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Theoretical Evaluation of the Substrate-Assisted Catalysis Mechanism for the Hydrolysis of Phosphate Monoester Dianions

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Abstract: Quantum chemistry methods coupled with a continuum solvation model have been applied to evaluate the substrate-assisted catalysis (SAC) mechanism recently proposed for the hydrolysis of phosphate monoester dianions. The SAC mechanism, in which a proton from the nucleophile is transferred to a nonbridging phosphoryl oxygen atom of the substrate prior to attack, has been proposed in opposition to the widely accepted mechanism of direct nucleophilic reaction. We have assessed the SAC proposal for the hydrolysis of three representative phosphate monoester dianions (2,4-dinitrophenyl phosphate, phenyl phosphate, and methyl phosphate) by considering the reactivity of the hydroxide ion toward the phosphorus center of the corresponding singly protonated monoesters. The reliability of the calculations was verified by comparing the calculated and the observed values of the activation free energies for the analogous $S_N2(P)$ reactions of F^- with the monoanion of the monoester 2,4-dinitrophenyl phosphate and its diester analogue, methyl 2,4-dinitrophenyl phosphate. It was found that the orientation of the phosphate hydrogen atom has important implications with regard to the nature of the transition state. Hard nucleophiles such as OH^- and F^- can attack the phosphorus atom of a singly protonated phosphate monoester only if the phosphate hydrogen atom is oriented toward the leaving-group oxygen atom. As a result of this proton orientation, the SAC mechanism in solution is characterized by a small Brønsted coefficient value ($\beta_{lg} = -0.25$). This mechanism is unlikely to apply to aryl phosphates, but becomes a likely possibility for alkyl phosphate esters. If oxyanionic nucleophiles of $pK_a < 11$ are involved, as in alkaline phosphatase, then the $S_N2(P)$ reaction may proceed with the phosphate hydrogen atom oriented toward the nucleophile. In this situation, a large negative value of β_{lg} (-0.95) is predicted for the substrate-assisted catalysis mechanism.

Keywords: density functional calculations · hydrolyses · phosphate esters · reaction mechanisms · substrate-assisted catalysis

Introduction

Phosphoryl transfer reactions are ubiquitous in biological chemistry.[1] The formation and hydrolysis reactions of phosphate monoesters and anhydrides are involved in crucial biochemical pathways and have been the subject of extensive investigation in both enzymatic and nonenzymatic systems for over 40 years.[2–4] Despite these studies, there is no universal agreement on the enzymatic and, to a lesser extent, nonenzymatic phosphoryl-transfer mechanisms. Currently, the main mechanistic debate is focused on the chemical mechanism of guanosine triphosphate (GTP) hydrolysis by GTP-binding proteins of the Ras superfamily. The debate centers on the nature of the transition state, and specifically, whether it is "associative" or "dissociative" in character and whether there is a common GTPase mechanism for Ras proteins. Recent findings suggest that a common mechanism might not exist and that G proteins might actually select a

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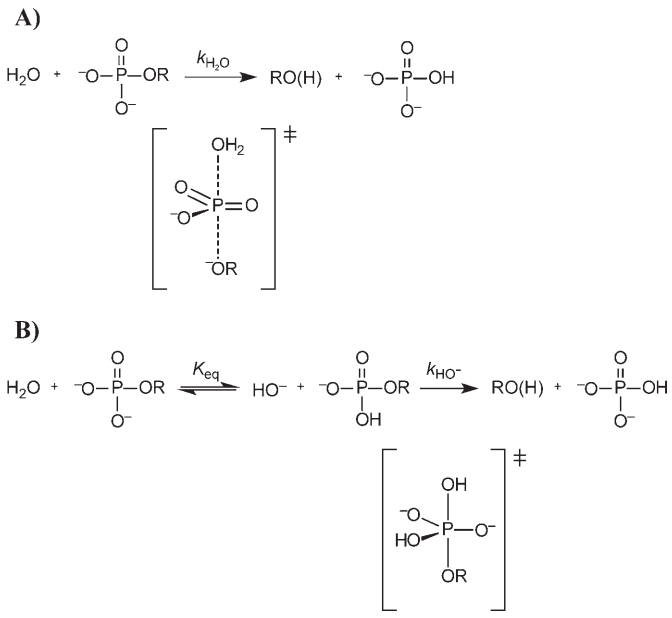
more or less associative/dissociative mechanism depending on the particular electrostatic environment of their active site.[5]

This conclusion is important as it suggests that, when considering the mechanisms of phosphoryl-transfer reactions in solution, the dissociative and associative alternatives should both be carefully examined.[6,7] It has often been suggested that the transition state for a reaction is intrinsically determined by the molecular properties of the reactant(s) and product(s), and that enzymes have evolved to stabilize these transition-state structures without significantly altering them. If this view is correct and if phosphoryl-transfer enzymes apparently stabilize associative as well as dissociative transition states, it therefore seems quite reasonable to envisage the possibility that the energetics of the associative and dissociative pathways are closely similar in solution. Yet, the currently dominating view regarding nonenzymatic phosphate monoester reactions is that phosphoryl transfer proceeds by means of a concerted A_ND_N (A_N: association to a nucleophile; D_N: dissociation of a nucleofuge) dissociative mechanism involving a loose transition state in which bond fission to the leaving group is extensive, and bond formation to the nucleophile is small (Scheme 1A).[3,4] If we assume



Scheme 1. Alternative, kinetically equivalent, mechanisms that have been proposed for phosphoryl transfer from a phosphate monoester dianion in aqueous solution. A) According to the currently dominating view, phosphoryl transfer occurs by means of a concerted A_ND_N dissociative-type mechanism involving a loose metaphosphate-like transition state in which P–O bond cleavage to the leaving group is much more advanced than P–O bond formation to the nucleophile. B) The recently proposed substrate-assisted catalysis mechanism consists of a preequilibrium proton transfer from water to the dianion (K_{eq}), followed by hydroxide attack on the resulting monoanion (k_{HO^-}). This mechanism corresponds either to an associative (A_N+D_N) or concerted (A_ND_N) pathway in which a pentacoordinate phosphorane intermediate or transition state is formed. Note that in ref. [12], the preequilibrium step has been considered to be an oversimplification, with the associative mechanism requiring only a prior proton transfer.

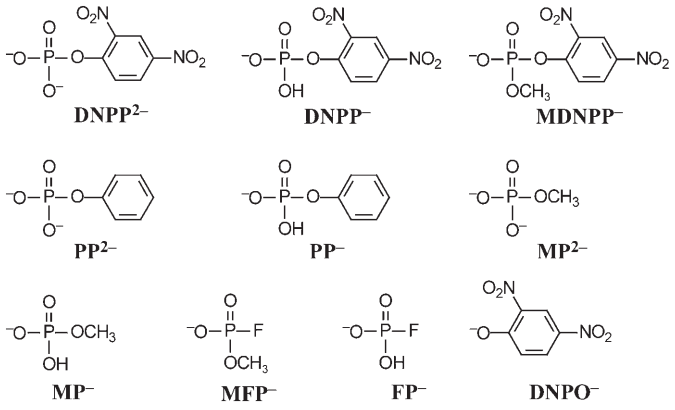
that the dissociative-type mechanism is unique in solution, then there seem to be situations in which phosphoryl-transfer enzymes (e.g., G proteins) actually alter the transition-state structure of the uncatalyzed reaction. This property of enzymes, if confirmed, would imply that we cannot safely use what we know about an uncatalyzed reaction to rationally design a transition-state analogue inhibitor; instead, we must deduce the mechanism and transition-state structure directly from the enzymatic reaction.

Although a substantial amount of experimental data could be interpreted to support a dissociative, metaphosphate-like transition state for the nonenzymatic hydrolysis reactions of phosphate monoesters, this mechanism has been recently challenged by ab initio calculations and other theoretical arguments that favor an alternative mechanism, substrate-assisted catalysis (SAC), in which an initial proton transfer from water to the phosphate dianion precedes hydroxy-phosphorus bond formation via an associative transition state (Scheme 1B). On the basis of various ab initio calculations performed on phosphate monoesters, it was concluded that the SAC mechanism could be operational both in enzymes and in solution.[6–9] This proposal has been controversial, with criticisms[10] and counter-arguments[11] presented. In a very recent theoretical work, Klähn et al.[12] have also questioned the tendency to conclusively associate experimental findings with the dissociative mechanism. Although the SAC proposal is being increasingly accepted as a possible effective mechanism of Ras-like GTP-binding proteins,[5] the idea that this mechanism could also be operational in solution is far from being adopted by the community of physical organic chemists.

