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(31) Assuming that the monoanion leaving-group β is also -0.27 at 39 °C.
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Metal-Ion Catalysis of Phosphoryl Transfer via a Ternary Complex. Effects of Changes in Leaving Group, Metal Ion, and Attacking Nucleophile

Chih-Min Hsu and Barry S. Cooperman*

Contribution from the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19174.

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Abstract: Our earlier work[2a] showing rapid phosphoryl transfer in the ternary complex pyridine-2-carbaldoxime–Zn^{2+}–phosphorylimidazole is here extended by studies of the effects of (a) substituting Ni^{2+} for Zn^{2+}, (b) substituting a series of phosphoramidates for phosphorylimidazole, and (c) substituting pyridine-2-aldehyde hydrazone for pyridine-2-carbaldoxime. The results with Ni^{2+} are qualitatively similar to those obtained previously with Zn^{2+} and allowed a clearer demonstration of the need for a protonated imidazole ring in the reactive ternary complex. The rates of transfer for a series of phosphoramidates permit calculation of a Bronsted leaving-group β which is similar to that obtained for simple phosphoramidate hydrolysis. No transfer to the hydrazone is detected. These results are interpreted as showing that the catalysis of phosphoryl transfer by Zn^{2+} results from both charge-shielding and template effects and that Zn^{2+} has little direct effect on the phosphoryl group itself.

Introduction

Phosphoryl-transfer reactions are among the most common in biological systems. The enzymes catalyzing these reactions generally require divalent metal ions for activity. In the last decade a great deal of effort has been directed toward understanding the mechanism of phosphoryl transfer[1] and elucidating the possible catalytic roles of metal ions in the reaction.[2] Several recent reviews have appeared summarizing work in this area.[1,3]

A previous publication from this laboratory reported on the Zn^{2+}-requiring phosphoryl transfer from phosphorylimidazole (PIm) to pyridine-2-carbaldoxime (PCA) anion and provided evidence that this transfer proceeded via the ternary complex PCA–Zn^{2+}–PIm.[2a] The importance of this reaction was that it represented a clear example of anionic oxygen attack on a phosphoryl dianion, a reaction which though common enzymatically had been difficult to demonstrate in model systems. This paper extends our earlier work by measuring the effect of varying the leaving-group amine on the rate of phosphoryl transfer. In addition, the effects of substituting Ni^{2+} for Zn^{2+} and pyridine-2-aldehyde hydrazone (PAH) for PCA have also been determined.

Experimental Section

Materials. Calcium phosphorylimidazole (PIm),[2a] *N*-methyl phosphorylimidazole (*N*-MePIm),[2a] potassium ammonium phosphoramidate (PA),[4,5] potassium *N*-(phenethyl)phosphoramidate (*N*-(phenethyl)PA),[6] potassium *N*-(benzyl)phosphoramidate[6] (*N*-(benzyl)PA), and pyridine-2-aldehyde hydrazone (PAH)[7] were prepared as previously described. *N*-(*n*-Butyl)phosphoramidate (*N*-(*n*-butyl)PA) was a gift of Professor S. J. Benkovic. Pyridine-2-carbaldoxime (PCA) and *N,N'*-dimethylpiperazine were obtained from Aldrich and the latter was redistilled before use. $ZnSO_4 \cdot 7H_2O$, $NiCl_2 \cdot 6H_2O$, and KCl were Baker Analyzed Reagents. 2-[*N*-Morpholino]ethanesulfonic acid (Mes) was obtained from Sigma.

Kinetic Procedures. Rates of phosphoryl transfer from phosphoramidates to Zn^{2+}–PCA and Ni^{2+}–PCA were measured by a modification of the modified Martin–Doty procedure of Jencks and Gilchrist, as described previously.[2a] Reaction mixtures were generally 0.6 mM in the phosphoramidate under study and were brought to an ionic strength of 0.5 with KCl. Temperature variation was within ±0.1 °C. The pH of reaction mixtures was measured before and after the kinetic runs and never varied by more than ±0.1 pH unit. All kinetic runs obeyed first-order kinetics. Reactions were generally followed for 2 half-lives and at least six points were taken per run. All rate constants were measured at least twice. Reproducibility was ±5%. Measurements of pH at 10 °C were by a standard procedure.[8]

