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Solvent Effects and Alkali Metal Ion Catalysis in Phosphodiester Hydrolysis

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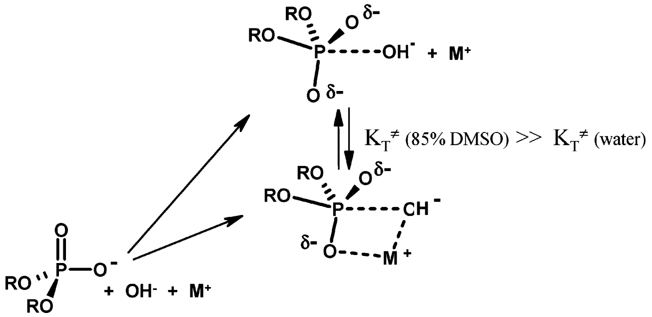

The kinetics of the alkaline hydrolysis of bis(*p*-nitrophenyl) phosphate (BNPP) have been studied in aqueous DMSO, dioxane, and MeCN. In all solvent mixtures the reaction rate steadily decreases to half of its value in pure water in the range of 0−70 vol % of organic cosolvent and sharply increases in mixtures with lower water content. Correlations based on different scales of solvent empirical parameters failed to describe the solvent effect in this system, but it can be satisfactorily treated in terms of a simplified stepwise solvent-exchange model. Alkali metal ions catalyze the BNPP hydrolysis but do not affect the rate of hydrolysis of neutral phosphotriester *p*-nitrophenyl diphenyl phosphate in DMSO-rich mixtures. The catalytic activity decreases in the order $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. For all cations except Na^+, the reaction rate is first-order in metal ion. With Na^+, both first- and second-order kinetics in metal ions are observed. Binding constants of cations to the dianionic transition state of BNPP alkaline hydrolysis are of the same order of magnitude and show a similar trend as their binding constants to *p*-nitrophenyl phosphate dianion employed as a transition-state model. The appearance of alkali metal ion catalysis in a medium, which solvates metal ions stronger than water, is attributed to the increased affinity of cations to dianions, which undergo a strong destabilization in the presence of an aprotic dipolar cosolvent.

Introduction

The catalytic role of metal ions in phosphodiester hydrolysis has been under extensive exploration due to the important role they play in enzyme active centers as well as the expectation of the development of practical efficient artificial catalysts. Extensive studies with model substrates have been accomplished with transition metal[1] and lanthanide complexes,[2] but very little has been done yet with s-block cations, alkali metal ions in particular, which nevertheless often serve as cofactors in hydrolytic and other metal enzymes.[3]

Alkali metal ions lack low-lying empty orbitals, so no coordination is expected with substrates or transition states. Significant catalytic effects were described only with specially designed substrates bearing fragments of crown ethers, which provide necessary affinity to the metal ion in organic solvents.[4] In water, small catalytic effects of alkali cations were observed only for reactions between highly charged anionic substrates, such as cyanometallates or heteropolyanions.[5] Also, modest

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catalysis by concentrated alkali metal ion solutions was described in the acylation of *o*-phosphate dianion by acetyl phosphate[6] and in the alkaline hydrolysis of methyl phosphate dianion.[7] Of course, much stronger electrostatic interactions are possible in media less polar than water. Large rate-enhancing effects of added salts were reported in organic solvents for reactions proceeding through highly polar transition states, e.g., S_N1 solvolysis reactions.[8] Recently, much attention has been directed to anhydrous methanol and ethanol as solvents for studies of the catalytic effects of alkali metal ions in the alcoholysis of phosphate esters of different structures.[9,10] Preassociation and multiple ion catalysis with Li^+, Na^+, and K^+ have been reported for phosphoryl transfer reactions in anhydrous ethanol[11] and for the ethanolysis of *p*-nitrophenyl diphenyl phosphate (NPDPP).[12] A role with specific geometrical constraints, rather than simple electrostatic shielding, has been suggested.[11] Importantly, ion pairing in these solvents, although much more significant than in water, is still not complete and there is a noticeable fraction of free cations and free alkoxo anions in solution. This allows one to quantify the catalytic effect of a metal ion by comparing the reactivities of free and metal-bound nucleophiles.[9−12]

It has been pointed out that a nonaqueous solvent may be considered as a model for the microenvironment of the active site groups in natural enzymes.[1e,13] Dehydration of the substrate and the active site groups is considered to be a very important factor in enzyme activity;[14] however, due to the chemical nature of proteins, this microenvironment with a low water content should be a rather polar medium. In particular, for metal enzymes the active site should be polar enough to allow the incorporation of metal ions from water. In this sense, a better model for the enzymatic microenvironment could be an organic-rich mixture of water and an organic solvent like DMF or DMSO, imitating the solvating properties of peptide groups. It should be noted that metal ions are spontaneously incorporated in proteins from water by a complex formation process accompanied by a negative change in free energy, whereas the transfer free energies of cations to alcohols are positive.[15] The increased free energy (a destabilization) of cations in alcohols is the reason for their higher tendency to form adducts with reactants and to serve as catalysts, but protein binding sites actually stabilize cations. Therefore, an interesting question is whether the catalytic activity of a metal ion can be improved by transferring it into a better solvating medium than water. Recently, we discovered a strong enhancement in the phosphodiesterolytic activity of alkaline earth cations in aqueous DMSO mixtures, which thermodynamically stabilize metal ions.[16] In this paper we report a catalytic effect of alkali metal ions on the hydrolysis of a model phosphodiester (BNPP) in aqueous DMSO as well as dioxane and acetonitrile mixtures.

During the course of this study we noticed that solvent effects on the alkaline hydrolysis of phosphodiesters had never been explored before, although solvent effects in reactions of monoesters[17] and triesters[18] were investigated in detail. Therefore, the first part of this paper is dedicated to a study of medium effects on the kinetics of alkaline hydrolysis of a model diester BNPP. For comparison, some measurements were performed also with a triester, NPDPP.

Results and Discussion

Kinetics of Alkaline Hydrolysis of Phosphate Esters in Aqueous Organic Mixtures. The second-order rate constants, k_{OH}, for BNPP alkaline hydrolysis in aqueous DMSO, acetonitrile, and dioxane are summarized in Table 1S (Supporting Information) and are shown as a function of water volume fraction in Figure 1. Qualitatively, the effects of all organic cosolvents are similar; as the volume percentage of an organic solvent increases, the second-order rate constants initially decrease by a factor of approximately 2, but above ca. 80% they sharply increase.