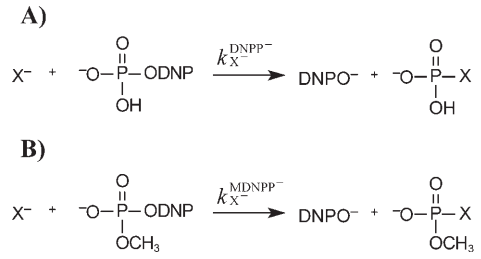
The main problem with this controversy is that much of the arguments put forward in favor of the dissociative mechanism have been inferred from experiments using phosphate esters with strongly activated leaving groups, that is, with leaving-group pK_a (pK_{lg}) values most often in the range 3–7. This limitation is due to the extremely slow rate with which unactivated phosphate monoester dianions are hydrolyzed, which in turn makes detailed studies of their reactions very difficult to measure experimentally.[13] However, we have no guaranty that the reactions of phosphate esters with highly activated leaving groups are good models for evaluating the actual mechanism of the background reactions of, for example, phosphatase enzymes whose substrates are most commonly tyrosine phosphate ($pK_{lg} \approx 10$), and serine and threonine phosphates ($pK_{lg} \geq 14$). On the other hand, the computational studies that have concluded in favor of the SAC mechanism have largely been, although not exclusively,[9] performed by using the methyl phosphate ester. Therefore, it cannot be excluded that the classical dissociative mechanism (Scheme 1A) is favored for monophosphate esters with good or moderately good leaving groups and that the SAC mechanism (Scheme 1B) becomes operational for monophosphate esters with alkoxy leaving groups. It is also possible that both mechanisms compete for the hydrolysis of triphosphate monoesters in aqueous solution, which would explain why phosphoryl-transfer enzymes such as GTP-binding

proteins apparently exhibit a continuum of transition states between the two limiting dissociative (D_N+A_N) and associative (A_N+D_N) mechanisms.[5]

Admiraal and Herschlag[10] have evaluated the SAC proposal (Scheme 1B) for the hydrolysis of the 2,4-dinitrophenyl phosphate dianion ($DNPP^{2-}$, $pK_{lg}=4.07$) by comparing



the reactivity of its protonated monoanionic form $DNPP^-$ (Scheme 2A), involved in the second step of the SAC mechanism, with that of its methylated diester analogue



Scheme 2. The analogous nucleophilic reactions of X^- with A) the protonated monoester monoanion $DNPP^-$ and B) the methylated diester monoanion $MDNPP^-$. The kinetic effect k_{rel} induced by H/Me substitution is given by the ratio of the second-order rate constant for the reaction of X^- with $DNPP^-$ and the second-order rate constant for the reaction of the same nucleophile with $MDNPP^-$ ($k_{rel}=k_{X^-}^{DNPP^-}/k_{X^-}^{MDNPP^-}$).

$MDNPP^-$ (Scheme 2B). By using existing data, these authors have shown that the protonated and methylated species have similar rate constants for reaction with fluoride ions and other neutral nucleophiles such as substituted pyridines ($k_{rel}=k_{nuc}^{DNPP^-}/k_{nuc}^{MDNPP^-}=3$–20). A relative rate constant of 4.3 was reported for fluoride ions at 39 °C. Unfortunately, there is no experimental way to determine the rate constant for the OH^-+DNPP^- reaction ($k_{OH^-}^{DNPP^-}$), as $DNPP^-$ ($pK_2=4.5$) will be deprotonated at the high pH required to measure the kinetics of the OH^- attack. The observed rate constant, k_{obs}, for hydrolysis of $DNPP^{2-}$ at 39 °C is $1.75\times10^{-4}\,s^{-1}$.[15] According to the stepwise associative mechanism shown in Scheme 1B, k_{obs} represents nucleophilic attack by OH^- upon $DNPP^-$, and the second-order rate constant for this reaction would be calculated as shown in Equation (1):[16]

$$k_{OH^-}^{DNPP^-}=\frac{k_{obs}}{K_{eq}[H_2O]}=\frac{1.75\times10^{-4}\,s^{-1}}{10^{-11.2}\times55\,M}=5.0\times10^{5}\,M^{-1}\,s^{-1} \quad (1)$$

A value $k_{OH^-}^{MDNPP^-}$ of $4.7\times10^{-4}\,M^{-1}s^{-1}$ has been experimentally determined for the reaction of hydroxide with $MDNPP^-$ (Scheme 2B).[18] Thus, a relative rate constant ($k_{OH^-}^{DNPP^-}/k_{OH^-}^{MDNPP^-}$) of $\approx10^9$ is predicted from the hypothetical and experimental second-order rate constants for reactions of $DNPP^-$ and $MDNPP^-$ with hydroxide, respectively. This value, which greatly exceeds the measured k_{rel} values of 3–20 for reactions with other nucleophiles, was considered by Admiraal and Herschlag as evidence against the SAC proposal.[10] However, these arguments were severely criticized by Glennon et al.,[11] who retorted that this reactivity analysis could not be used to refute the substrate-assisted catalysis proposal because the kinetics of the attack of nucleophiles such as F^- on protonated phosphate monoesters lack the crucial P-O-H···OH^- interaction that was considered to provide the major stabilization for the OH^- attack of the second step of the SAC mechanism.

The specific role that the phosphate hydrogen atom may play in the kinetics of OH^- attack at the P atom of $ROPO_3H^-$ is therefore at the heart of the mechanistic debate concerning the hydrolysis reaction of phosphate monoester dianions. Physical organic chemists have traditionally assumed that a methyl group is a valid substitute for a phosphate hydrogen atom and have accordingly often speculated, in order to reject the SAC alternative, that the rate constant for OH^- attack on $ROPO_3H^-$ should be roughly the same as that for OH^- attack on the corresponding methylated diester monoanion.[15,19] Whether or not this assumption is justified is an important question that can only be resolved by careful quantum studies. However, when reactions in aqueous solution are considered and when charged reactants with hydrogen-bond-donating and -accepting ability, such as OH^- and $ROPO_3H^-$, are involved, it seems reasonable to assume that quantum approaches will not be a faithful comparison to experiment unless the calculations include an appropriate (large) number of explicit solvent molecules and extensive dynamics. Unfortunately, despite the gradual emergence of stronger computers and the recent advances in the development of efficient codes for ab initio molecular dynamics,[20] the use of quantum mechanics (QM) techniques in the study of such reacting systems in a complete hydration shell remains unrealistic. Thus, one is faced with making approximations in order to obtain the most accurate description of the chemical system of interest.

Water constitutes a high-dielectric medium that plays a large role in reactions involving phosphate esters.[21] Therefore, for evaluating the effect of solvation, one approach consists of representing the solvent as an isotropic medium with the dielectric constant of pure water ($\varepsilon\approx78$). Some of us have recently shown that density functional theory (DFT) calculations coupled with such a model could re-

markably reproduce the activation enthalpies reported for well-documented hydroxide-catalyzed P–O cleavage reactions of representative phosphate triesters and diesters bearing good and poor leaving groups.[22] The primary objective of these preliminary calculations was to validate a proper modeling approach to determine realistic free-energy pathways in solution for $S_N2(P)$ reactions involving OH^- attack on protonated phosphate esters. Here, we have applied this methodology to evaluate the SAC proposal for the hydrolysis of three representative phosphate monoester dianions: 2,4-dinitrophenyl phosphate ($pK_{lg}=4.07$), phenyl phosphate ($pK_{lg}=9.95$), and methyl phosphate ($pK_{lg}=15.5$). Our results indicate that although the SAC mechanism is clearly unlikely for esters with good or moderately good leaving groups, it becomes a very likely alternative for esters with poorer leaving groups such as methanol.

