the pseudorotational process, all taking place within the "lifetime" of the transition state, thus lowering the activation energy. This possibility exists if the particular substrate following a retention path requires the new rearrangement to complete its reaction sequence relative to a prior one for enzymes that exhibit multiple roles at a single active site. (as discussed in the next section, on Enzyme Mechanisms).

Enzyme Mechanisms

As pointed out in the Introduction, the study of the isomerization of β-glucose 1-phosphate to β-glucose 6-phosphate catalyzed by β-phosphoglucomutase was reported to give a trigonal bipyramidal *intermediate* isolated by X-ray analysis,[15] therefore suggesting an associative reaction for phosphoryl transfer enzymes. The result was upsetting to some in the biochemical community in that solution chemistry indicated phosphoryl transfer enzymatic reactions leaned toward a dissociative process.[46] A report[47] quickly followed offering an additional interpretation of the X-ray study accompanied by a rebuttal by the principal authors of the X-ray study[48] Whether or not time will tell, the final outcome is not the point here. Rather the point is the firmness with which scientists guard "accepted" lines of reasoning. In reaction mechanisms of phosphorus compounds in the absence of an enzyme, particularly when the reactant is tetracoordinate, an associative process leading to a pentacoordinate activated state is generally envisioned.[2] The much more complex problem in an enzyme reaction gives rise to additional factors that must be taken into account, some of which have been discussed in this Account. However, both areas are subject to the same problems when one must obtain X-ray results on trapped substrates and compare this behavior with deductions from solution chemistry. As stated by James and Tawfik,[49] 'The crystal structure of a protein might not be the only conformation adopted in solution and, owing to the influence of crystal-

packing forces, might not even be the most representative." In both the enzymatic and the nonenzymatic studies, the same uncertainties arise regarding the fact that these are at best approaches to solving the problem at hand, but not a final solution. However, the phosphorus chemistry that has been developed over the past quarter of a century also applies to both areas of investigation and must be taken into account.

As an example, consider the pseudorotation mechanism[50] that is well established for nonrigid pentacoordinate molecules of phosphorus, usually existing in a trigonal bipyramidal geometry (Figure 2).[2,5a,44,45,50] This rapid reorientational process has been applied successfully in interpreting mechanisms of many nonenzymatic reactions of phosphorus but is invariably absent from active site considerations of phosphoryl transfer reactions dealing with chirality. As mentioned in the previous section, an enzymatic chiral reaction involving phosphoryl transfer with retention of configuration is depicted as proceeding by two sequential inline transformations involving trigonal bipyramidal activated states. Even the premise that inline or adjacent attack is involved in forming a trigonal bipyramidal transition state in an enzymatic reaction leading to retention or inversion of configuration is not without problems. Pentacoordinated phosphorus is a member of a class of nonrigid molecules of main group elements whose geometries span the spectrum of structures from trigonal bipyramidal to square or tetragonal pyramidal.[51,52] Thus, in an enzyme with its structured active site, the possibility arises that an activated state may be displaced along the coordinate connecting these two pentacoordinate geometries, assuming we are dealing with pentacoordinate activated states in the first place.

There is a large body of information concerning enzyme promiscuity and enzymes' modes of evolution,[53−56] that is, the ability of an enzyme to catalyze different reactions at the same active site. The article by O'Brien and Herschlag[53] cites a number of phosphoryl transfer enzymes exhibiting catalytic promiscuity. Some discussions center on a switch in enzyme activity brought about by a different active site residue acting on the substrate[54] In another type of enzyme promiscuity, the same active site nucleophile is found to catalyze the reaction of different substrates. For example,[53] chymotrypsin catalyzes the hydrolysis of a number of different substrates initiated by attack of a serine residue at a carbon atom that is thought to lead to similar tetrahedral activated states. Chymotrypsin also catalyzes the reaction at a tetrahedral phosphoryl group that leads to a proposed trigonal bipyramidal activated state.[53] Thus the active site of chymotrypsin is able to catalyze both amidase and phosphotriesterase reactions. There is a variety of other types of enzyme reactions that lead to other structures and functions in addition to their original catalytic activity in the realm of evolution, some of which may involve more than one active site of the protein.[57] The latter are referred to as moonlighting enzymes.[58−60]

With this diversity in enzyme activity, it is not unlikely that pseudorotation of a phosphorus transition state or intermediate formed at a phosphoryl transfer active site takes place. This could facilitate placement of a different nucleophile for attack in concert with an active site reorientation resulting in a different enzymatic reaction.

Summary

Recent chemistry of hypervalent phosphorus has been outlined with reference to active site interactions of phosphoryl transfer enzymes. Discussed is the influence of anionicity, hydrogen bonding, and van der Waals forces in relation to their role on donor atom coordination at the phosphorus atom. The potential exists for similar interactions involving donor atoms from nearby residues at enzyme active sites, both with the phosphate substrate and the phosphorane activated state. The energies of hydrogen bonding and van der Waals effects compete with the energies associated with donor interactions. Depending on substituent makeup, one may become dominant in controlling the extent of donor action and influence the course of nucleophilic substitution. Pseudorotation, as an aspect of phosphorus hypervalency, is also envisioned in certain cases to possibly facilitate phosphoryl transfer reactions.

Further, the energies associated with the conversion of five to six coordinate phosphorus is found to be small, such that the formation of a hexacoordinated activated state in phosphoryl transfer reactions becomes a likely possibility. As shown, the hexacoordinated state exhibits greater reactivity than pentacoordinated analogues. This suggests that enzyme action would be facilitated by an increase in coordination at the active site. The increase or partial increase in coordination geometry will result in a loosening of all bonds to phosphorus and allow the leaving group to depart more readily as a result of the lowered energy barrier. Also, it is found that donor action from oxygen or nitrogen moieties is stronger in pentaoxyphosphoranes compared to that in phosphate substrate compositions. As a consequence, a rate enhancement effect would be realized as a result of donor coordination caused by an amino acid residue at an enzyme-pentacoordinated activated state leading to hexacoordinated phosphorus. This has been termed a nucleophilic-assisted nucleophilic attack by Ramirez and co-workers[61−65] in their work long ago dealing with nonenzymatic reactions involving phosphoryl transfer that were proposed to proceed via hexacoordinated phosphorus.

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