Increased reactivity of anionic nucleophiles, including hydroxide, in dipolar aprotic solvents is a well-known phenomenon principally attributed to nucleophile dehydration.[19] The increase in k_{OH} for conditions above 80% of the solvent is in line with this general rule. A quantitative treatment in terms of solvation energy changes requires the knowledge of the free energy of transfer, $\Delta G°_{tr}$, from water to the corresponding aqueous mixture for each of the involved species. There is not much literature data for the transfer of OH^- free energy from water to aqueous DMSO, acetonitrile, and dioxane mixtures at 25 °C (up to 80% organic solvent)[20,21] which are collected in Table 2S (Supporting

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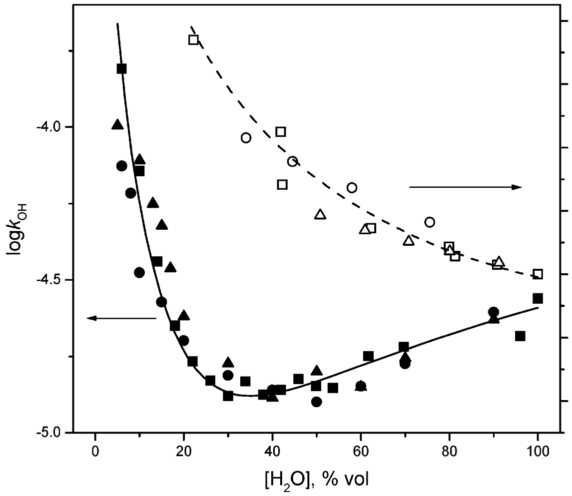

FIGURE 1. Logarithms of rate constants of alkaline hydrolysis of BNPP, 37 °C (solid symbols, left axis), and of activity coefficients of hydroxide anion, 25 °C (open symbols, right axis), vs volume fraction of water in mixtures with DMSO (squares), dioxane (triangles), or MeCN (circles). The solid line is the fitting curve to eq 3.

Information), but there are no transfer free energy data for BNPP or similar anions. Due to the highly dispersed negative charge on BNPP, the transfer free energy for this anion should be much smaller than that for hydroxide. However, the solvent effect in BNPP hydrolysis cannot be entirely attributed to the change in the free energy of hydroxide. The change in the activation free energy on going from water to a mixture with a maximum content of 95% organic cosolvent is about -5 kJ/mol, whereas ΔG°_{tr} for hydroxide anion reaches 43 kJ/mol in 80% solvent (Table 2S). This means that the transfer free energy of the transition state must be also a large positive number, which compensates for the main contribution of hydroxide destabilization. In addition, the logarithmic plot of the activity coefficients of hydroxide anions, $\log \gamma_{OH} = \Delta G^{\circ}_{tr}/2.3RT$, vs the amount of water in solvent mixtures shows an opposite trend to that of $\log k_{OH}$ in the range from 40 to 100% water, Figure 1 (open symbols).

In order to identify the factors ruling the observed medium effects on rate constants we analyzed the results in terms of the empirical solvent parameters reported in the literature for a large number of pure solvents and solvent mixtures. Attempts to correlate $\log k_{OH}$ with single parameter scales as Z[22] and E_T(30)[23] or single parameters for specific contributions (π^*,[24] α and β,[25] AN,[26] ETN, and BKT[27]) were unsuccessful. A multiparameter correlation with a set of parameters α, β, and π^* given in ref 28 was also unsuccessful. However, since these parameters were not available for all solvent mixtures employed, a similar more recent and more-complete scale was used.[29] This scale for solvent effects is dissected into three orthogonal parameters, basicity (SB), acidity (SA), and polarity/polarizability (SPP).[29a] For acetonitrile and dioxane mixtures our kinetic data can be satisfactorily fit to a linear combination of all three parameters in terms of eq 1, but for DMSO the fitting quality was poor.

$$\log k_{OH} = \log k_0 + a(SA) + b(SB) + p(SPP) \qquad (1)$$

The numerical values for the coefficients a, b, and p of eq 1 together with statistical parameters for correlations are given in Table 1, and the fitting results are illustrated graphically in Supporting Information, Figure 1S.

Although for individual solvent mixtures this treatment seems to be statistically satisfactory, the comparison of coefficients obtained from the fitting results for different mixtures clearly shows that they are physically meaningless. If each parameter truly reflects a certain contribution to solvation free energy of reactant and transition state originating from a certain type of intermolecular interactions, the respective coefficient must be the same for all mixtures. Instead, we see that contributions from basicity and polarity/polarizability can be either negative (dioxane and DMSO) or positive (MeCN) and the contribution from acidity, although always negative, varies by a factor of more than 5. Not unexpectedly, when eq 1 was applied to all experimental results for three solvent mixtures together, the correlation was unacceptably poor (Table 1, line 4).

If only the results for mixtures containing less than 80% v/v of organic solvent are considered, the correlation becomes quite satisfactory (Table 1, line 5). For this region the coefficient at SPP is statistically insignificant and the correlation equation takes the form

$$\log k_{OH} = \log k_0 + a(SA) + b(SB) \qquad (2)$$

A similar equation describes the solvent effect on the hydrolysis of the triester substrate NPDPP (see below), and its interpretation will be discussed later. The essential point is that the approach based on multiparametric correlation with empirical solvent parameters fails to describe the most important part of the solvent effect on BNPP hydrolysis, the reaction rate increase at low water content.

Bunton and co-workers studied solvent effects on the kinetics of alkaline hydrolysis of a triester NPDPP, a neutral substrate mechanistically related to BNPP, and observed that rate constants showed a minimum similar to that of BNPP[18a,b] upon the addition of acetonitrile and *tert*-butyl alcohol to water. They proposed that the reaction rate decreased on the addition of these organic solvents due to a decrease in the activity coefficient (stabilization) of the hydrophobic neutral substrate while changes in the activity coefficients of anionic nucleophile and transition states canceled out. We extended this study to the aqueous mixtures with dioxane and DMSO employed in this work and observed a similar behavior for the former solvent but no minimum for the latter (Table 3S in the Supporting Information and Figure 2).

