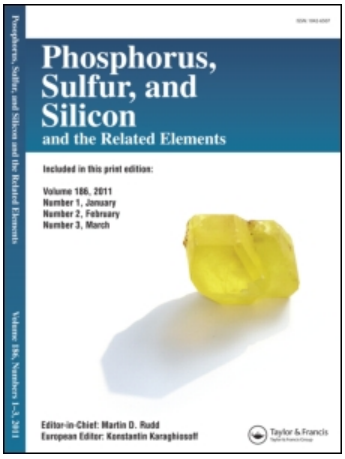

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Quantum-Chemical Insights into Mechanisms of the Nonenzymatic Hydrolysis of Phosphate Monoesters

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The interpretation of a large number of experiments that are generally considered to support the dissociative or metaphosphate mechanism of phosphate monoesters in aqueous solution is scrutinized using results of ab initio quantum chemical calculations. It is concluded that while it is currently not clear whether the associative or dissociative mechanism of phosphate monoester hydrolysis is operating in aqueous solution, the associative transition state can be better stabilized by the interaction between the equatorial oxygens and the proton donor groups in the enzyme active sites.

Keywords: dimethyl phosphate; rate constant; activation barrier; ab initio calculations

INTRODUCTION

The transfer of the phosphoryl group from phosphate monoesters and anhydrides to the water molecule or another nucleophile represents a key step in the regulation of biochemical processes. In the literature on phosphate monoester hydrolysis it is usually said that a considerable amount of evidence in favor of the dissociative or metaphosphate mechanism has accumulated over the years [1-4]. The evidence mentioned involves the comparison of the overall rate between the mono- di- and triesters, and between monoanions and dianions of monoesters [5-7], the linear free-energy relationships, the kinetic isotope effects, and the activation entropies. In this paper, a part of this evidence is analyzed using the results of ab initio quantum mechanical calculations in aqueous solution. It is shown that in most cases the assumptions that led to the exclusion of the associative mechanism are not fully justified, and that the experimental data mentioned as a direct support for the dissociative mechanisms can be interpreted equally well in terms of the associative mechanism.

ACTIVATION BARRIERS FOR DIFFERENT SUBSTRATES

The experimental kinetic studies [5-9] showed that the activation free energy ($\Delta G^{\neq}$) for the nonenzymatic hydrolysis of monomethyl phosphate in aqueous solution is smallest for

its monoanionic form near pH=4.4 and increases by 2 and 5 kcal/mol for the neutral and dianionic forms, respectively. The 5 kcal/mol difference between $\Delta G^{\neq}$ values for the monoanion and dianion hydrolysis was explained in terms of the dissociative mechanism [2, 5, 8] (Figure 1).

Our quantum chemical calculations (MP2/6-31+G**) coupled with the Langevin dipoles (LD) solvation model showed that $\Delta G^{\neq}$ for the monoanion is indeed lower by 5 - 6 kcal/mol than $\Delta G^{\neq}$ for the dianion [10] (Figure 1). However, these calculations indicated a large difference in $\Delta G^{\neq}$ for both the the dissociative and associative mechanisms.

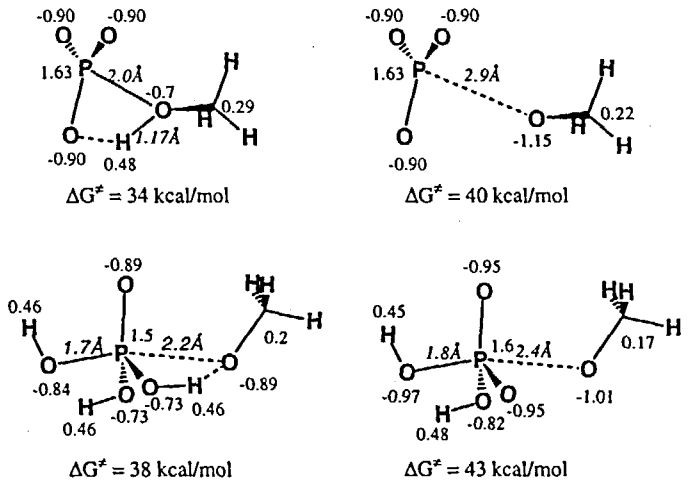

FIGURE 1: Transition states for the dissociative (top) and associative (bottom) hydrolysis of methyl phosphate monoanion (left) and dianion (right) and the calculated (ESP PCM/HF/6-31G*) atomic charges and the activation free energies (MP2+LD [10]) at 25°C.