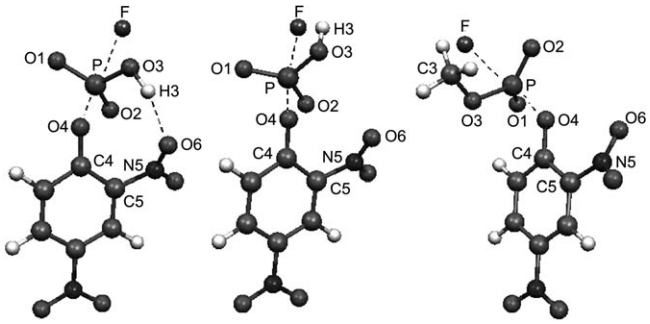


Figure 1. Solution-phase-optimized structures of transition states involved in the $S_N2(P)$ reactions $F^- + DNPP^-$ (**TS1** and **TS1***) and $F^- + MDNPP^-$ (**TS2**). The main geometric parameters are given in Table S12 in the Supporting Information.

Computational Methods

All calculations were performed with the Gaussian 98 set of programs[23] using the hybrid density functional method B3LYP[24,25] as a result of its previous success in similar calculations involving charged phosphates.[26] A double-ζ plus polarization valence basis set[27] was employed for each atom including hydrogen. s and p diffuse functions were added for oxygen ($\zeta_s=0.108151$ and $\zeta_p=0.070214$) and fluorine ($\zeta_s=0.064231$ and $\zeta_p=0.078814$) atoms. Standard pseudopotentials developed in Toulouse[27] were used to describe the atomic cores of all non-hydrogen atoms.[28] Structural parameters and associated energies for reactants and products resulted from full-geometry optimization procedures in the solution phase, with no imposed constraints. Harmonic frequencies of these B3LYP-optimized solution structures were calculated with the solvation correction in order to verify that the stationary points found were minima, and to obtain zero-point energies (ZPE) and thermal corrections to enthalpies and free energies at 298.15 K in the usual rigid-rotor harmonic oscillator approximation.

The shape and the height of the relevant energy barriers between fully optimized reactants and products were determined by means of geometry optimizations in solution with constraints on the reaction coordinate (the relevant reaction coordinate was constrained at a specific value while the remaining degrees of freedom were fully optimized). The reason for resorting to constrained optimizations to evaluate the barrier heights is that full geometry optimizations of transition states turned out to be impractical for most of the reactions investigated here. In cases where the transition-state structure could be fully optimized and characterized (only one imaginary frequency), for example, **TS1** (Figure 1), we noticed no significant structural change between the fully and partially optimized structures (see Table S12, in the Supporting Information). We used the distance from the phosphorus atom to the incoming hydroxy oxygen (or fluorine) atom and, when necessary (i.e., for **TS6***), the distance from the phosphorus atom to the departing alkoxy oxygen atom as the reaction coordinate for the initial and final parts of the $S_N2(P)$ reaction profiles, respectively. For the purpose of comparing experimental and calculated activation energies (ΔH^{+} and $T\Delta S^{+}$) in solution at a temperature of 298 or 312 K, a normal-mode analysis (with the solvation correction) was performed at each point. Typically, the variations of the solution energies ΔE, ΔH, and ΔG along the selected reaction coordinate gave a curve with one maximum. The maxima obtained from the enthalpy profiles, which may slightly differ from the maxima arising from ΔE or ΔG profiles, were used to locate the relevant transition-state structures in solution.[22]

All calculations (including geometry optimizations) were performed in a dielectric continuum by using the multipolar expansion (MPE)[30–33] model developed at Nancy, with which we did not encounter the convergence problems recently described in geometry optimizations with the polarizable continuum model (PCM).[34,35] Probably, the higher efficiency of the MPE method over the PCM method in geometry optimization calculations comes from the fact that the basic equations of the former model are well adapted to analytically compute the energy derivatives.[33] Moreover, the MPE method leads to results basically identical to PCM, as shown by Curutchet et al.,[36] provided that the same cavity characteristics are assumed, although the MPE model demands significantly less computational time.[33] Preliminary calculations[22] indicated that the MPE solvation code is especially efficient and accurate to model $S_N2(P)$ reactions in solution by providing activation energies (ΔH^{+}) within good chemical accuracy (<1 kcal mol^{-1}).[22] Therefore, all calculations throughout this work were done by using this model. In this self-consistent reaction field (SCRF) approach, the solvent is represented by a dielectric continuum and the electrostatic properties of the solute by an atomic distribution of multipoles. The method, implemented in Gaussian 98, offers the possibility of three different distributions. In the present work, we consider multicentric expansions with molecular-shaped cavities represented as superpositions of overlapping nuclear-centered spheres with radii equal to Bondi values multiplied by a constant factor of 1.3 (note that hydrogen atoms also bear a sphere, that is, we do not use the united-atom approximation). For all the reaction systems investigated, we carried out standard MPE computations, that is, the multipole expansion is truncated at $l=4$, which provided acceptable convergence of the electrostatic potential. The dielectric constant of the continuum was set to $\varepsilon=78.39$ (i.e., water).

The proposed computational approach has some advantages with respect to explicit-solvent models such as the combined quantum mechanics and molecular mechanics (QM/MM) or the Car-Parrinello simulations. In particular, it demands much less computational time and allows the rigorous location of transition structures and reaction paths. Indeed, computational time is not much longer in solution than in the gas phase: for instance, the energy computation of structure $DNPP^-$ takes 482 s for the isolated molecule and 489 s for the molecule in solution (computations were carried out in a SGI computer with 4 processors). However, free energies of solvation are, in general, less accurate. QM/MM computations have recently been carried out to compute free energies of solvation in the hydrolysis mechanism of phosphate monoester dianions.[12]

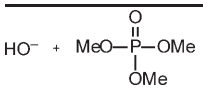
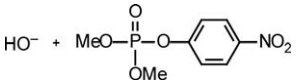
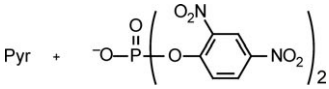
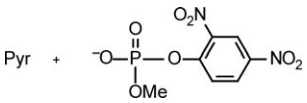
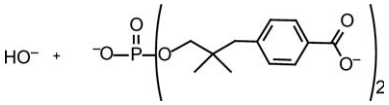
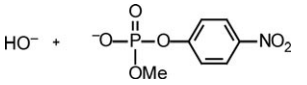
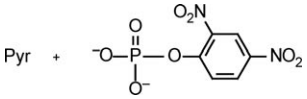
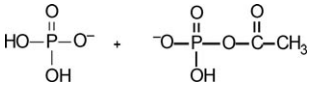
Results and Discussion

Although implicit solvation models have proven to be extremely useful in the study of solvent effects, numerous examples are known for which the specific interactions of the solute and solvent molecules cannot be neglected. In this

case, an appropriate number of explicit solvent molecules must be considered, preferably at the same level of theory as the system of interest. Therefore, when modeling chemical reactions in solution, it is especially important to first determine whether specific solvation effects can contribute significantly to the observed free-energy barrier. This is not an easy task as solvation effects often make a significant positive or negative contribution to either the entropic ($T\Delta S^{\neq}$) or enthalpic ($\Delta H^{\neq}$) component of the free-energy barrier, or to both of them. Moreover, the overall effect may not appear at all in the resulting free energy of activation if compensating entropy and enthalpy changes are involved. Because significant errors can result from the harmonic oscillator approximation commonly used to calculate intrinsic activation entropies,[22,37] and because specific solvation effects can play a major contribution to the observed activation entropies in solution, there is no reason to expect accurate values for this parameter when calculations are performed in a continuum model. Note that solvation entropies can be computed by using the Langevin dipoles model of Florián and Warshel[38] for instance, but here we chose a different (more simple) approach, as explained below. In contrast, the harmonic oscillator approximation is expected to have a much smaller impact in the calculation of the enthalpy term $\Delta H^{\neq}$. Therefore, one way to determine whether microscopic effects that are improperly described by continuum models are likely to give a significant contribution to this latter parameter, is to simply compare the activation enthalpies calculated in the dielectric medium to the reported experimental values.