Table I. Relevant Equilibria

	Measured values	Values used in calculations[a] 29.2 °C	10 °C
$PCAH_2^+ \rightleftarrows PCAH + H^+$	1.5×10^{-4} M[b]	1.5×10^{-4} M	4×10^{-5} M
$PCAH \rightleftarrows PCA^- + H^+$	9×10^{-11},[c] 7×10^{-11} M[d]		
$PImH^- \rightleftarrows PIm^{2-} + H^+$	1×10^{-7},[e] 1.6×10^{-7} M[d]	1.12×10^{-7} M	
$PAH^- \rightleftarrows PA^{2-} + H^+$	7.1×10^{-9} M[f]		
N-(benzyl)$PAH^- \rightleftarrows N$-(benzyl)$PA^{2-} + H^+$	1.4×10^{-9} M[f]		
N-(phenethyl)$PAH^- \rightleftarrows N$-(phenethyl)$PA^{2-} + H^+$	7.2×10^{-10} M[f]		
N-(n-butyl) $PAH^- \rightleftarrows N$-(n-butyl)$PA^{2-} + H^+$	1.1×10^{-10} M[f]		
Zn^{2+}-$PCAH \rightleftarrows Zn^{2+}$-$PCA^- + H^+$	3×10^{-7},[c] 1×10^{-6} M[g]	1×10^{-6} M	7.8×10^{-7} M
Ni^{2+}-$PCAH \rightleftarrows Ni^{2+}$-$PCA^- + H^+$	1.5×10^{-6},[h] 3.6×10^{-6} M[i]	3.6×10^{-6} M	
$PCAH + Zn^{2+} \rightleftarrows Zn^{2+}$-PCAH	150 M^{-1} [c]	150 M^{-1}	340 M^{-1}
$PCAH + Ni^{2+} \rightleftarrows Ni^{2+}$-PCAH	8600 M^{-1} [h]	8600 M^{-1}	

[a] Where appropriate, values used in calculations were estimated from measured values using known ΔH values for similar processes. [b] This work, $\mu = 0.5$, 25 °C. Determined by spectrophotometric titration at 295 nm. [c] 25 °C, see ref 9. [d] $\mu = 0.5$, 40.1 °C, see ref 2a. [e] $\mu = 1.0$, 39 °C, see ref 10. [f] $\mu = 0.2$, 20 °C, see ref 6a. [g] $\mu = 0.5$, 25 °C, see ref 2a. [h] 25 °C, see ref 11. [i] This work, $\mu = 0.5$, 25 °C. Determined by spectrophotometric titration at 315 nm.

Equilibrium constants relevant to this work are summarized in Table I.

Results

Rates of phosphoryl transfer from several phosphoramidates to Zn^{2+} and Ni^{2+} complexes of PCA were measured as a function both of pH and M^{2+}-PCA concentration. In all experiments the total metal-ion concentration ($[M^{2+}]_T$) was always equal to the total PCA concentration ($[PCA]_T$). The concentration of the M^{2+}-PCA complex was calculated from eq 7 in Scheme I. M^{2+}-PCA was always in large excess over

Scheme I. Phosphoryl Transfer from Phosphoramidates to M^{2+}-PCA

$$\begin{bmatrix} PCAH_2^+ \\ \Updownarrow K_{H_2A} \\ PCAH \end{bmatrix} + M^{2+} \xrightleftharpoons{K_{M_{app}}} \begin{bmatrix} M^{2+}\text{-}PCAH \\ \Updownarrow K_{HAM} \\ M^{2+}\text{-}PCA^- \end{bmatrix} \quad (1)$$

$$\begin{bmatrix} M^{2+}\text{-}PCAH \\ \Updownarrow K_{HAM} \\ M^{2+}\text{-}PCA^- \end{bmatrix} + \begin{bmatrix} R_2NH^+PO_3^{2-} \\ \Updownarrow K_{HNP} \\ R_2NPO_3^{2-} \end{bmatrix} \xrightleftharpoons{K_{A_{app}}} \begin{bmatrix} H_2X^+ \\ \Updownarrow K_{H_2X} \\ HX \\ \Updownarrow K_{HX} \\ X^- \end{bmatrix} \quad (2)$$