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TABLE 1. Coefficients for Eq 1 Obtained by the Multiple Regression of Second-Order Rate Constants for the Alkaline Hydrolysis of BNPP and NPDPP in Aqueous−Organic Mixtures at 37 °C

substrate	cosolvent	log k_0	a	b	p	R^2	SD	F
BNPP	dioxane	0.25(1.1)	−0.97(0.4)	−2.2(0.4)	−3.9(1.5)	0.916	0.098	$<1 \times 10^{-4}$
	DMSO	21.8(5.8)	−6.6(1.3)	−8.2(1.6)	20.0(4.6)	0.731	0.168	5.2×10^{-4}
	MeCN	−16.6(4.8)	−1.5(0.1)	0.49(1.1)	14.2(5.0)	0.959	0.059	$<1 \times 10^{-4}$
	all data	−3.0(0.4)	−1.5(0.3)	−1.7(0.7)	0[a]	0.390	0.308	2.1×10^{-4}
	below 80%	−3.7(0.4)	−0.5(0.1)	−1.2(0.2)	0[a]	0.714	0.061	$<1 \times 10^{-4}$
NPDPP	dioxane	2.3(0.2)	−2.2(0.2)	−2.8(0.3)	0[a]	0.979	0.081	$<1 \times 10^{-4}$
	DMSO	2.3(0.6)	−2.2(0.4)	−1.6(0.8)	0[a]	0.939	0.138	$<1 \times 10^{-4}$
	MeCN[b]	1.8(0.5)	−1.8(0.4)	−4.5(0.6)	0[a]	0.941	0.094	8.5×10^{-4}
	dioxane and DMSO	2.3(0.5)	−2.2(0.4)	−2.0(0.8)	0[a]	0.775	0.258	$<1 \times 10^{-4}$

[a] The coefficient was statistically insignificant. [b] At 25 °C; experimental data from ref 18b.

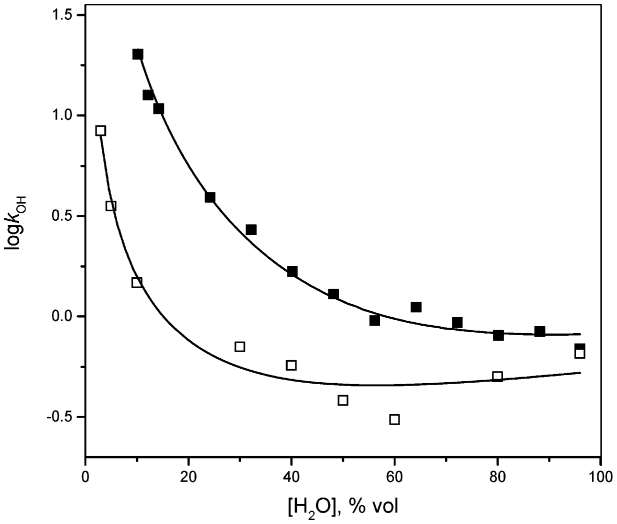

FIGURE 2. Logarithms of rate constants of alkaline hydrolysis of NPDPP at 37 °C vs volume fraction of water in mixtures with DMSO (solid squares) and dioxane (open squares). Solid lines are the fitting curves to eqs 4 and 5.

Evidently, all three solvents should stabilize the hydrophobic substrate, and the largest effect is expected for dioxane, possessing the smallest value of the solvophobic parameter (S_p = 1 for water, 0.2268 for DMSO, 0.2167 for MeCN, and 0.0794 for dioxane).[30] In fact, the minimum is most pronounced with MeCN as cosolvent (a 7-fold decrease in 60% MeCN)[18a,b] and is not observed with DMSO. Apparently, other factors are responsible for this effect.

Comparison of results in Figures 1 and 2 reveals generally similar tendencies for both substrates: reaction rates barely change or even decrease slightly on an increase of the organic cosolvent content from 0 to 60−70 vol % but then start to increase, and finally the reaction becomes 10−40 times faster in organic-rich media than in water. This similarity of solvent effects is in line with the similarity in mechanisms of hydrolysis of both substrates. However, the quantitative description of the solvent effects on the hydrolysis of diester and triester substrates requires a different approache.

The analysis of the solvent effect on NPDPP hydrolysis in terms of eq 1, in contrast to that of BNPP, provided an acceptable fit even for results in low water content mixtures. Table 1 lists the respective coefficients and statistical parameters, and Figure 2S (Supporting Information) illustrates the fit graphically. Within error limits, the independent term log k_0 and coefficient a coincide in DMSO and dioxane, and although the coefficient b is different for these solvents, it has at least the same sign. Parameter SPP was found to be insignificant for all three solvents. Probably because of different reaction conditions (25 °C instead of 37 °C), results for MeCN deviate from those in the two other solvents. Thus, the results for NPDPP actually fit eq 2, which also provides a satisfactory fit for BNPP in mixtures containing less than 80% v/v of organic solvent. The negative coefficient a, the contribution of SA, evidently reflects the hydroxide stabilization by hydrogen bonding to water. The negative coefficient b, the contribution of SB, is more difficult to interpret. However, it should be noted that the basicity parameter of SB = 0.025 assigned to pure water in Catalan's scale[29] is surprisingly low, much lower than the value of $\beta = 0.47$ for water.[28] It may therefore happen that the basicity contribution is strongly overestimated when this scale is applied to aqueous−organic mixtures.

Equation 1 fails to describe results for BNPP at high content of the organic cosolvent because k_{OH} for this substrate starts to increase rapidly in a narrow range of cosolvent concentration between 85 and 95 vol %, when all of the empirical parameters undergo very small changes. Obviously, in this range of solvent composition, changes in solvation of reactants and/or the transition state of the reaction do not follow the changes in solvation of the indicator molecules employed for measurement of the solvent parameters. Such incongruence in the preferential solvation of reacting species and indicators chosen as standards for the quantification of different types of intermolecular interactions in the solution is quite expectable and often leads to an unsatisfactory fit of kinetic data to an apparently universal eq 1.[31] Engberts et al. pointed out that, instead, one may obtain a more successful correlation simply with the molarity or proportional-to-molarity volume fraction (f_V) of water in the mixed solvent[31] ([H_2O] = 55.5f_V, the exact volume fraction of water was calculated from known volumes of mixed components and densities of pure liquids and respective mixtures). Such an approach is logical for BNPP since rate constants for the hydrolysis of this substrate principally depend on the volume fraction of water and are essentially independent of the type of organic cosolvent.