When such comparisons were made for well-documented monoanionic and dianionic reactions involving OH^- attack at the P center of di- and trisubstituted phosphate esters with poor and good leaving groups, we always observed a remarkable agreement between the experimental and calculated $\Delta H^{\neq}$ values.[22] We believe that this general agreement is not coincidental but rather reflects the fact that the microscopic structure of the solvent can safely be neglected to reproduce, within reasonable chemical accuracy, the activation enthalpies of $S_N2(P)$ reactions for which OH^- attack is rate-determining.[39] As anticipated, our calculated $\Delta S^{\neq}$ values exhibited significant discrepancies when compared with the experimental data. Overestimations (in absolute magnitude) of 5–15 cal mol^{-1} deg^{-1} were typically observed. Thus, to avoid obscuring the enthalpy results with inaccurate entropy contributions, the free-energy values considered in the present study were evaluated by combining the calculated enthalpy changes with a consensus activation entropy term, $\Delta S^{\neq}_{cons}$, derived from experimental data. The entropies of activation for a number of representative anionic $S_N2(P)$ reactions involving various nucleophiles and phosphate esters are collected in Table 1. As can be seen in this table, the reported entropies of activation have values typically between -19 and -31 cal mol^{-1} deg^{-1} with an average of -24.5 cal mol^{-1} deg^{-1}. In view of these and other[40] collected data, we arbitrarily picked (-24 ± 5) cal mol^{-1} deg^{-1} as the "best" consensus activation entropy for the $S_N2(P)$ reactions investigated here. We believe that a combination of experimentally determined trends and careful quantum calculations should help in providing realistic free-energy profiles for the proposed substrate-assisted catalysis mechanism.

Table 1. Entropies of activation[a] [cal mol^{-1} deg^{-1}] for representative monoanionic and dianionic $S_N2(P)$ reactions in aqueous solution.

$S_N2(P)$ reaction	Reaction class	$\Delta S^{\neq}$ [cal mol^{-1} deg^{-1}]
	monoanionic (triester)	-23.6[b]
		-26.0 ± 0.7[c]
	monoanionic (diester)	-23.1[d]
		-31.3[e]
	dianionic (diester)	-24.4 ± 2[f]
		-28.0 ± 1.7[g]
	dianionic (monoester)	-19.4[d]
		-20 ± 6[h]
		av -24.5

[a] Calculated from data in references cited. Pyr: pyridine. [b] Ref. [69]. [c] Ref. [70]. [d] Ref. [41]. [e] Ref. [18]. [f] Ref. [71]. [g] Ref. [72]. [h] Ref. [73].

A final remark seems necessary. Computed enthalpy changes within the continuum model approximation are obtained as the sum of variations in electronic energy, zero-point energy, thermal corrections, and electrostatic solvation energy. Because, formally, the latter term is a free-energy contribution, it may include some solvation entropy. Though

it has been recently argued that the corresponding contribution might not be negligible,[41] the precise entropy amount included in a continuum model calculation is not yet clear and requires further analysis. Some efforts in this direction have been done by using the Born model.[42–44] Within the standard model, in which one neglects the variations of the ionic radius with temperature, solvation entropy comes from the temperature dependence of the dielectric constant. Such a term represents a small contribution to the total free energy (below 2%).[42] Therefore, we shall assume that this contribution is negligible and that main contributions to the activation entropy for the processes investigated below come from 1) translational, rotational, and vibrational reactants degrees of freedom and 2) solvation entropy terms not correctly accounted for in the standard continuum model. Note that a method to separate configurational and solvation entropy has been proposed for reactions in solution[45] or in enzymes.[46]

Hydrolysis of 2,4-dinitrophenyl phosphate dianion: As discussed in the Introduction, to be valid, the SAC mechanism requires that the attack of OH^- on $DNPP^-$ be $\approx 10^9$ faster than that on $MDNPP^-$ (Scheme 2). In order to determine whether this enormous rate constant ratio is likely, we first tried to reproduce, using the methodology described above, the corresponding rate constant ratio reported for fluoride ions at 39°C ($k_{rel} = k_{F^-}^{DNPP^-}/k_{F^-}^{MDNPP^-} = 4.3$). The accurate reproduction of this ratio, by means of reasonably accurate second-order rate constants, would give further credibility to our computational approach and its relevance in providing realistic free-energy profiles for OH^- attack on singly protonated phosphate monoesters. As in our previous work,[22] we selected the distance between the fluorine (or oxygen in OH^-) and phosphorus atoms as the reaction coordinate and chose the maximum of the corresponding enthalpy profiles instead of the free-energy profiles to localize the relevant transition-state structures (Tables S1–11, in the Supporting Information). Enthalpies of infinitely separated species F^- (or OH^-) + $DNPP^-$ and F^-(or OH^-) + $MDNPP^-$ were taken as reference points in these profiles.

The reported second-order rate constant for the attack of F^- on $DNPP^-$ ($k_{F^-}^{DNPP^-} = 1.3 \times 10^{-4}\,M^{-1}s^{-1}$ at 39°C)[47] represents a unique opportunity to explore the role played by the phosphate hydrogen atom in the observed kinetics of $DNPP^-$. Our geometry optimizations in the dielectric field ($\varepsilon = 78.39$) indicate that F^- can attack the phosphorus atom of $DNPP^-$ without capturing its proton only when the O–H bond of $DNPP^-$ is oriented opposite the attacking fluoride ion. Any other orientation of the proton at any reacting P–F distance greater than ≈ 2.3 Å invariably leads to proton transfer between F^- and $DNPP^-$. Additional calculations performed in the dielectric continuum show that proton transfer still occurs when two explicit water molecules are included in the system. Note that in the gas phase, this proton-transfer step is always observed regardless of whether the proton of $DNPP^-$ is initially oriented toward the attacking F^- or opposite to it (i.e., toward the oxygen atom of $DNPP^-$ that will become axially oriented upon reaching the transition-state structure). Proton transfer to F^- in solution is unexpected on the basis of pK_a values: 3.17 for HF, 4.5 for $DNPP^-$[17] ($\Delta pK_a = +1.33$). One could be tempted to attribute this result to errors in the continuum solvation model. However, further tests demonstrate that this is not the case. Indeed, by using the MPE model, one obtains $\Delta pK_a = +2.18$, which is in good agreement with the experimental quantity and, in particular, exhibits the correct sign. Rather, the explanation lies in the modification of the effective acidity/basicity strengths upon F^- attack on $DNPP^-$, which is necessarily accompanied by substantial ion desolvation. Thus, as the ions come close to each other, they are destabilized, the effect being particularly noticeable for the smaller fluoride anion whose effective basicity increases and becomes large enough to deprotonate $DNPP^-$. These calculations confirm our preliminary results,[22] namely, that the key structural feature of $S_N2(P)$ reactions between hard, charged nucleophiles and protonated phosphates is the *syn* orientation between the phosphate hydrogen atom and the leaving oxygen atom. Under these conditions, the energetically most favorable enthalpy profile found for F^- attack at the P atom of $DNPP^-$ yields the phosphorane-like transition state **TS1** depicted in Figure 1. This transition state is reached at a P–F distance of 2.56 Å coupled with an axial P–O4 bond of 1.87 Å, which drives the reaction directly to the products FP^- and $DNPO^-$. An interesting characteristic of **TS1** is that the O3–H3 bond is not precisely aligned with the axial P–O4 bond (O4-P-O3-H3 = 51.6°). The hydroxyl group of **TS1** preferably points toward the *ortho*-nitro group rather than toward the axially oriented P–O4 bond (**TS1a**, Figure S1 and Table S12 in the Supporting Information) because of the more favorable hydrogen-bond geometry between O5 and H3 than between O4 and H3. The other specificity of **TS1** originates from its resonance structures. Natural bond order (NBO) calculations[48] indicate that, as a result of a high degree of charge delocalization within the 2,4-dinitrophenyl framework, there is not a significantly large charge buildup on the leaving-group oxygen atom. Interestingly, the negative charge borne by this oxygen atom decreases on going from the transition state ($q_{O4} = -0.79$) to the 2,4-dinitrophenolate ion ($q_{O4} = -0.67$). As a result of the low charge buildup at the phenolate oxygen, P–O bond fission occurs without proton transfer to the leaving group.[49]

To the best of our knowledge, the activation parameters for the $F^- + DNPP^-$ reaction have not been published, and hence our calculated activation enthalpy cannot be directly compared to experiment. Nevertheless, as can been seen in Table 2, when the calculated activation enthalpy of **TS1** (16.9 kcal mol^{-1}) is combined with the consensus activation entropy ((-24 ± 5) cal mol^{-1} deg^{-1}), we obtain a corrected free energy of activation at 39°C ((24.4 ± 1.6) kcal mol^{-1}) that is in very good agreement with the experimentally determined value at the same temperature (23.8 kcal mol^{-1}).[50] A satisfactory agreement with experiment is also obtained when the phosphate hydrogen atom of $DNPP^-$ is replaced by the methyl group. The measured rate constant for the re-