$$HX \xrightarrow{k} M^{2+}\text{-}PCAP + R_2NH \quad (3)$$

$$\text{rate} = k_{obsd}[R_2NHPO_3]_T \quad (4)$$

$$k_{obsd} = \frac{k/f_1}{1 + \dfrac{1}{K_{A_{app}}[M^{2+}\text{-}PCA]_t}} \quad (5)$$

$$k_{obsd} = \frac{k/q}{1 + (a_{H^+}/K_I) + (K_{II}/a_{H^+})} \quad (5a)$$

where

$$K_{M_{app}} = [M^{2+}\text{-}PCA]_t/[M^{2+}][PCA]_t = K_M \frac{1 + (K_{HAM}/a_{H^+})}{1 + (a_{H^+}/K_{H_2A})} \quad (6)$$

$$[M^{2+}\text{-}PCA]_t = [M^{2+}\text{-}PCAH] + [M^{2+}\text{-}PCA^-] = [M^{2+}]_T - \frac{\sqrt{1 + (4K_{M_{app}}[M^{2+}]_T)} - 1}{2K_{M_{app}}} \quad (7)$$

$$[M^{2+}]_T = [PCA]_T = [M^{2+}] + [M^{2+}\text{-}PCA]_t = [PCA]_t + [M^{2+}\text{-}PCA]_t \quad (8)$$

$$[PCA]_t = [PCAH_2^+] + [PCAH] \quad (9)$$

$$K_M = [M^{2+}\text{-}PCAH]/[M^{2+}][PCAH] \quad (10)$$

$$K_{A_{app}} = [X]_t/[M^{2+}\text{-}PCA]_t[R_2NHPO_3]_t = \frac{f_1 K_A}{(1 + (a_{H^+}/K_{HAM}))(1 + (K_{HNP}/a_{H^+}))} \quad (11)$$

$$[X]_t = [H_2X^+] + [HX] + [X^-] \quad (12)$$

$$[R_2NHPO_3]_t = [R_2\overset{+}{N}HPO_3^{2-}] + [R_2NPO_3^{2-}] \quad (13)$$

$$f_1 = 1 + (a_{H^+}/K_{H_2X}) + (K_{HX}/a_{H^+}) \quad (14)$$

$$K_A = [HX]/[M^{2+}\text{-}PCA^-][R_2\overset{+}{N}HPO_3^{2-}] \quad (15)$$

$$[R_2NHPO_3]_T = [R_2NHPO_3]_t + [X]_t \quad (16)$$

$$q = 1 + \frac{(1 + (K_{HNP}/K_{HAM}))}{K_A[M^{2+}\text{-}PCA]_t} \quad (17)$$

$$K_I = qK_{HAM}K_A[M^{2+}\text{-}PCA]_t/(1 + (K_A[M^{2+}\text{-}PCA]_tK_{HAM}/K_{H_2X})) \quad (18)$$

$$K_{II} = (K_{HX} + (K_{HNP}/K_A[M^{2+}\text{-}PCA]_t))/q \quad (19)$$

phosphoramidate so that pseudo-first-order rate constants were obtained. Rate data are summarized in Tables II and III.

Reliable k_{obsd} values could only be obtained below pH 6.5 because precipitates were formed above this pH.

Phosphoryl Transfer to Ni^{2+}-PCA. The results for the transfer from PIm and N-MePIm to Ni^{2+}-PCA are qualitatively similar to what was found earlier with Zn^{2+}-PCA.[2a] The rate of transfer from PIm reaches a saturating value with respect to Ni^{2+}-PCA concentration (Figure 1), providing evidence that transfer proceeds via a ternary PIm-Ni^{2+}-PCA complex, and the pH-rate profile at close to saturating Ni^{2+}-PCA shows a well-defined optimum at pH 4.0-4.5 (Figure 2), consistent with the idea that the reactive form of the ternary complex requires both a protonated imidazole ring and an oxime anion. Further evidence for this is that the rate of phosphoryl transfer from N-MePIm, where the imidazole ring must remain positive when the pH is increased, shows no downturn in rate above pH 4.5. The Ni^{2+}-PCA results differ quantitatively from the Zn^{2+}-PCA results in two important respects. First, the pH optimum is almost two pH units lower than for Zn^{2+}-PCA (6.0) and second, k_{obsd} at the pH optimum (0.066 min^{-1}) is only one-third k_{obsd} at the pH optimum for Zn^{2+}-PCA (0.195 min^{-1}).