Figure 1 shows the plot of all results for BNPP as a function of volume fraction of water (solid symbols), which fits

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satisfactorily to the simple polynomial in eq 3, where $[H_2O]$ is the molarity of water.

$$k_{OH} = ((1.67 \pm 0.12) \times 10^{-3})[H_2O]^{-2} + ((4.5 \pm 0.3) \times 10^{-7})[H_2O] \quad (3)$$

The correlation with water molarity implies the participation of water as a stoichiometric component of the reaction elementary steps involving, e.g., preequilibrium changes in the solvation shells of reactants. A long time ago it was demonstrated that solvent effects on many equilibrium parameters measured in mixed solvents, such as ionic activity coefficients or ion pairing formation constants, can be successfully described by including the molar concentration of water in the expression for the equilibrium constant without inducing a change in the macroscopic dielectric constant of the medium.[32] Modern coordination or stepwise solvent-exchange models describe the transfer free energies of solutes to a mixed solvent in terms of the equilibrium exchange of solvent molecules in the coordination sphere of a solute.[33] For an anionic solute in aqueous mixtures with aprotic organic solvents, solvation by water should be much stronger than that by organic solvent molecules. Therefore, as an approximation one can consider the organic cosolvent as an inert diluent and use the water molar concentration as a single variable. From this point of view, eq 3 may be interpreted such that the acceleration of the reaction in organic-rich media represented by the $[H_2O]^{-2}$ term is due to the loss of two water molecules from the hydration shell of the hydroxide ion, which remains unchanged until this region due to the preferential solvation of hydroxide by water; a minimum of around 40% water is observed because of the existence of a linear in the $[H_2O]$ term, which reflects favorable reaction solvation of the substrate PO_2^- moiety by a water molecule. Since the total hydration number of hydroxide is 3,[34] the loss of two water molecules does not actually represent a complete desolvation of the nucleophile; however, we cannot find a simple explanation of why two and not, for example, just one water molecule is lost in the transition state.

This approach also works for the triester hydrolysis. The results in DMSO−water mixtures fit satisfactorily to eq 4, which involves the same $[H_2O]^{-2}$ term as that of BNPP hydrolysis but has no water catalysis for the reaction with the fully hydrated hydroxide ion.

$$k_{OH} = (610 \pm 30)[H_2O]^{-2} + (0.51 \pm 0.04) \quad (4)$$

In dioxane−water mixtures the best fit is observed with eq 5, which implies that in this solvent a monodehydrated hydroxide ion is the principal reactive species in organic-rich medium and the reaction is assisted by two water molecules in the water-rich medium. The fit to eqs 4 and 5 is illustrated in Figure 2.

$$k_{OH} = (7 \pm 5)[H_2O]^{-2} + (8 \pm 1)[H_2O]^{-1} + (0.00015 \pm 0.00004)[H_2O]^2 \quad (5)$$

The fact that the reaction with NPDPP is more sensitive to the nature of an organic cosolvent is in line with the expected stronger solvation of this neutral substrate as compared with the BNPP anion by the organic component of the mixture. For this reason a simplified description considering the organic cosolvent as an inert diluent is less appropriate for NPDPP hydrolysis. However, in the case of BNPP, the treatment considering water as a stoichiometric solvating component provides a better numerical and conceptual description of the solvent effect on the reaction rate than is possible to obtain by the use of empirical solvent descriptors.

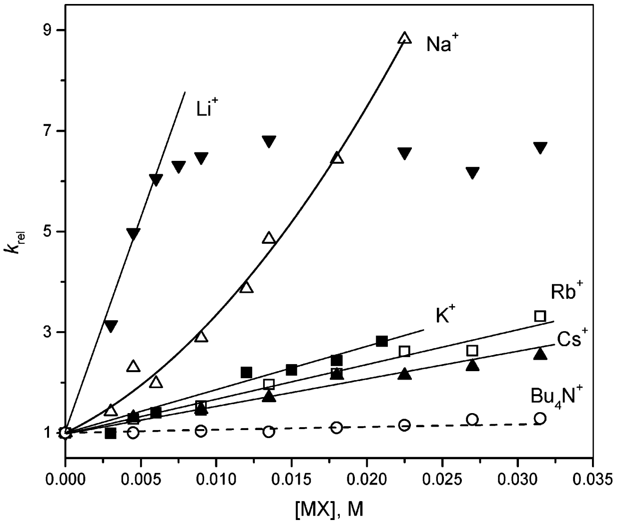

FIGURE 3. Salt effects on the BNPP hydrolysis in 85% v/v DMSO in the presence of 25 mM Bu_4NOH at 37 °C. Dashed line is the theoretically expected profile calculated from the Debye−Hückel equation.

Catalysis by Alkali Metal Ions. In aqueous solution, neutral salts can accelerate the BNPP hydrolysis due to a positive salt effect expected for a reaction between two similarly charged ions. Therefore, reaction rates were first measured in water in the presence of variable concentrations of different alkali metal and Bu_4N^+ salts including Bu_4NOH employed as a base. The observed rate constants increased as expected on increase in salt concentration (Figure 3S, Supporting Information), following very closely the "normal" salt effect predicted on the basis of the Debye−Hückel equation with a small positive deviation for LiCl at higher concentrations (addition of 0.15 M LiCl to a solution containing 0.05 M base enhances the reaction rate by 60% while other salts at the same concentration produce a 40% increase). Since the activity coefficients in more concentrated solutions do depend on the chemical nature of the electrolyte, such deviation is not unexpected. Salt effects created by sodium and tetrabutylammonium cations are the same, within experimental error. The k_{OH} value extrapolated to zero ionic strength equals 6.3×10^{-6} M^{-1} s^{-1} at 37 °C.

In aqueous organic solvent mixtures the situation is quite different. Kinetic studies were limited by the strongly decreased solubility of metal hydroxides in media with low water content. This effect was smaller in DMSO/water mixtures, but even in this case measurements were possible only below 90 vol % of DMSO. Figure 3 illustrates the effects of added salts on the BNPP hydrolysis in 85% DMSO; in this and following figures k_{rel} is the k_{obs} in the presence of added salt divided by the k_{obs} value measured in the absence of salt. Here, additions of low concentrations of salts, which essentially do not affect the reaction rate in water, produce much larger acceleration effects far surpassing those predicted by application of the Debye−Hückel equation shown as a dashed line (in this and other mixed

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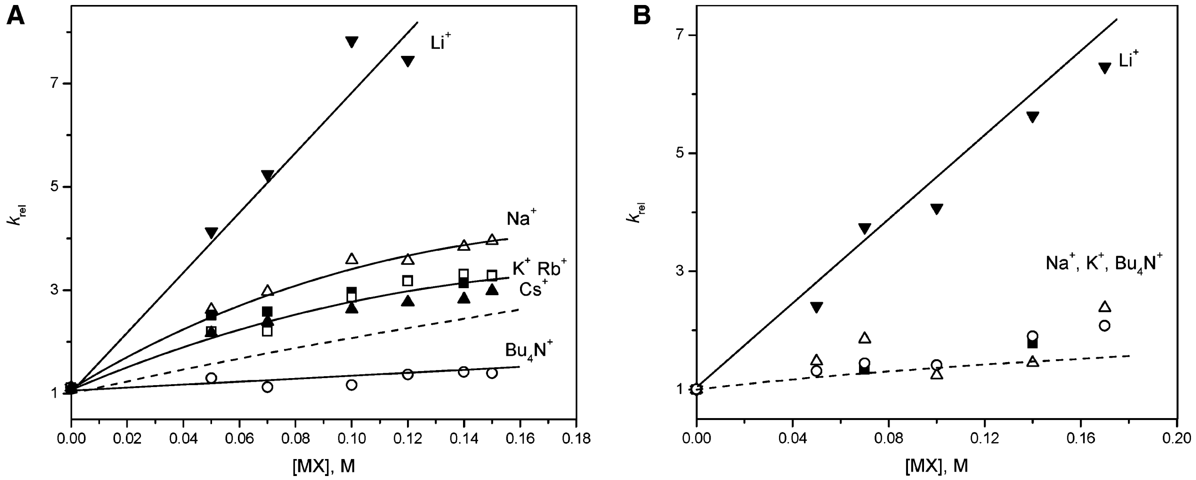

