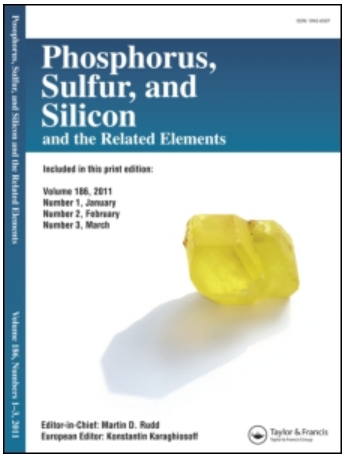

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Active Site Model for Phosphoryl Transfer Enzymes Via Hexacoordination

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ACTIVE SITE MODEL FOR PHOSPHORYL TRANSFER ENZYMES VIA HEXACOORDINATION

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Previous work has demonstrated the ease with which phosphorus can increase its coordination geometry. The present study has more closely modeled active sites of phosphoryl transfer enzymes by the inclusion of anionic phosphorus, which allows for oxygen atom donor coordination at phosphorus in the presence of a hydrogen bonding network. The resulting increase in phosphorus–oxygen donor coordination compared to analogous systems containing neutral phosphorus compounds serves as a model applicable to proposed mechanisms at active sites of phosphoryl transfer enzymes.

Keywords: Anionic phosphorus; hexacoordinate phosphorus; phosphoryl transfer enzymes

Biochemists studying phosphoryl transfer enzymes outline mechanisms of nucleophilic attack at phosphate centers that proceed by in-line displacement reactions that take place by way of proposed trigonal bipyramidal intermediates or transition states. If a chiral reaction is involved, these reactions occur with inversion of configuration. If the chirality shows retention of configuration, then multiple in-line displacement steps are suggested, again all by way of trigonal bipyramidal active states. In-line attack, that is, apical entry of the incoming nucleophile and apical departure of the leaving group, agrees with definitive chemistry of pentacoordinate phosphorus, both structurally and in terms of reaction mechanisms 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 departure of the leaving group

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positioned at an equatorial site, 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 an apical site. The latter reasoning seems valid in view of the relatively high energy that would be required to cause rearrangement of the active-site residues necessary to accommodate the repositioning of the ligands attached to phosphorus.[1]

The description just given outlines what was reasonable relative to the advances made regarding higher coordinate phosphorus chemistry in the absence of an enzyme environment over 30 years ago. However, the area has experienced major advances since then, particularly in the ease of formation of hexacoordinated phosphorus and its relationship to pentacoordinated phosphorus.[2] At present, biochemists have not included such knowledge in their considerations of active-site mechanisms of phosphoryl transfer enzymes. In this paper, we outline developments in higher valent phosphorus chemistry that reasonably may be utilized by biochemists in refining their current proposals concerning mechanistic models of nucleophilic displacements at active sites of phosphoryl transfer enzymes.

HEXACOORDINATE PHOSPHORUS

Previous Studies: Cyclic Oxyphosphoranes[2a]

Previously, we have carried out studies with pentaoxyphosphoranes with flexible eight-membered ring systems capable of assuming different structural orientations if coordination does not take place, for example, **2** and **3** compared to coordination for **1** (Figure 1). Series of these phosphoranes contained donor sulfur, oxygen, or nitrogen atoms capable of coordinating to the central phosphorus atom. The compounds were synthesized primarily by oxidative addition reactions employing phosphates interacting with diols. X-ray analysis gave ranges of the

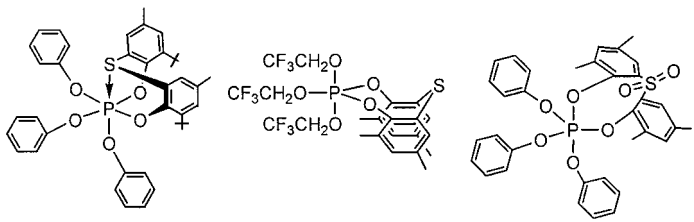


FIGURE 1

phosphorus—donor bond which varied from 3.041(3) Å to 2.373(5) Å for sulfur as the donor atom and from 2.646(5) Å to 1.936(7) Å for oxygen as the donor atom. Using a linear interpolation between the sum of the single-bond covalent radii and the sum of the van der Waals radii, the extent of displacement toward an octahedon from a trigonal bipyramid varied from 24% to 73% for the phosphoranes containing sulfur as the donor atom and from 28% to 82% when oxygen was the donor atom. The variation in donor ability was largely associated with substituent effects and to a lesser extent attributed to steric interactions.