Table 2. Relative B3LYP-MPE energies (ΔE), zero-point vibrational energies (ΔZPE), enthalpies (ΔH), entropies (ΔS), and free energies (ΔG and ΔG_{cor}) for the $S_N2(P)$ reactions of F^- and OH^- with $DNPP^-$ and $MDNPP^-$ with respect to the reactants separated at infinity in the solution phase.[a]

Species	ΔE	ΔZPE	ΔH	ΔS	ΔG[b]	ΔG_{cor}[c]
$F^- + DNPP^-$	0.0	0.0	0.0	0.0	0.0	0.0
TS1	17.5	0.1	16.9	−31.1	26.2	24.4
TS1*	12.6	−0.2	11.5	−30.9	20.7	19.0
$FP^- + DNPO^-$	−18.8	−0.9	−20.7	10.7	−23.9	
exptl[d]						23.8
$F^- + MDNPP^-$	0.0	0.0	0.0	0.0	0.0	0.0
TS2	16.9	0.0	16.3	−29.3	25.0	23.8
$MFP^- + DNPO^-$	−21.2	−0.4	−22.4	11.0	−25.7	
exptl[e]						24.7
$HO^- + DNPP^-$	0.0	0.0	0.0	0.0	0.0	0.0
TS3	14.4	1.0	15.7	−34.2	25.9	23.2
TS3*	9.4	0.0	8.8	−43.0	21.6	16.3
$H_2PO_4^- + DNPO^-$	−37.6	1.4	−37.3	7.2	−39.4	
$HO^- + MDNPP^-$	0.0	0.0	0.0	0.0	0.0	0.0
TS4	14.3	1.0	14.6	−30.0	23.5	22.1
$MP^- + DNPO^-$	−37.8	1.5	−37.5	5.8	−39.2	
exptl[e]						23.0

[a] Units are kcal mol^{-1} for energies and cal mol^{-1} deg^{-1} for entropies. [b] Calculated at 298 K according to the equation $\Delta G = \Delta H - T\Delta S$. [c] Calculated at 312 K according to the equation $\Delta G_{cor} = \Delta H - T\Delta S_{cor}$ by using the consensus activation entropy $\Delta S_{cor} = (-24 \pm 5)$ cal mol^{-1} deg^{-1} and neglecting the entropy change contribution contained in the SCRF computation (see text). [d] Experimental free energy of activation at 39 °C taken from ref. [47]. [e] Experimental free energy of activation at 39 °C taken from ref. [18].

action of fluoride and MDNPP$^-$, $k_{F^-}^{MDNPP^-} = 3.2 \times 10^{-5}\,M^{-1}\,s^{-1}$ at 39 °C,[18] corresponds to a free energy of activation of 24.7 kcal mol^{-1}. This value is in good accord with the calculated value derived from the transition-state structure **TS2** shown in Figure 1 (23.8 kcal mol^{-1}, Table 2). Thus, as observed experimentally, our calculations indicate that replacing the phosphate hydrogen atom of $DNPP^-$ with the methyl group is kinetically insignificant for the nucleophilic reaction of F^-at the P center.

A series of constrained geometry optimizations in which a phosphate hydrogen atom points toward the attacking OH^- nucleophile were used to support the SAC proposal.[8] It is therefore instructive to examine the energy error introduced by such an approximation in our case. We have carried out a calculation for a configuration in which the proton is directed towards F^- and the O3–H3 bond length is constrained at 0.95 Å, thus preventing proton transfer from $DNPP^-$ to F^-. Under these conditions, an energy maximum is reached at a significantly shorter P–F distance of 2.1 Å (**TS1***, Figure 1). The free energy of activation estimated in this way (19.0 kcal mol^{-1}, Table 2) is significantly underestimated. Indeed, it is almost 5 kcal mol^{-1} lower than both the experimentally determined value and the value computed by using a full transition-state optimization (**TS1**). This strongly suggests that the reacting configuration posited to support the SAC mechanism (associated with a crucial P-O-H···OH^- interaction) is an unsuitable mechanistic picture for F^- attack at the P center of $DNPP^-$ (Figure 2).

The same calculations performed with hydroxide show that this nucleophile attacks the phosphorus atom of

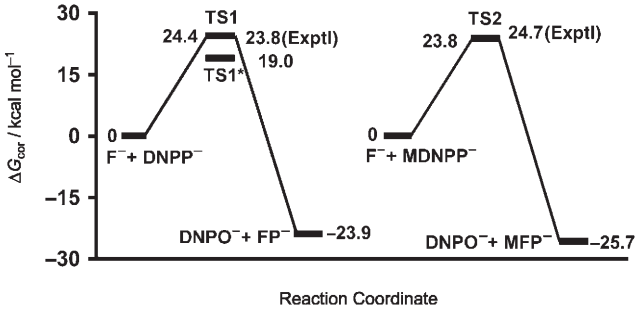


Figure 2. Calculated free-energy profiles in solution for the $S_N2(P)$ reactions of F^- with $DNPP^-$ (**TS1** and **TS1***) and $MDNPP^-$ (**TS2**).

$DNPP^-$ and $MDNPP^-$ in a very similar way as a fluoride ion. Both nucleophiles lead to similar transition-state structures (Figures 1 and 3) and activation free energies

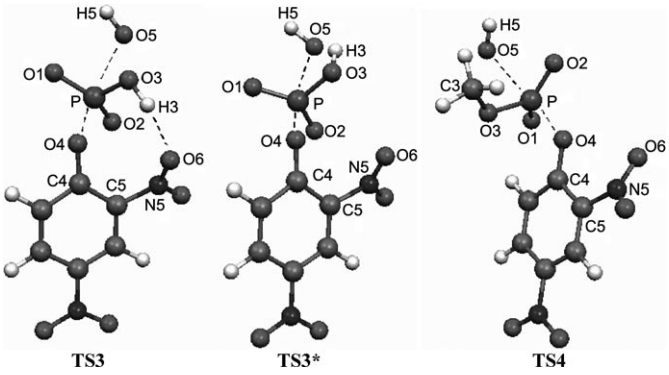


Figure 3. Solution-phase-optimized structures of transition states involved in the $S_N2(P)$ reactions $HO^- + DNPP^-$ (**TS3** and **TS3***) and $HO^- + MDNPP^-$ (**TS4**).

(Table 2). In line with previous results obtained with other phosphate esters,[22] MPE calculations excellently reproduce the reported free energy of activation for the hydroxide-catalyzed P–O cleavage reaction of $MDNPP^-$ (**TS4**, Table 2). However, the calculated free-energy barrier for OH^- attack on $DNPP^-$ (**TS3**, 23.2 kcal mol^{-1}) is considerably greater than the barrier required by the substrate-assisted catalysis mechanism ($\approx$10 kcal mol^{-1}, Figure 4). Further calculations performed with the phosphate hydrogen atom oriented toward the attacking hydroxide ion (with the O3–H3 bond length constrained at 0.95 Å to prevent proton transfer) indicate that even in this reacting configuration, the free energy of activation for the second step of the SAC mechanism (**TS3***, Table 2) would still be too high (by $\approx$6 kcal mol^{-1}) to be consistent with the observed rate constant for hydrolysis of $DNPP^{2-}$. Overall, these findings do not support

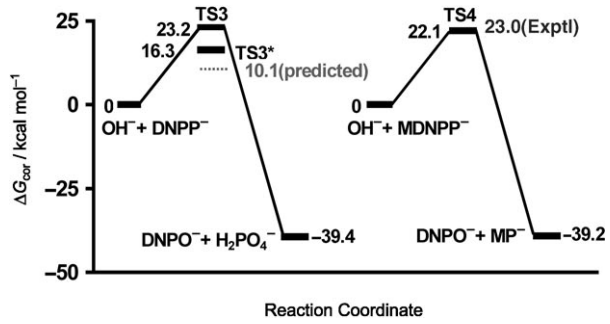


Figure 4. Calculated free-energy profiles in solution for the $S_N2(P)$ reactions of HO^- with $DNPP^-$ (**TS3** and **TS3***) and $MDNPP^-$ (**TS4**). The free-energy barrier required by the SAC mechanism to account for the overall hydrolysis rate of $DNPP^{2-}$ is indicated in gray as a dotted line.