FIGURE 4. Salt effects on BNPP hydrolysis in 60% v/v dioxane (A) and 60% v/v MeCN (B) in the presence of 50 mM Bu_4NOH at 37 °C. Dashed line is the theoretically expected profile calculated from the Debye−Hückel equation.

solvents the Debye−Hückel parameters were corrected for the change in the dielectric constant in accordance with known theoretical expressions).[35] Also, there is a strong differentiation of salt effects, the reactivity order being $Li^+ > Na^+ > K^+ \sim Rb^+ \sim Cs^+ \gg Bu_4N^+$, and the effect of Bu_4N^+ being close to that predicted from the Debye−Hückel equation. Results with Li^+ show an unexpected "saturation" above 5 mM salt and that the effect of Na^+ is nonlinear. Both of these peculiarities will be discussed below.

The experiments with sodium salts of different anions (chloride, bromide, nitrate, and acetate) showed that with all salts the same rate acceleration was observed, indicating that the catalysis is due to the cation. Cation catalysis in BNPP alkaline hydrolysis was also confirmed by adding 18-crown-6 ether to a solution containing KCl and Bu_4NOH, which reduced the reaction rate to the level observed with Bu_4NOH alone (Figure 4S, Supporting Information). In order to see how the effect of alkali cations depends on the solvent composition, the effects of Na^+ and Li^+ were measured at increased amounts of DMSO. With both cations the effect starts to strongly increase above 80% DMSO, which corresponds to a 0.5 mol fraction of the organic cosolvent; already in the range of 50−60% DMSO, the effects of these cations significantly surpass those of water (Figure 5S, Supporting Information).

Measurements of salt effects in mixtures of water with dioxane and MeCN were limited by the too low solubility of alkali metal hydroxides in media containing more than 60 vol % of organic cosolvent. Results in 60% solvents are shown in parts A and B of Figure 4.

For the mixture with dioxane, with the lowest dielectric constant, $\epsilon = 3.5$, among all three solvent mixtures studied, the Debye−Hückel parameters and predicted salt effect have the largest values. Results in Figure 4A show that the effect of Bu_4NCl is even smaller than expected and only LiCl significantly surpasses the normal salt effect. In MeCN (Figure 4B), the effects of NaCl, KCl, and Bu_4NCl are close to the Debye−Hückel prediction and the effect of LiCl again is significantly higher. The effects observed in these mixtures cannot be quantitatively compared with those in 85% DMSO, which contains less water. However, one can see that in all cases the reduction in water content makes the effect of lithium salts stronger than others.

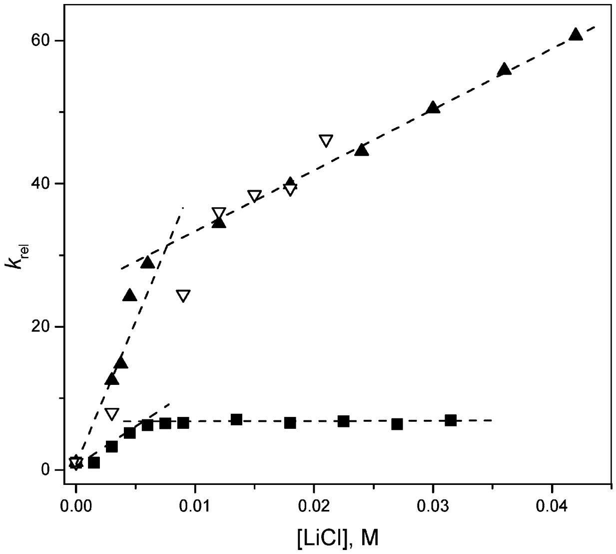

FIGURE 5. Effect of LiCl on the rate of BNPP hydrolysis at 37 °C in 85% v/v DMSO in the presence of 25 mM (solid squares), 6 mM (solid triangles), and 3 mM (open triangles) Bu_4NOH.

A possible reason for the "saturation" observed in the catalytic effect of LiCl in Figure 3 is a strong self-aggregation of LiOH above 5 mM. Indeed, at lower Bu_4NOH concentrations the catalytic effect is larger and the "saturation" is less pronounced (Figure 5).

This conclusion is confirmed by conductance measurements. In the concentration range below 0.02 M, the conductance of LiCl, NaCl, NaOH, Bu_4NCl, or Bu_4NOH in 80−90% DMSO is a linear function of the salt concentration. Figure 6S (Supporting Information) shows as an example results for LiCl and Bu_4NOH in 85% DMSO. There is no indication of ion pairing or any other type of self-aggregation of these electrolytes in the given concentration range. However, with LiOH the conductance increases linearly to approximately 0.005 M and then becomes independent of the electrolyte concentration. Such sharp change in the slope indicates the formation of neutral polymeric aggregates of the type $(LiOH)_n$ with a large aggregation number n reminiscent of a micellization process. When LiCl

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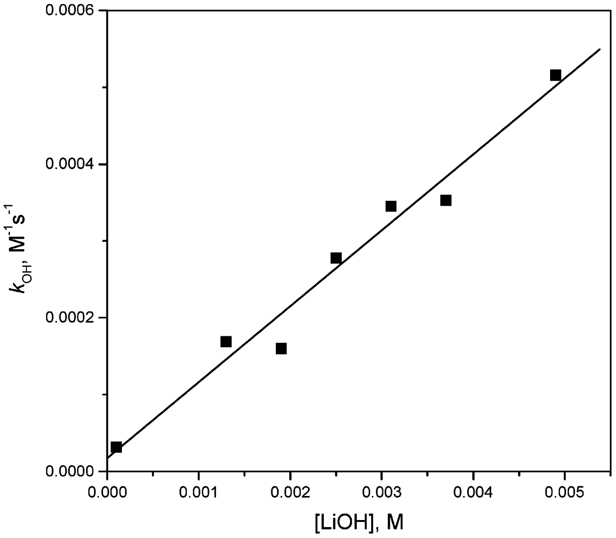

FIGURE 6. The second-order rate constant k_{OH} for BNPP hydrolysis in 85% aqueous DMSO at 37 °C by LiOH as a function of LiOH concentration.