Attempted Phosphoryl Transfer to PAH and Zn^{2+}-PAH. Several attempts, all unsuccessful, were made to demonstrate

Table II. k_{obsd} (min^{-1}) for Phosphoryl Transfer to 0.1 M PCA in the Presence of 0.1 M Zn^{2+} or 0.1 M Ni^{2+} as a Function of pH[a]

pH	PA Zn^{2+} 29.2 °C	*N*-(*n*-butyl)PA Zn^{2+} 29.2 °C	*N*-(*n*-butyl)PA Zn^{2+} 10.0 °C	*N*-(phenethyl)PA Zn^{2+} 10.0 °C	*N*-(benzyl)PA Zn^{2+} 10.0 °C	Plm Ni^{2+} 10.0 °C	*N*-MePlm Ni^{2+} 29.2 °C
3.55						0.030	0.034
4.0	0.0245	0.0156	0.0070	0.0077	0.0064	0.051	0.053
4.5	0.055	0.0345	0.0100	0.020	0.0174	0.066	0.073
5.0	0.113	0.090	0.0161	0.046	0.057	0.046	0.067
5.5	0.247	0.224	0.0244	0.174	0.167	0.022	0.066
5.75	0.277	0.333	0.0392	0.195	0.233		
6.0	0.283	0.364	0.0488	0.231	0.272		
6.25	0.247	0.420	0.0518	0.283	0.310		
6.5	0.239	0.485	0.0542	0.289	0.310		

[a] For Zn^{2+}-PCA in the pH range 5.5–6.5 and Ni^{2+}-PCA in the pH range 5.0–5.5 kinetic runs were self-buffered. All other runs were buffered with added 0.05 M *N,N'*-dimethylpiperazine.

Table III. k_{obsd} for Phosphoryl Transfer at Fixed pH and Varying $[PCA]_T = [M^{2+}]_T$

$[M^{2+}]_T$ or $[PCA]_T$, M	PA Zn^{2+} 29.2 °C pH 5.0[a]	PA Zn^{2+} 29.2 °C pH 6.5[b]	*N*-(*n*-butyl)PA Zn^{2+} 29.2 °C pH 5.0[a]	*N*-(*n*-butyl)PA Zn^{2+} 29.2 °C pH 6.5[b]	*N*-(phenethyl) PA Zn^{2+} 10.0 °C pH 5.0[a]	*N*-(phenethyl) PA Zn^{2+} 29.2 °C pH 5.0[a]	*N*-(benzyl)PA Zn^{2+} 10.0 °C pH 6.5[b]	Plm Ni^{2+} 29.2 °C pH 5.5[b]	*N*-MePlm Ni^{2+} 29.2 °C pH 5.5[b]
0.01	0.015	0.086	0.0030	0.0446		0.019	0.031	0.0059	
0.020								0.0108	
0.025	0.024	0.193	0.0229	0.140	0.0040	0.045	0.105		
0.030								0.0159	0.033
0.050	0.056	0.216	0.045	0.277	0.0059	0.072	0.204	0.0182	0.046
0.075	0.083	0.238	0.066	0.38	0.0098	0.095	0.350		
0.080								0.0220	0.058
0.100	0.114	0.239	0.090	0.49	0.0161	0.120	0.311	0.0220	0.066
0.120	0.127		0.083	0.44	0.0169				
0.140				0.45					
0.150	0.132		0.100		0.0154			0.0190	0.082

[a] For $[M^{2+}]_T \geq 0.05$ M, runs were self-buffered. Below this concentration runs were buffered with added *N,N'*-dimethylpiperazine (0.05 M). [b] For $[M^{2+}]_T \geq 0.05$ M, runs were self-buffered. Below this concentration runs were buffered with added 0.05 M Mes.