the substrate-assisted catalysis mechanism for phosphate monoester dianions with highly activated leaving groups like 2,4-dinitrophenol. For these esters, our results indicate that the methyl group can indeed be used as a surrogate for protonation of the phosphate to aid in resolving kinetic ambiguities such as that depicted in Scheme 1.[52]

Hydrolysis of phenyl phosphate dianion: Could the SAC mechanism nevertheless apply to phosphate monoesters having less-activated leaving groups than the 2,4-dinitrophenol leaving group? The phenyl phosphate dianion (PP^{2-}) appears to be, to the best of our knowledge, the less-reactive phosphate monoester whose activation parameters have been experimentally determined.[14,53] It is therefore an interesting compound to use to address the validity of the SAC mechanism when a significantly more basic leaving group is involved, as in biological phosphate esters. In this regard, PP^{2-} can be considered as typical of phosphotyrosine residues. An Eyring plot obtained at very high temperatures indicates that the spontaneous hydrolysis of PP^{2-} occurs with $\Delta H^{\neq} = (37 \pm 1)\ \text{kcal mol}^{-1}$ and $\Delta S^{\neq} = (+7.3 \pm 0.6)\ \text{cal mol}^{-1}\,\text{deg}^{-1}$.[53] Extrapolating these data to 39 °C yields a pseudo-first-order rate constant, k_{obs}, of $3.2 \times 10^{-12}\ \text{s}^{-1}$, which is very close to the value predicted by the Brønsted correlation established forty years ago for the hydrolysis of activated dianionic species at 39 °C [Eq. (2)].[15] In Equation (2), pK_{lg} refers to the pK_a of the conjugate acid of the leaving group ($pK_{lg} = 9.95$ for PP^{2-}).

$$\log\left(k_{obs}\ [\text{s}^{-1}]\right) = -1.23\,pK_{lg} + 0.86 \qquad (2)$$

To account for this observed rate constant, the second-order rate constant $k_{OH^-}^{PP^-}$ of the second step of the SAC mechanism (Scheme 1B) must be $4.6 \times 10^{-4}\ \text{M}^{-1}\,\text{s}^{-1}$,[54] which corresponds to a free energy of activation, $\Delta G^{\neq}$, of $23.1\ \text{kcal mol}^{-1}$.[51] The calculated free energy of activation for the OH^- nucleophilic attack at the phosphorus atom of PP^- exceeds this value by $6\ \text{kcal mol}^{-1}$ (**TS5**, Table 3), which again argues against the substrate-assisted catalysis mechanism. However, the discrepancy found between the required and calculated free-energy barriers is significantly smaller for PP^- ($6\ \text{kcal mol}^{-1}$) than for $DNPP^-$ ($\approx 12\ \text{kcal mol}^{-1}$), which suggests that the SAC mechanism becomes more and more likely (in terms of free-energy barrier) as the basicity of the leaving group increases.

Table 3. Relative B3LYP-MPE energies (ΔE), zero-point vibrational energies (ΔZPE), enthalpies (ΔH), entropies (ΔS), and free energies (ΔG and ΔG_{cor}) for the $S_N2(P)$ reactions of OH^- with PP^- and MP^- with respect to the reactants separated at infinity in the solution phase.[a]

Species	ΔE	ΔZPE	ΔH	ΔS	ΔG[b]	ΔG_{cor}[c]
$HO^- + PP^-$	0.0	0.0	0.0	0.0	0.0	0.0
TS5	21.7	1.4	21.6	−39.6	33.4	29.1
TS5*	16.6	1.9	16.9	−39.4	28.6	24.4
$HO^- + MP^-$	0.0	0.0	0.0	0.0	0.0	0.0
TS6	22.6	1.2	22.4	−36.6	33.3	29.9
TS6*	27.7	0.9	26.8	−39.6	38.6	34.3

[a]–[c] See footnotes of Table 2.

The transition state **TS5** (Figure 5) is characterized by the axial P–O bond distances P–O5 = 2.4 Å and P–O4 = 1.80 Å. Structurally, **TS5** is very similar to **TS1** and **TS3**. In these

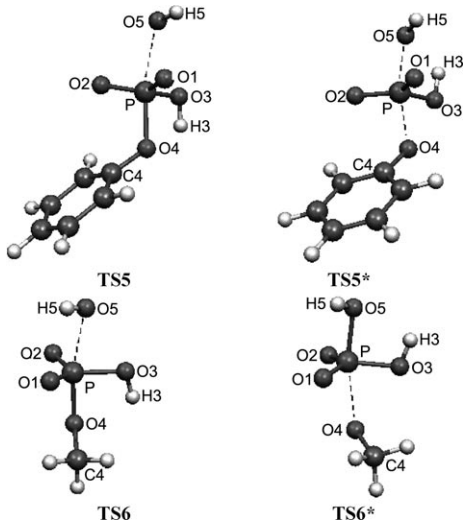


Figure 5. Solution-phase-optimized structures of transition states involved in the $S_N2(P)$ reactions $HO^- + PP^-$ (**TS5** and **TS5***) and $HO^- + MP^-$ (**TS6** and **TS6***).

structures the P–O bond lengths are roughly similar and, notably, the proton is attached to a phosphoryl oxygen atom. As in the 2,4-dinitrophenyl phosphate case, the charge buildup on the phenolic oxygen atom peaks in the transition state ($q_{O4} = -0.90$ in **TS5** vs. −0.86 in the phenolate ion) and P–O bond fission occurs without proton transfer to the leaving group.

To assess how the orientation of the phosphate hydroxyl group might affect the free-energy barrier, we extended our analysis to the hypothetical case in which OH^- would attack the P center of PP^- with the assistance of the P-O-H···OH^- interaction (again, with the PO–H bond kept at 0.95 Å to

prevent proton transfer). As observed with $DNPP^-$, we found that this reacting configuration leads to a significantly later transition state with the axial P–O bond lengths P–O5 = 2.0 Å and P–O4 = 1.87 Å (**TS5***, Figure 5). This structural change produces a stabilizing effect of 4.7 kcal mol^{-1} in free energy (compare the calculated free energies of **TS5*** and **TS5** in Table 3). Thus the stabilizing effect caused by the P-O-H···OH^- interaction in this system is noticeably less than that observed for OH^- attack on $DNPP^-$ (6.9 kcal mol). This difference certainly reflects the increasing requirement for the phosphate hydrogen atom to orient toward the leaving oxygen atom (to assist P–O bond scission) with increasing basicity of the leaving group.

Hydrolysis of methyl phosphate dianion: The hydrolysis of phosphate monoester dianions with alkoxy leaving groups is extremely slow in the absence of efficient catalysts.[13] In terms of leaving-group pK_a, the methyl phosphate dianion (MP^{2-}, pK_{lg} = 15.5) can be considered as an analogue of serine/threonine phosphates, the substrates of protein phosphatases. In an effort to evaluate the approximate rate enhancement produced by phosphatases, Lad et al.[14] have recently sought to establish the value of the pseudo-first-order rate constant for the spontaneous P–O cleavage of MP^{2-}. This study revealed that MP^{2-} actually reacts much more slowly than was previously reported.[56] By extrapolating rate data acquired at the limit of detection (over a range of very high temperatures in 1 M KOH), a rate constant of 2×10^{-17} s^{-1} was predicted at 39 °C. However, varying the KOH concentration in the range 0.05–1 M showed that the reaction was actually acid catalyzed, which meant that the monoanion form of the ester (MP^-) was still the kinetically active species. Thus, these data can only be used to establish an approximate upper limit for the reactivity of MP^{2-}. The estimated upper limit of 2×10^{-17} s^{-1} means that the corresponding free energy of activation should be at least 42.1 kcal mol^{-1}.[51] As these data are in fairly good agreement with those predicted by Equation (2) when pK_{lg} = 15.5 (k_{obs} = 6.2×10^{-19} s^{-1}, $\Delta G^{\neq}$ = 44.3 kcal mol^{-1}), it is tempting to speculate that aryl and alkyl phosphate dianions undergo hydrolysis by means of a similar reaction mechanism.