TABLE 2. Third-Order Catalytic Rate Constants for BNPP Hydrolysis in the Presence of Alkali Metal Ions and Transition-State Binding Constants $K_T^{\neq}$ and Association Constants K_{ass} of Metal Ions with NPP Dianion in 85% DMSO[a]

cation	$k_{M,OH}$, M^{-2} s^{-1}	$K_T^{\neq}$, M^{-1}	K_{ass} (NPP^{2-}) M^{-1}
Li^+	0.104	3 150	740
Na^+	0.00452	137	150
Na^+	0.324[b]		
K^+	0.00254	77	46
Rb^+	0.00200	61	
Cs^+	0.00134	41	

[a] Relative errors in $K_T^{\neq}$ and K_{ass} ± 10%. [b] The rate constant is $k_{2M,OH}$ (M^{-3} s^{-1}) for the second-order reaction in the Na^+ reaction path.

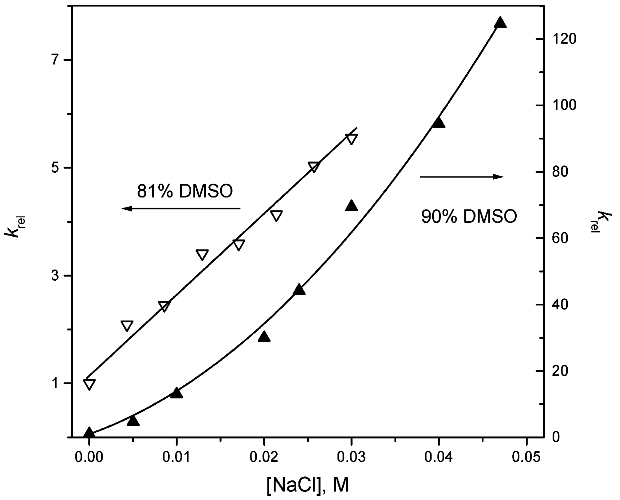

FIGURE 7. The effect of NaCl on the rate of hydrolysis of BNPP in the presence of 25 mM Bu_4NOH at 37 °C in 81% (open triangles, left scale) and 90% (solid triangles, right scale) DMSO.

is added to a 0.025 M solution of Bu_4NOH employed in the kinetic experiments of Figure 3, a very small increase in the conductance is observed (Figure 6S), indicating that the major portion of added Li^+ is incorporated in uncharged aggregates. In contrast to this, addition of NaCl to 0.025 M Bu_4NOH leads to an increase in the conductance with the same slope as the one observed for NaCl alone (Figure 6S), indicating the absence of ion pairing between Na^+ and OH^- under these conditions.

In view of strong self-aggregation of LiOH, the best way to study the catalytic effect of Li^+ in BNPP hydrolysis is to employ LiOH as a base at possibly low concentrations. Since the reaction is first-order with respect to OH^-, one can analyze the second-order rate constant k_{OH} calculated as k_{obs}/[LiOH] as a function of LiOH concentration in terms of the catalytic effect of the cation. Figure 6 shows that the plot of k_{OH} vs [LiOH] is linear with the intercept close to $k_{OH} = 3.3 \times 10^{-5}$ M^{-1} s^{-1} measured with Bu_4NOH as a base. Thus, the reaction is first-order in Li^+, and the slope of the line in Figure 6 gives the third-order rate constant $k_{M,OH} = 0.104 \pm 0.008$ M^{-2} s^{-1}.

Catalytic effects of K^+, Rb^+, and Cs^+ cations in 85% DMSO are linear in metal concentration (Figure 3). Therefore, for these cations one may calculate the third-order rate constant $k_{M,OH}$, similar to that for Li^+, which corresponds to eq 6

$$BNPP^- + OH^- + M^+ \xrightarrow{k_{M,OH}} \text{Products} \tag{6}$$

The values of $k_{M,OH}$ for all cations are collected in Table 2.

The catalytic effect of Na^+ in 85% DMSO fits eq 7, which involves both first- and second-order reactions in metal ion terms. The respective rate constants are given in Table 2.

$$k_{obs} = k_{M,OH}[Na^+][OH^-] + k_{2M,OH}[Na^+]^2[OH^-] \tag{7}$$

The second-order reaction in the metal ion reaction is observed only at sufficiently high content of DMSO in the solvent mixture. Figure 7 shows the effects of NaCl on the rate of BNPP hydrolysis at lower (81%) and higher (90%) DMSO content. In 81% DMSO the catalytic effect is smaller and linear within the same concentration range of the salt, with $k_{M,OH} = 0.0034$ M^{-2} s^{-1}. In 90% DMSO the contribution of the quadratic term is evident, and the respective rate constants equal $k_{M,OH} = 0.068$ M^{-2} s^{-1} and $k_{2M,OH} = 2.6$ M^{-3} s^{-1}. The ratio $k_{2M,OH}/k_{M,OH}$, which reflects the relative contribution of the second-order term, is approximately the same in 85 and 90% DMSO, but the catalytic effect in 90% solvent is 1 order of magnitude higher.

The addition of alkali metal or Bu_4N^+ chlorides caused no change in the observed rate constants for the NPDPP hydrolysis in aqueous dioxane (60%) and DMSO (85%). Even Li^+, the most active cation in BNPP hydrolysis, did not affect the reaction rate.

Comparing the catalytic effects of alkali cations in aqueous DMSO and in anhydrous ethanol,[9−12] one observes a similar reactivity order ($Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$) but also several important differences. First, the catalysis in ethanol is observed with both neutral phosphates or phopshinates and with the anionic 4-nitrophenyl phenylphosphonate. In aqueous DMSO the catalysis is observed only with the anionic substrate. Second, in the case of the anionic substrate in anhydrous ethanol, significant catalytic effects are observed at high metal ion concentrations in the 0.1−1.0 M range and high reaction orders in the cation are displayed: third for K^+, fourth for Na^+, and fifth order in the case of Li^+.[11] In aqueous DMSO the catalysis is already observed in dilute salt solutions and it is principally first-order in metal ion with a second-order reaction contribution only for Na^+.