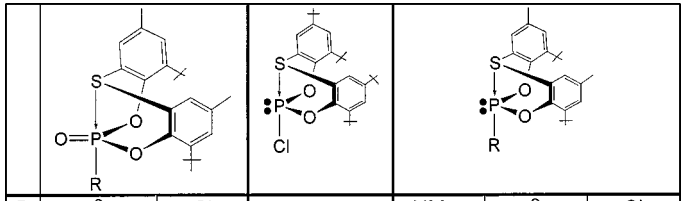
In the presence of five attached oxygen atoms, the electrophilicity at phosphorus is high, similar to what might be expected for a pentacoordinated transition state formed at an active site from a phosphate substrate undergoing reaction by a phosphoryl transfer enzymes.

PENTACOORDINATION

Previous Studies: Cyclic Phosphates and Phosphites[2a]

Related structural studies were carried out on phosphates **4** and **5** and phosphates **6–9** using the same type of flexible ring system that was used with oxyphosphoranes. These were synthesized from PCl_3 or $POCl_3$ with the appropriate diol using sulfur as the donating agent. Due to the lower electrophilicity supplied by the reduced number of attached ligands, the degree of coordination by the sulfur donor atom was markedly less compared to that observed with oxyphosphoranes. The range covered by this series varied from 3.177(2) Å to 2.816(2) Å with a corresponding displacement toward a trigonal bipyramid varying from 31% to 55% (Figure 2).

One may conclude from these observations that donor action from nearby residues at active sites of phosphoryl transfer enzymes (should they be positioned to enter into effective coordination at phosphorus) is expected to play a greater role at the transition state than by interaction at the phosphorus substrate undergoing incipient attack. The donor action at the substrate would be energetically weak in inducing its displacement toward a trigonal bipyramid, compared to a much larger energy effect in causing displacement of the transition state toward a hexacoordinated entity. As such, a rate enhancement of the enzymatic action would result. The latter effect has been demonstrated in the absence of an enzyme. In reaction with catechol, a series of hexacoordinated pentaoxyphosphoranes formed via oxygen or sulfur donor action underwent rapid reaction ($t_{1/2}$, 12 min to 60 min at 90°C), whereas similar trigonal bipyramidal pentaoxyphosphoranes like **3** were inert (no reaction in 48 h at 110°C).



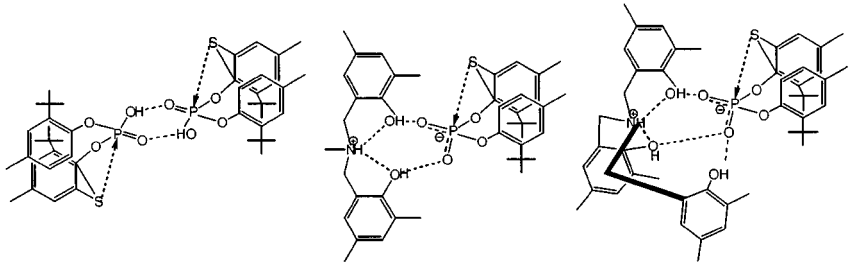
R =		Cl		NMe_2		Cl
	3.177(2) Å 30.9% TBP	3.114(2) Å 35.0% TBP	3.043(2) Å 39.7% TBP	2.952(9) Å 47.1% TBP	2.876(2) Å 50.6% TBP	2.816(2) Å 54.5% TBP
	4	**5**	**6**	**7**	**8**	**9**

FIGURE 2

DONOR ACTION IN THE PRESENCE OF HYDROGEN BONDING AND ANIONIC PHOSPHORUS

Recent Studies

Figure 3 shows that with the inclusion of hydrogen bonding sulfur donor interaction persists with a weakly electrophilic phosphate structure **10** to a similar degree as found in related phosphates lacking any hydrogen bonding interactions[3] (cf. **4** in Figure 2). Furthermore, for formation of anionic phosphates **11** and **12** in the presence of hydrogen bonding, donor action at phosphorus persists and is reduced to only a modest extent.