Our calculations show that the nucleophilic attack of hydroxide on the methyl phosphate monoanion (MP^-) proceeds as on $DNPP^-$ and PP^-, that is, by a concerted A_ND_N mechanism with the phosphate hydrogen atom oriented toward the leaving-group oxygen atom (**TS6**, Figure 5). Subtle differences, however, can be observed in terms of charge distributions as the reaction proceeds to the product state. In contrast to aryl phosphates, the charge buildup on the alkoxy oxygen atom of **TS6** ($q_{O4} = -0.95$) increases continuously in progressing to the methoxide ion ($q_{O4} = -1.0$). We performed a NBO analysis on various S_N2(P) reactions with protonated phosphate esters that reveals that proton transfer to the leaving group occurs only when the charge buildup on the leaving oxygen atom amounts to at least −0.96. We found that this situation arises in the course of the reaction only for alkoxy leaving groups, after the transition state is reached (viewed in the forward direction). Therefore, central to the SAC mechanism is that a proton resides on the transferred phosphoryl group in the transition state, regardless of the leaving-group pK_a. This picture clearly contrasts with the classical mechanism in which the transferred phosphoryl group resembles the metaphosphate monoanion.

In terms of the bonding between the incoming nucleophile and outgoing leaving group, **TS6** has, like **TS3** and **TS5**, the characteristics of an early associative transition state with relatively little bond formation to the nucleophile (P–O5 = 2.2 Å) and almost no bond fission to the leaving group (P–O4 = 1.775 Å). The calculated free-energy barrier associated with **TS6** is 29.9 kcal mol^{-1} (Table 3). When this value is combined with the free energy required to transfer a proton from H_2O to MP^{2-}, ΔG_{eq} = 12.8 kcal mol^{-1},[57] an overall free energy of activation of 40.2 kcal mol^{-1} (29.9 + 12.8 − 2.5)[59] is obtained for the substrate-assisted catalysis mechanism. This value is appreciably lower than the value predicted by using Equation (2) (44.3 kcal mol^{-1}), and is in satisfactory agreement with the experimentally estimated lower limit of 42.1 kcal mol^{-1} mentioned above. From these data and the associated uncertainties, we therefore conclude that there is no compelling evidence, based on overall reaction rates, that justifies the exclusion of the substrate-assisted catalysis mechanism for the spontaneous hydrolysis of alkyl phosphate dianions.

In summary, when applied to aryl phosphates, the substrate-assisted catalysis mechanism leads to hydrolysis rates that are clearly too slow to account for the observed rates (Figure 6). However, when applied to alkyl phosphates with a leaving-group p$K_a \geq \approx 13$, our calculations indicate that this mechanism leads to hydrolysis rates that are similar or

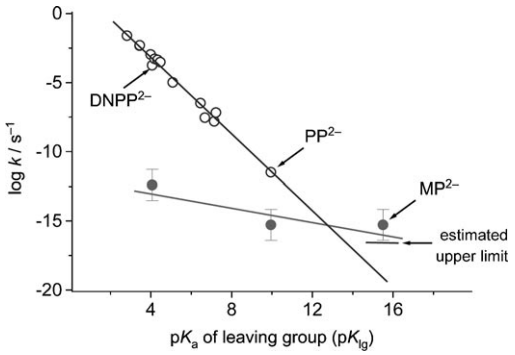


Figure 6. Comparison of the experimental and calculated linear free-energy relationship between leaving-group pK_a values and first-order rate constants for hydrolysis of phosphate monoester dianions at 39 °C. The experimental line (○) corresponds to that in ref. [15] and is described by Equation (2). The plot of the calculated first-order rate constants for the SAC mechanism versus pK_a of the leaving group (●) is described by the equation $\log(k\,[s^{-1}]) = (-0.25 \pm 0.14)pK_{lg} - (11.8 \pm 1.5)$. The error bars reflect the uncertainty associated with the consensus activation entropy ((-24 ± 5) cal mol^{-1} deg^{-1}) chosen to calculate the free-energy of activation values for the nucleophilic step of the SAC mechanism. The upper limit estimated by Wolfenden et al.[14] for the first-order rate constant for hydrolysis of the methyl phosphate dianion is also shown.

even greater than those predicted by the Brønsted correlation of Equation (2). Consequently, in the absence of rate data examining the kinetic effect of the leaving-group pK_a in the range 13–16, it will be difficult to conclusively determine which mechanism (if any) dominates the reaction of alkyl esters. The slope of the line correlating the log of the rate constants derived from the SAC mechanism with the pK_a of the leaving group (the Brønsted coefficient, β_{lg}) is calculated to be not larger than -0.25 (Figure 6). This weak dependence upon the pK_a of the leaving-group contrasts strongly with the strong Brønsted leaving-group dependence ($\beta_{lg}=-1.23$) found for the reaction of aryl phosphate dianions. Unfortunately, the determination of the Brønsted coefficient in the pK_{lg} region 13–16 appears to be unpractical as it requires rate measurements that are below the threshold of detectability.[13]

Kinetic studies on alkyl esters with a leaving-group pK_a of about 13 might provide a unique opportunity to assess the reactivity of an alkyl phosphate dianion. Would the reported activation parameters then allow a clear choice to be made between the mechanistic alternatives shown in Scheme 1? The near-zero values of entropies of activation reported for the hydrolysis of phosphate monoester dianions have been widely,[3,4] although not universally,[7] interpreted as being consistent with a transition state that is dissociative in nature, as that depicted in Scheme 1A. To be consistent with these entropy data, the SAC mechanism, on its part, requires that the preequilibrium proton-transfer step between H_2O and a phosphate dianion be associated with a significantly positive entropy change, $\Delta S_{eq}\approx+24\ cal\,mol^{-1}\,deg^{-1}$, so as to counterbalance the negative activation entropy of the subsequent bimolecular step (Table 1). The contribution made by specific solvation effects to the sign and overall magnitude of ΔS_{eq} is likely to be large, but difficult to predict. The activation parameters $\Delta H^{\pm}_{obs}$ ((50.1 ± 0.6) kcal mol^{-1}) and $\Delta S^{\pm}_{obs}$ ($(+23\pm1)\ cal\,mol^{-1}\,deg^{-1}$) reported recently for the hydrolysis of the methyl phosphate ester in 1 M KOH[14] offer an excellent opportunity to determine the enthalpic and entropic components of the free-energy change ΔG_{eq}. As already mentioned, the reaction that is observed in these conditions is specific acid catalyzed, and MP^-, not MP^{2-}, is the kinetically active species. Thus, the observed activation parameters in 1 M KOH can be expressed as shown in Equations (3) and (4):

$$\Delta H^{\pm}_{obs}=\Delta H_{eq}+\Delta H^{\pm}_{MP^-} \quad (3)$$

$$\Delta S^{\pm}_{obs}=\Delta S_{eq}+\Delta S^{\pm}_{MP^-} \quad (4)$$

in which $\Delta H^{\pm}_{MP^-}$ ($29.9\ kcal\,mol^{-1}$) and $\Delta S^{\pm}_{MP^-}$ ($-2.2\ cal\,mol^{-1}\,deg^{-1}$) are the activation parameters for the spontaneous P–O cleavage reaction of MP^-;[60] this yields $\Delta H_{eq}=20.2\ kcal\,mol^{-1}$ and $\Delta S_{eq}=+25.2\ cal\,mol^{-1}\,deg^{-1}$. From these values, ΔG_{eq} ($=\Delta H_{eq}-T\Delta S_{eq}$) is calculated to be $12.7\ kcal\,mol^{-1}$ at 25 °C, which is in excellent agreement with the value of $12.8\ kcal\,mol^{-1}$ derived from the difference in the pK_a constants of MP^- and water.[57] Thus, as previously suspected,[7] it is found that ΔS_{eq} can completely counterbalance the negative activation entropy associated with the nucleophilic step of the substrate-assisted catalysis mechanism. The near-zero values of entropies of activation reported for the hydrolysis of phosphate monoester dianions are therefore entirely consistent with this mechanism.

Implications for enzymatic phosphoryl-transfer reactions: Although the reactions of alkyl phosphate dianions have not been systematically studied, it has often been speculated that alkyl esters undergo phosphoryl transfer as their aryl counterparts, that is, by means of a concerted process with a loose metaphosphate-like transition state (Scheme 1A). However, the results obtained in this work suggest that alkyl phosphates might possibly react through an alternative, substrate-assisted mechanism with a phosphorane-like transition state, raising the possibility that mechanisms of the SAC type could be operational both in enzymes and in solution.