We have somewhat simplified the real situation by considering only transition states dominated by the cleavage of the leaving group, but the overall faster hydrolysis of the monoanionic species is obtained even when all the TS along the associative and dissociative pathways are considered [10]. In this case, activation barriers for the associative and dissociative mechanism were found to differ by less than 2 kcal/mol [10].

The dissociative mechanism also seemed to be intuitively more reasonable to rationalize the slower observed rate of hydrolysis of dimethyl phosphate monoanion compared to the monomethyl phosphate monoanion [6]. However, our computer simulation showed that this rate difference can be explained in terms of the associative mechanism [11].

An interesting analogy between the monomethyl and trimethyl phosphate hydrolysis was raised by Bunton et al. [5,7] in order to exclude the associative mechanism, which involves the attack by the OH^- nucleophile. Here, again, calculations predicted the rate constants for OH^- attack on the neutral monomethyl and trimethyl phosphate that reproduced the experimental data [12]

BRØNSTED LINEAR FREE-ENERGY RELATIONSHIPS (LFER)

The rate constants for the hydrolysis of phosphate monoester dianions have been found to be very sensitive to the pKa constant of the leaving group, but practically independent of the pKa of the nucleophile [13]. This finding was interpreted as an evidence for the dissociative mechanism [13]. In contrast, our calculations for the associative hydrolysis of substituted phenyl phosphate dianions found that the experimental LFER will be observed if the rate limiting step for this reaction would involve a late TS [14]. This condition can be satisfied even for good leaving groups since the proton transferred from the attacking nucleophile greatly facilitates the nucleophilic attack. Furthermore, the detailed analysis using Marcus formalism indicated mechanistic ambiguity of the observed LFER [15].

DEUTERIUM ISOTOPE EFFECTS

The rate of hydrolysis of methyl phosphate monoanion is practically unchanged in deuterium oxide ($k_{H2O}/k_{D2O} = 0.87$) [5]. Since the proton transfer occurs in the dissociative transition state (Figure 1), the observed solvent isotope effect should naturally support the associative mechanism. This conclusion is consistent with the calculated solvent isotope effects for the dissociative and associative transition states. No solvent deuterium isotope effect was measured also for the hydrolysis of dianion of acetyl phosphate [1], which is consistent with both the associative and dissociative transition states (Figure 1).

STRUCTURAL BASIS FOR THE ENZYMATIC STABILIZATION OF TRANSITION STATES FOR THE PHOSPHATE MONOESTER HYDROLYSIS

The calculated charge distributions for both the monoanionic and dianionic TS are presented in Figures 1 and 2. Here, let us note the small differences between the atomic charges for the TS corresponding to the associative and dissociative hydrolysis of the dianionic substrate. Thus, the electrostatic stabilization of both TS in a polar enzyme active site can be expected to be of similar magnitude. On the other hand, we found large differences in the proton affinities and pK_a constants of metaphosphate (PO_3^-) and the dianionic phenylphosphorane intermediate, that are structurally very close to the TS for the dissociative and associative mechanisms, respectively (Table 1). As a result, the associative TS can be selectively stabilized by the interaction of equatorial oxygens with proton donors, such as arginine finger found in the GTPase-activating proteins [16].

TABLE 1: Comparison of the basicities of the substrate and unstable intermediates for the hydrolysis of phosphate monoester dianion in gas-phase and aqueous solution.

	$PhOPO_3^{2-}$	$MeOP(OH)(O_2)OPh^{2-}$	PO_3^-
PA (kcal/mol)[a]	440.8	441.8	315.9
pKa[b]	5.7	10.3	-10.2

[a] Proton affinity calculated at the MP2/6-31+G**//HF/6-31G* level. The geometry with the P-OMe and P-OPh distances of 1.8 and 2.0 Å, respectively, was used for $MeOP(OH)(O_2)OPh^{2-}$ [14]. Ph and Me denote the phenyl and methyl groups, respectively. [b] pK_a of the conjugated acid (BH) was calculated as follows: $pK_a(BH) = [PA(B^-) - 7.5 + \Delta G_{solv}(B^-) - \Delta G_{solv}(BH) + \Delta G_{solv}(H^+)]/1.37$, where the solvation free energies $\Delta G_{solv}(B^-)$ and $\Delta G_{solv}(BH)$ were determined using the iterative Langevin dipoles solvation model [17], and $\Delta G_{solv}(H^+) = 259.5$ kcal/mol.

ACKNOWLEDGEMENT

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