To the best of our knowledge, this is the first time that the catalytic effect of alkali metal cations applied in the millimolar concentration range has been reported for a phosphodiester hydrolysis in solvents containing significant amounts of water mixed with another even more polar solvent. Alkali metal ion catalysis was unexpected in mixtures of water with DMSO, which solvates metal ions stronger than water. The observed reactivity order for metal ions is in agreement with electrostatic interactions dominating solvation changes when the cation is changed; neglecting the association of alkali metal cations with the substrate (see below), transition-state stabilization has a strong preference for smaller ions.

There are different ways to interpret the mechanism of catalysis. One possible approach is to consider alkali metal cations as templates accelerating the reaction between BNPP and hydroxide anions by bringing them together in an ionic associate. In line with this explanation is the lack of catalysis with neutral NPDPP. Indeed, although addition of DMSO to water stabilizes cations, at the same time it strongly destabilizes anions. As a result, the ionic association in DMSO becomes stronger than that in water.[36,37] It should be noted that the association of Li^+ and Na^+ with alkoxide anions in pure DMSO is extremely strong: equilibrium constants for the association of *tert*-butoxides of these cations equal 10^8 and 10^6 M^{-1}, respectively.[37] By analogy, one should expect also a very strong association of these cations with highly basic hydroxide anions. However, solvation of the alkoxide anion by just one alcohol molecule reduces these constants to the level of 100 M^{-1}, [37] and the presence of more than 10 vol % of water in DMSO should have an even stronger effect on ion pairing with hydroxide.

In order to estimate possible metal ion−substrate association, we attempted to measure the interaction of BNPP with Li^+ and Na^+ by ^1H and ^{31}P NMR titrations in 85% DMSO-d_6/D_2O (binding of Li^+ to phosphate esters in acetone induces detectable shifts in proton and phosphorus NMR spectra)[38] but did not observe any spectral changes in the concentration range of 0−0.06 M of LiCl or NaCl. The conductivity measurements (see above) also indicate the absence of ion pairing between hydroxide and metal ions at concentrations below ca. 0.02 M. These results do not exclude possible ionic association with equilibrium constants below ca. 10 M^{-1}, but failure to estimate the association constants makes it impossible to discuss the catalysis quantitatively in terms of this approach.

Instead of considering catalysis as a ground-state effect one may interpret it in terms of a transition-state stabilization. The transition state for the alkaline hydrolysis of BNPP is a dianion. The transfer free energy of dianions to DMSO is much more positive than that of monoanions; e.g., for SO_4^{2-}, it equals 131.3 kJ/mol,[39] whereas for a smaller but monovalent I^-, the value is only 10 kJ/mol.[40] Therefore, one may expect much stronger association of alkali metal ions with the dianionic transition state of BNPP hydrolysis than with monoanionic reactants or the monoanionic transition state of NPDPP hydrolysis. The quantification of the catalytic effect in terms of this approach is based on an estimation of the binding constant $K_T^{\neq}$ of the catalyst (metal ion) to the transition state of the noncatalyzed reaction (alkaline hydrolysis), which may be performed by application of the transition-state theory to a hypothetical cycle involving the reactants and transition states of both catalytic and noncatalyzed reactions.[41,42] Equation 8 is applied for the catalytic reaction proceeding through step 6.[1a]

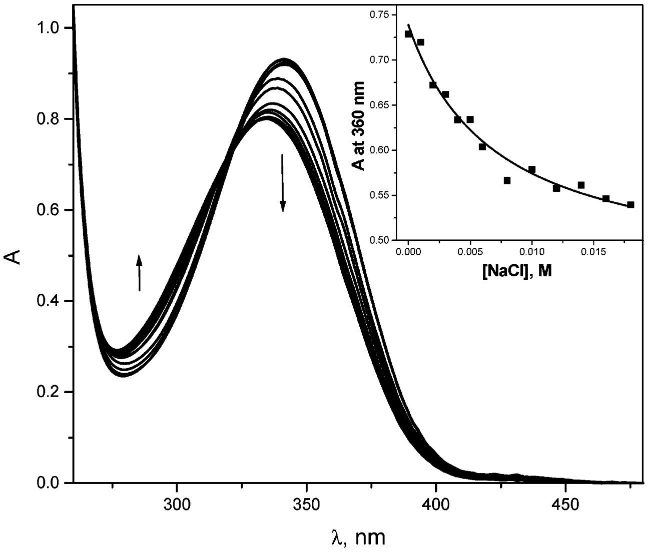

FIGURE 8. Spectrophotometric titration of NPP by NaCl in 85% DMSO at 37 °C. Arrows indicate the direction of spectral changes on addition of increasing amounts of NaCl (1−20 mM). Inset shows the fit of the titration results at 360 nm to eq 9.

$$K_T^{\neq} = k_{M,OH}/k_{OH} \qquad (8)$$

This approach was also applied for the analysis of alkali metal ion catalysis in anhydrous ethanol.[12]

Table 2 gives the values of $K_T^{\neq}$ for all cations for BNPP hydrolysis in 85% DMSO. It has been proposed that dianions of phosphate monoesters $ROPO_3^{2-}$ can be considered as crude models of dianionic transition states of the alkaline hydrolysis of phosphodiesters.[43] Following this idea, we spectrophotometrically measured the binding constants of alkali metal ions to $4\text{-}O_2NC_6H_4OPO_3^{2-}$ (NPP) in 85% DMSO . Figure 8 illustrates the results for titration of NPP by NaCl.

Addition of the salt induces a decrease in absorbance at 340 nm and a shift of the maximum to shorter wavelengths with an isosbestic point at 321 nm. Plots of the absorbance (A) at a fixed wavelength of 360 nm vs NaCl concentration followed simple hyperbolic equation (9) derived for a 1:1 complex formation process (A_0 is the absorbance of free NPP, and A_B is the absorbance of NPP bound to the metal ion M^+).[44] The inset in Figure 8 illustrates the fit of results at 360 nm.

$$A = (A_0 + K_{ass}[M^+]A_B)/(1 + K_{ass}[M^+]) \qquad (9)$$

Similar behavior was observed with lithium and potassium salts (Figures 7S, 8S, Supporting Information). The binding constants for all three cations are given in Table 2.