P-S = 3.187(1) Å, 3.192(1) Å	P-S = 3.281(2) Å	P-S = 3.254(9) Å
10	**11**	**12**

FIGURE 3 Schematic representations of phosphate structures exhibiting hydrogen bonding and P–S interactions.[3]

Additional studies were conducted on a series of phosphorus compounds **13–15** containing carboxyl groups[4] and their anionic counterparts **16–18**[5] obtained by treatment of the precursor acid forms with amines which also served to introduce hydrogen bonding interactions. The structures, a hexacoordinated anionic phoshoranate **16A** and **16B**, a trigonal bipyramidal anionic phosphine **17**, and a trigonal bipyramidal phosphine oxide **18**, revealed the presence of P—O donor coordination that was stronger for all members than what existed in the precursor acid forms **13** to **15**. The extent of the P—O bond distance shortening is expressed by the Δ values listed at the bottom of Figure 4.

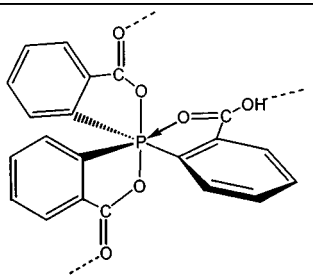
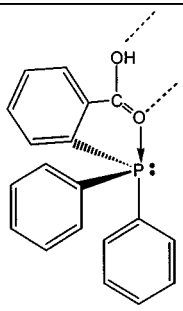
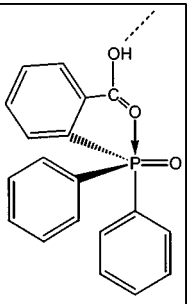
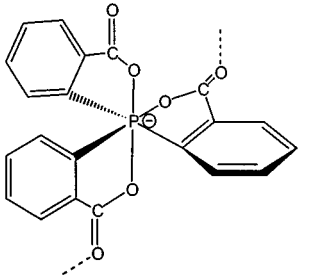
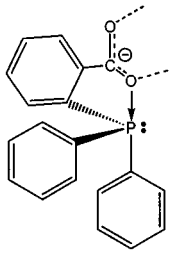
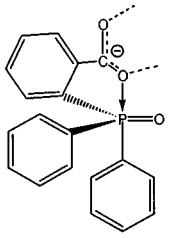
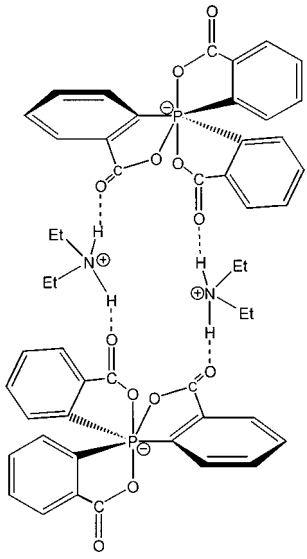
				
^{31}P, ppm P-O, Å	-56.9 2.984(2) Ave.		-4.8 2.806(1)	38.3 3.075(2)
	13		**14**	**15**
				
^{31}P, ppm P-O, Å	$Et_2NH_2^+$ -135.5 1.901(1)	Et_3NH^+ -124.9 1.898(1)	$Et_2NH_2^+$ -5.7 2.696(2)	$Et_2NH_2^+$ 30.3 2.863(2)
	16A	**16B**	**17**	**18**
Δ, Å	1.083	1.086	0.110	0.212

FIGURE 4



16A

FIGURE 5

The hydrogen bonded dimer arrangement for the phosphoranate **16A** is shown in the schematic above. Evaluation of the energies of the donor interactions for **16–18** revealed that they were stronger than the hydrogen bond energies. In kilocalories per mole, these are estimated to be approximately 80 for **16**, 16 for **17**, and 6.5 for **18**.