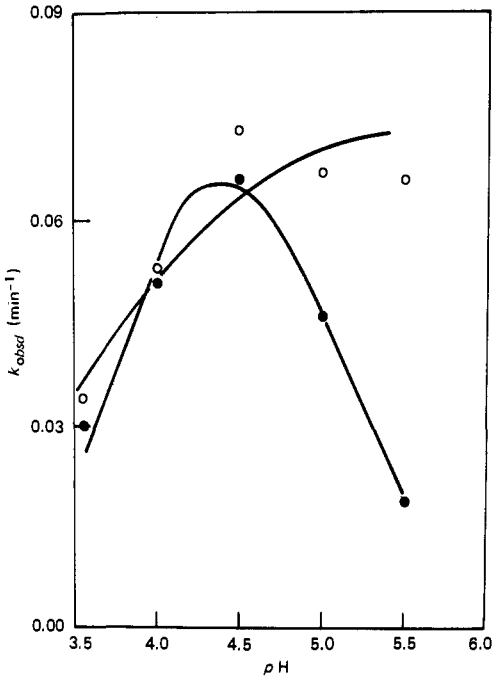

Figure 1. Plot of k_{obsd} for phosphoryl transfer to Ni^{2+}-PCA from PIm (●) and *N*-MePlm (○) at 29.2 °C. $[Ni^{2+}]_T = [PCA]_T = 0.1$ M.

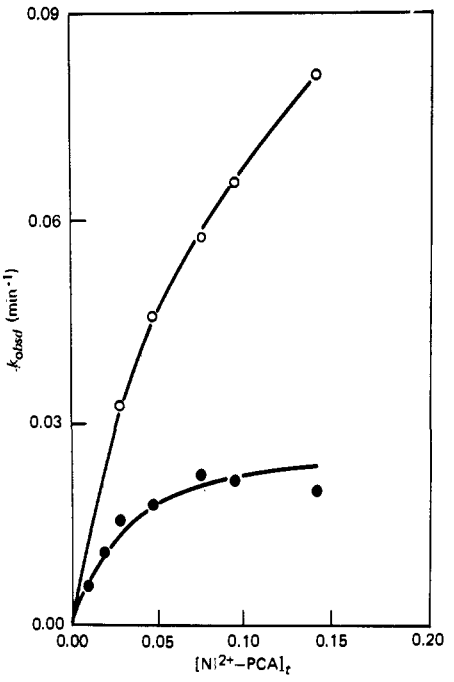

Figure 2. Plot of k_{obsd} for phosphoryl transfer to Ni^{2+}-PCA from Plm (●) and *N*-MePlm (○) at 29.2 °C at pH 5.5.

phosphoryl transfer from PIm to PCH or Zn^{2+}-PAH in reaction mixtures ranging from equimolar (0.04 M) to a tenfold excess of either species (0.1 M/0.01 M) at pH 4–5.5 at 40 °C for periods up to 20 h. In these experiments we looked for phosphorylated PAH by methods similar to that used to detect the formation of PCAP (see Methods), by thin layer chro-

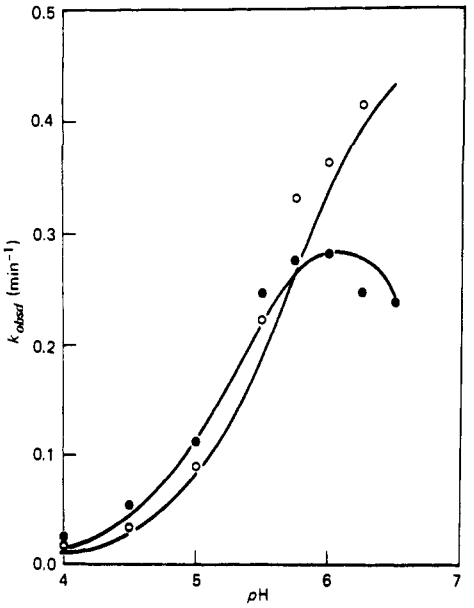

Figure 3. Plot of k_{obsd} for phosphoryl transfer to Zn^{2+}-PCA from PA (●) and *N*-(*n*-butyl)PA (○) at 29.2 °C. $[Zn^{2+}]_T = [PCA]_T = 0.1$ M. Lines are theoretical and are drawn from eq 5 using parameter values listed in Tables I and IV.