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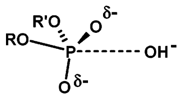
The comparison of $K_T^{\neq}$ and K_{ass} shows that in general the values of calculated transition-state binding constants are consistent with interactions of alkali metal ions with a dianionic phosphate species. In fact, the salt effect observed in water also can be treated in terms of direct binding of cations to the transition state of the alkaline hydrolysis reaction.[41] The respective $K_T^{\neq}$ values are numerically equal to the slopes of the plots of k_{rel} vs metal ion concentration given in Figure 3S (Supporting Information). Such an estimate gives a value of $K_T^{\neq} = 4.3 \pm 0.3$ M^{-1} for Li^+ and 2.6 ± 0.5 M^{-1} for Na^+. These values are remarkably close to the stability constants of complexes of Li^+ and Na^+ with HPO_4^{2-} in water equaling 5.2 and 4.0 M^{-1}, respectively.[45]

A discussion of a possible mode of metal ion binding to the transition state requires at least an approximate structural model of the latter. Recent high-level quantum mechanical calculations of the structures of the transition states for alkaline hydrolysis of phosphodiesters[46] show that the entering hydroxide forms a rather extended bond with phosphorus, which exceeds by ca. 0.7 Å the length of a normal P−O bond. At the same time, the bond length with the leaving group extends by only 0.1 Å and both P−O bonds in the phosphoryl group undergo a very small extension of less than 0.02 Å. Schematically, the transition state can by presented as **1**.



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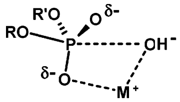

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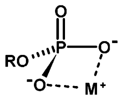

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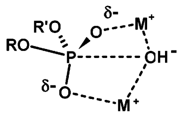

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These structural features indicate that the degree of bonding with the entering hydroxide in the transition state is rather small and the negative charge is principally retained on the entering hydroxide ion. This makes it the most plausible site for the metal ion binding. An additional stabilization may be gained through chelation to one of the phosphoryl oxygens, as illustrated in structure **2**. A similar mode of alkali metal ion transition-state binding was proposed in ref 12 on the basis of quantum mechanical calculations using a much simplified transition-state model in the form of an anionic phosphorane $H_3PO_2(OH)^-$. The structure **3** illustrates the complexation with a phosphate monoester dianion used as a transition-state model. A possible binding mode for the second sodium cation is shown as **4**.

In conclusion, the appearance of alkali metal ion catalysis in a medium, which stabilizes metal ions stronger than water, can be attributed to the increased affinity of cations to dianions, which undergo even stronger destabilization in the presence of an aprotic dipolar cosolvent. The transition-state binding formalism serves very well in this case even with such a simple transition-state model as the $ROPO_3^{2-}$ species; similar $K_T^{\neq}$ and K_{ass} values, within an order of magnitude, are observed in both water and a mixed solvent. This is probably due to the purely electrostatic nature of binding to alkaline metal ions, which primarily depend on the charge density on interacting atoms and much less on the three-dimensional structure of interacting species. For example, in the case of Zn(II) complexes used as catalysts for the phosphodiester hydrolysis, the affinity to the transition state of BNPP hydrolysis is ca. 3 orders of magnitude higher than the affinity to phosphate monoester dianions.[1a] In general, the results of this study indicate a specific role of a polar medium with low water content, which allows even very weak Lewis acids to efficiently promote the reaction with an anionic nucleophile, as may occur in biological systems.

Experimental Section

Chemicals. Alkali metal salts, sodium, and tetrabutylammonium hydroxide were used as supplied. DMSO of spectroscopic grade was used fresh after fractional distillation under vacuum and drying over molecular sieves.[47] Dioxane and acetonitrile were used fresh after distillation and drying over molecular sieves. BNPP was recrystallized from water in acidic media; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.34 (d, 2H, $J = 9.3$ Hz), 8.162 (d, 2H, $J = 9.3$ Hz). *p*-Nitrophenyl diphenyl phosphate (PNPDPP) was synthesized and purified as previously described in the literature (mp 50 °C; the yield of 4-nitrophenol was spectrophotometrically determined after complete hydrolysis of a sample of PNPDPP was 99 ± 1% of the theoretically calculated value).[48] Distilled and deionized water was used in all experiments.

Kinetics. Kinetic measurements were performed using a diode array spectrophotometer equipped with a multicell transport unit and thermostated at ±0.1 °C with a recirculating water bath. Reaction solutions were prepared by combining the appropriate amounts of solvent, water, and aqueous salt stock solutions, and reactions were initiated by adding an aliquot of the substrate solution. The course of BNPP and PNPDPP cleavage was spectrophotometrically monitored by the appearance of the 4-nitrophenolate anion. Since the 4-nitrophenolate molar absorptivity and maximum absorption wavelength were changed with media composition, a standard addition of 4-nitrophenol was applied to each sample to determine the molar absorptivity under given conditions. The standard 1 M aqueous solution of tetrabutylammonium hydroxide was used as the source of hydroxide ions. Pseudo-first-order conditions with base concentrations (5−50 mM) at least 25 times greater than substrate concentration (0.01−0.02 mM) were used. Under these conditions the concentration of free hydroxide was considered equal to the total Bu_4NOH concentration. The observed first-order rate constants (k_{obs}) were calculated by the integral method or, for slow reactions, from initial rates. Second-order rate constants for k_{OH} were obtained from k_{obs} vs $[OH^-]$ profiles.

Conductance Measurements. Unless otherwise specified, conductances were measured at 25.0 ± 0.1 °C using a dip-type cell with lightly platinated electrodes and a cell constant of $K = 1.00 \pm 0.01$ cm^{-1}, connected to a conductance meter. All conductivity measurements were repeated at least twice to get concordant results.

Spectrophotometric Titrations. The binding of alkali metal cations to NPP was studied in 85% DMSO at 37 °C by UV absorption spectrophotometry. Aliquots of the alkali cation stock solution were added to a 0.08 mM NPP solution containing 5 mM Bu_4NOH to ensure complete deprotonation of the NPP dianion. The concentration of alkali metal salt ranged from ca. 0.6 to 20 mM (except for lithium, where the maximum concentration was 7.0 mM). The binding constants, represented by K_{ass}, were calculated from the changes of absorbance in the range of 340−

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380 nm at five different wavelengths and averaged. The degree of NPP hydrolysis during the titration experiment was less than 1%, and the appearance of 4-nitrophenolate anion did not interfere with titration results.

Acknowledgment. The financial support of DGAPA-UNAM (Project IN 204805) is gratefully acknowledged.

Supporting Information Available: Tables of rate constants for BNPP and NPDPP hydrolyses in aqueous−organic mixtures; table of transfer free energies for OH^-; illustrations of fitting to multiparameter eqs 1 and 2; figures showing salt effects on BNPP alkaline hydrolysis in water, effects of 18-crown-6 ether on the BNPP hydrolysis in the presence of KCl, effects of NaCl and LiCl on BNPP hydrolysis at variable DMSO content, conductance measurements in 85% DMSO, and spectrophotometric titrations of NPP by LiCl and KCl. This material is available free of charge via the Internet at http://pubs.acs.org.

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