In the dielectric field, we have shown that hard nucleophiles such as OH^- and F^- can attack the phosphorus atom of a singly protonated phosphate monoester only if the phosphate hydrogen atom is oriented toward the leaving-group oxygen atom. According to our findings, this orientation of the proton has important implications with regard to the nature of the phosphorane-like transition state. If the phosphate hydrogen atom is oriented toward the leaving group, then the $S_N2(P)$ reaction proceeds through an early associative transition state (**TS3**, **TS5**, and **TS6**). In this reacting configuration, the hydrogen bond formed between the phosphate hydrogen atom and the leaving-group oxygen atom limits charge delocalization within the leaving-group framework.[61] As a consequence of this electrostatic interaction, the SAC mechanism in solution is characterized by a small β_{lg} value (-0.25).[62]

On the other hand, our calculations also predict that if the phosphate hydrogen atom points toward the nucleophile, then the SAC reaction should proceed through a late associative transition state (**TS3***, **TS5***, and **TS6***) characterized by substantial bond fission to the leaving group and substantial bond formation to the nucleophile. The Brønsted plot obtained in this case gives a β_{lg} value of -0.95 ± 0.12 (Figure 7), which means that, as noted earlier,[9] a large negative β_{lg} value is not necessarily inconsistent with an associative phosphorane-like transition state. This finding raises an important question: could this reacting configuration occur at the active site of phosphoryl-transfer enzymes?

The results obtained from the dielectric continuum suggest that this proton orientation becomes a likely alternative for the SAC mechanism if hydroxy nucleophiles of $pK_a\leq\approx10$ are involved. Examination of **TS3** and **TS5** in the reverse direction ($ArO^-+H_2PO_4^-\rightarrow OH^-+ArOPO_3H^-$) reveals that the nucleophilic attack at the P center of a protonated phosphate can take place without proton transfer even though the phosphate hydrogen atom is oriented toward the attacking phenoxide species $DNPO^-$ ($pK_a=4.07$) and PhO^- ($pK_a=9.95$), respectively. At the active site of nu-

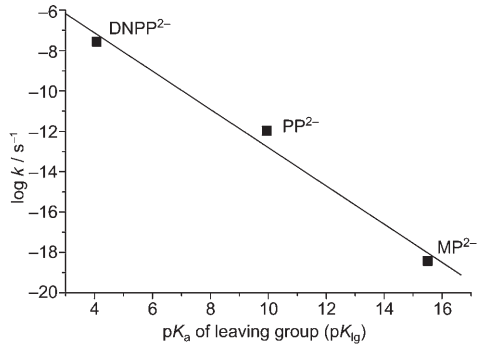


Figure 7. Calculated Brønsted plot for the spontaneous hydrolysis reaction of phosphate monoester dianions according to the SAC mechanism shown in Scheme 1B with the phosphate hydrogen atom oriented toward the attacking HO^- species (**TS3***, **TS5***, and **TS6***). The line ($R=-0.992$) is described by the equation: $\log(k\,[s^{-1}])=(-0.95\pm0.12)pK_{lg}-(3.3\pm1.3)$.

merous enzymes, the nucleophilic species that must be deprotonated for activity to occur usually have their negative charge strongly stabilized by positively charged amino acids or metal ions.[63] For instance, two zinc ions have been shown to simultaneously activate the Ser-102 nucleophile and stabilize the leaving group at the catalytic center of *Escherichia coli* alkaline phosphatase (AP).[64,65] The pK_a of the $Ser{-}O^-\cdots Zn^{2+}$ nucleophile in the E•S complex is estimated to be ≈ 7.[66,67] Thus, OH^- ($pK_a=15.7$), the nucleophile in the nonenzymatic SAC reaction, is a much stronger nucleophile than $Ser{-}O^-\cdots Zn^{2+}$ in the AP-catalyzed reaction. Therefore, it is likely that in the active site of enzymes, the SAC reaction proceeds through a late associative transition state with the phosphoryl proton oriented toward the nucleophile. In this reacting configuration, the β_{lg} value is calculated to be -0.95 ± 0.12 for the nonenzymatic SAC reaction. A similar β_{lg} value has been reported for the AP-catalyzed phosphate monoester hydrolysis ($\beta_{lg}=-0.85\pm0.13$).[67] Therefore, it appears that the SAC mechanism involving a late associative transition state is compatible with the reported β_{lg} value for AP. Thus, this parameter alone should not be interpreted in a unique way as it is found to be equally consistent with an associative or dissociative concerted mechanism.[7,68] Conversely, the determination of the Brønsted nucleophile coefficient (β_{nuc}), a measure of the role of the enzymatic nucleophile in the transition state, would allow a clear distinction between the two mechanistic alternatives. A small (positive) value of β_{nuc} would be consistent with a loose metaphosphate-like transition state, whereas a significantly larger value of β_{nuc} would reflect a late phosphorane-like transition state. Unfortunately, enzymatic studies directly assessing the degree of nucleophile bond formation in the transition state are scarce, leaving the picture of the transition state incomplete in many cases.[63]

Conclusions

In this work, we have applied quantum chemistry methods coupled with a continuum solvation model to evaluate the substrate-assisted catalysis mechanism recently proposed for the spontaneous hydrolysis of phosphate monoester dianions. Our results indicate that although this mechanism is unlikely to apply to aryl phosphates, it is a likely possibility for alkyl phosphate esters that have a leaving-group pK_a value greater than ≈ 13.

The substrate-assisted catalysis proposal consists of a preequilibrium proton transfer from water to the dianion, followed by hydroxide attack on the resulting monoanion. We have found that the latter reaction proceeds in a one-step process through an associative phosphorane-like transition state. However, we have also found that the orientation of the phosphate hydrogen atom relative to the attacking or leaving group has important implications with regard to the early or late nature of the transition state. Our calculations suggest that oxyanionic nucleophiles of $pK_a>11$ cannot attack the phosphorus atom of a protonated phosphate monoester if the phosphoryl proton points in their direction. Therefore, when the nucleophile is hydroxide (pK_a 15.7), the phosphate hydrogen atom must be oriented toward the leaving group. In this configuration, the $S_N2(P)$ reaction proceeds through an early phosphorane-like transition state characterized by little bond cleavage to the leaving group and relatively little bond formation to the nucleophile. The hydrogen bond formed between the phosphate hydrogen atom and the leaving-group oxygen atom intrinsically limits the sensitivity of the transition state to the basicity of the leaving group. Accordingly, a small negative β_{lg} value is predicted for the substrate-assisted hydrolysis reaction in aqueous solution.

An important conclusion that can also be drawn from the present study is that the nucleophilic reactions between charged nucleophiles and protonated phosphates may, under certain circumstances, take place with the phosphate hydrogen atom oriented toward the attacking group. According to our findings, this situation is likely to arise in aqueous solution if oxyanionic nucleophiles of $pK_a<11$ are involved. In this case, the reaction is predicted to proceed via a late phosphorane-like transition state involving a largely broken bond between the phosphorus atom and the leaving-group oxygen atom. In such a transition state, the phosphate hydrogen atom can no longer offset the charge accumulated at the position of bond cleavage, and a large negative value of β_{lg} is therefore predicted for the substrate-assisted catalysis mechanism. Because the nucleophilic character of RO^- species is usually much lower at the active site of phosphoryl-transfer enzymes than in aqueous solution, there may be enzymatic situations in which the substrate-assisted catalysis mechanism would preferably proceed through a late phosphorane-like transition state. Therefore, when large negative values of β_{lg} are reported in enzymatic phosphoryl-transfer reactions, care should be taken in their interpretation, especially when drawing conclusions about the associative/dissociative nature of the transition state.

To further support our conclusions, it is interesting to mention that the recent work by Klähn et al.[12] on related processes succeeded in reproducing the experimentally ob-

served linear free-energy relationships showing that the hydrolysis reaction becomes more dissociative with increasing acidity of the leaving groups.

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[51] The classical transition-state theory expresses the rate constant for a reaction as $k=(k_BT/h)\exp(-\Delta G/RT)$, in which k_B is the Boltzmann

constant, R is the gas constant, T is the absolute temperature, h is the Planck constant, and ΔG is the free energy of activation.

[52] According to our results, this is because the low pK_a of such leaving groups renders bond scission without proton transfer favorable. Because the proton has no significant catalytic activity to expel the leaving group, the methyl group appears as an appropriate substitute for it. This loss of the catalytic activity of the proton is reflected by the observation that when the leaving-group pK_a of aryl phosphates drops below $\approx$5.5, the dianion form reacts faster than the monoanion form.[15]

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