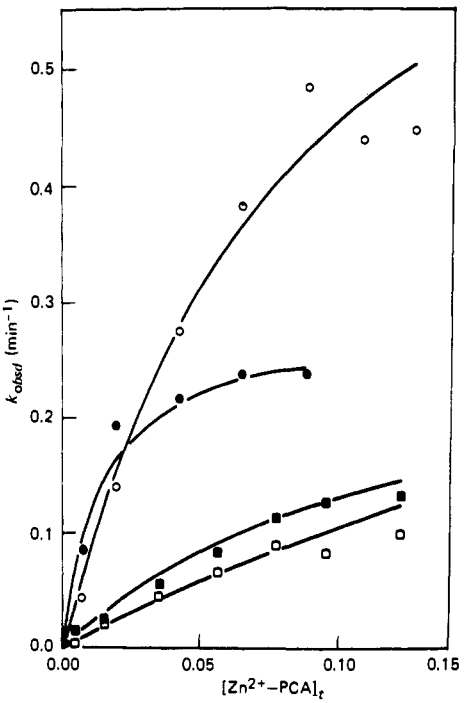

Figure 4. Plot of k_{obsd} for phosphoryl transfer to Zn^{2+}-PCA at 29.2 °C from PA at pH 5 (■) and 6.5 (●), and from *N*-(*n*-butyl)PA at pH 5 (□) and 6.5 (○). Lines are theoretical and are drawn from eq 5 using parameter values listed in Tables I and IV.

matography, and by looking for an increase in the rate of inorganic phosphate release.

Phosphoryl Transfer to Zn^{2+}-PCA. Data obtained for phosphoryl transfer from a given phosphoramidate to Zn^{2+}-PCA may be adequately fitted according to the general model presented in Scheme I, in which transfer proceeds via formation of the ternary complex phosphoramidate-M^{2+}-PCA, denoted X, and the reactive form of the complex has a net neutral charge. The appropriateness of the model is illustrated in Figures 3 and 4, where the data obtained for transfer from PA and *N*-(*n*-butyl)PA to Zn^{2+}-PCA at 29.2 °C are plotted together with lines predicted from eq 5.

Similar agreement of the data to the theoretical lines was found for PIm and *N*-MePIm at 29.2 °C (using data obtained previously[2a]) and *N*-(*n*-butyl)PA, *N*-(phenethyl)PA, and *N*-(benzyl)PA at 10 °C. Values of K_{HAM} and K_{HNP} used for the construction of these lines are listed in Table I. Values of k, K_A, K_{H_2X}, and where appropriate K_{HX} were determined by a computerized nonlinear least-squares fitting of the data to eq 5 and are summarized in Table IV. K_{HX} is evaluated for PIm and phosphoramidate, but for the other phosphoramidates no downturn in rate was seen up to pH 6.5 and it was assumed that in these cases X^- is present in negligible concentrations below pH 6.5, so that K_{HX} could not be determined. From the results summarized in Table IV, it can be seen that the fitting procedures yielded reasonably reliable values for K_A and k, but with the exception of PIm, K_{XH_2} was not well determined.

This can be understood as follows. If eq 5 is rewritten as eq 5a, then the only term containing K_{XH_2} is K_I. From eq 18 it is clear that at intermediate values of $K_A[Zn^{2+}\text{-PCA}]_t(K_{HAM}/K_{XH_2})$, K_I depends on both K_{XH_2} and K_{HAM}, while at extreme high values such that $K_A[Zn^{2+}\text{-PCA}]_t(K_{HAM}/K_{XH_2}) \gg 1$, K_I becomes essentially independent of K_{HAM} and at extreme low values, such that $K_A[Zn^{2+}\text{-PCA}]_t(K_{HAM}/K_{XH_2}) \ll 1$, K_I, and thus k_{obsd}, becomes independent of K_{XH_2}. Since pH-rate profiles for all of the phosphoramidates were done at the same $[Zn^{2+}\text{-PCA}]_t$, variation in dependence of K_I on K_{XH_2} depends on the value of K_A. Thus, for PIm at 29.2 °C, K_A is 150 M^{-1} and K_{XH_2} is well determined, while for *N*-benzyl phosphoramidate, K_A is 10 M^{-1} and K_{XH_2} cannot be estimated. For the other phosphoramidates with K_A in the range 20–50 M^{-1}, estimates for K_{XH_2} are very uncertain.