The anionic series **16–18** and their precursor acid forms **13** to **15** all possess three aryl bonds to phosphorus and as such are not the most electrophilic. Even so, the degree of donor coordination found in this series is comparable to that obtained with the cyclic phosphites and phosphates possessing flexible ring systems (Figure 2), where the ligand electronegativity is considerably higher. Thus, the inclusion of enzyme active-site constraints, phosphate anionicity, and hydrogen bonding interactions does not serve to diminish the strength of donor interactions. In a similar fashion, one would expect the greater donor ability found for pentaoxyphosphoranes to be increased or at least maintained in transforming trigonal bipyramidal phosphoranes to the hexacoordinated state in the presence of anionic phosphorus and hydrogen bonding.

APPLICATION TO ENZYME ACTIVE SITES

Recent enzymatic studies continue to focus attention solely on proposed TBP transition states, such as the mechanistic study of the cleavage

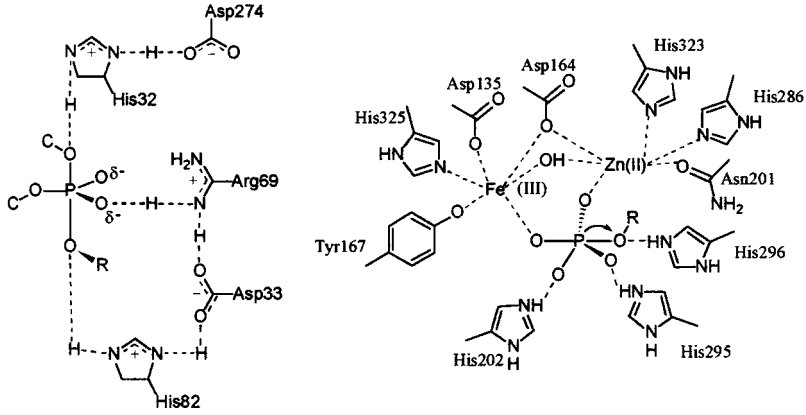


FIGURE 6 Active site model for (a) PI-PLC and (b) the hydrolysis of phosphomonoesters by PAP.

of the phosphate P—O bond by phosphatidylinositol-specific phospholipase C (PI-PLC)[6] and the hydrolysis of phosphomonoesters by purple acid phosphatase (PAP).[7] Potential donor oxygens from active site residues exist in these enzymes as they do in many other phosphoryl transfer enzymes.[1b,2] In the former system, carboxylate groups are present in the form of an aspartate (Asp) residue (Figure 6a). In the latter enzyme, in addition to four Asp residues at the active site, there is a carboxylate containing glutamate (Glu), a tyrosine (Tyr), and an asparagine (Asn) residue (Figure 6b). Also, one may not easily discount the role of water molecules, which are prevalent and serve as potential donor molecules as well as in their role as nucleophiles in initiating attack of a phosphate substrate. Previously, reference was made to the proposed transition state in the activation of tyrosine by the tyrosyl–tRNA synthetase system,[8] where we suggested that donor action by a carboxylate oxygen atom promotes the formation of a hexacoordinate structure.[9]

SUMMARY AND CONCLUSION

Previous work has demonstrated the ease with which phosphorus can increase its coordination geometry. This study has more closely modeled active sites of phosphoryl transfer enzymes by the inclusion of anionic phosphorus, which allows for oxygen atom donor coordination at phosphorus in the presence of a hydrogen-bonding network. The resulting increase in phosphorus—oxygen donor coordination compared to analogous systems containing neutral phosphorus compounds serves as a

model applicable to proposed mechanisms at active sites of phosphoryl transfer enzymes.

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