Values of log k are plotted against the pK_a of the leaving-group amine in Figure 5. A line drawn through the points for *N*-(*n*-butyl)PA, *N*-(phenethyl)PA, and *N*-(benzyl)PA gives a Bronsted β of 0.8 ± 0.1. The negative deviation from the Bronsted line seen for PIm and *N*-MePIm is similar to what was seen for phosphoramidate hydrolysis[6a] and may be attributed, as previously,[6a] to a delocalization of positive charge over the aromatic ring system. However, the negative deviation of the k value for PA from the Bronsted line for phosphoryl

Table IV. Fitted Parameters

No.	Phosphoramidate	pK_a of leaving amine [a]	Temp, °C	k, min^{-1}	K_A, M^{-1}	$K_{XH_2} \times 10^5$, M	$K_{XH} \times 10^7$, M
1	PA	9.38	29.2	0.50 ± 0.08	52 ± 12	1.4 ± 1.0	2.4 ± 1.1
2	PIm	7.2	29.2	0.285 ± 0.037	147 ± 25	0.54 ± 0.16	1.3 ± 0.7
3	*N*-MePIm	7.2	29.2	0.98 ± 0.17	26 ± 8	0.75–2.0	
4	*N*-(*n*-butyl)PA	10.77	29.2	0.86 ± 0.10	16 ± 4	8.8 ± 85	
4	*N*-(*n*-butyl)PA	10.77	10.0	0.074 ± 0.011	47 ± 22	1.3 ± 2.0	
5	*N*-(phenethyl)PA	9.83	10.0	0.50 ± 0.13	35 ± 15	>0.5	
6	*N*-(benzyl)PA	9.33	10.0	0.97± 0.27	10 ± 5	>0.5	

[a] See ref 6a.

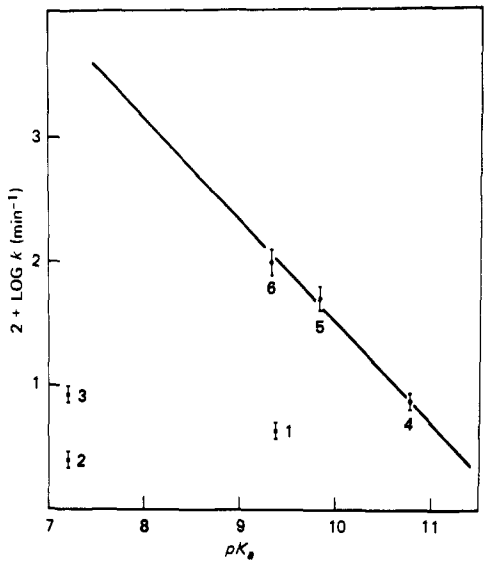

Figure 5. Bronsted plot of log k (Table IV) at 10.0 °C vs. the pK_a of the leaving amine. Values for PA, PIm, and N-MePIm are estimated by multiplying the values obtained at 29.2 °C by the $k_{10.0°}/k_{29.2°}$ ratio obtained for N-(n-butyl)PA (equal to 0.086).

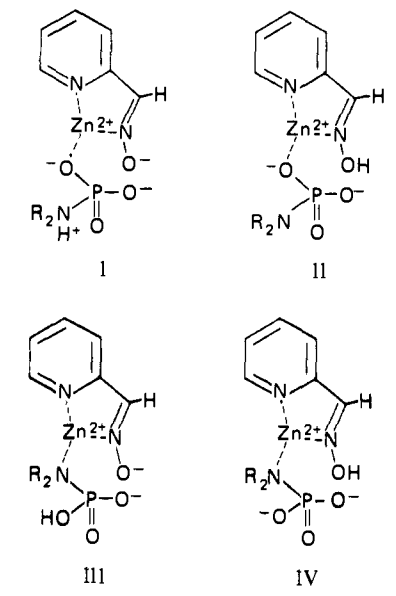

Figure 6. Plausible isomers for the neutral form of the ternary complex phosphoramidate-Zn^{2+}-PCA.

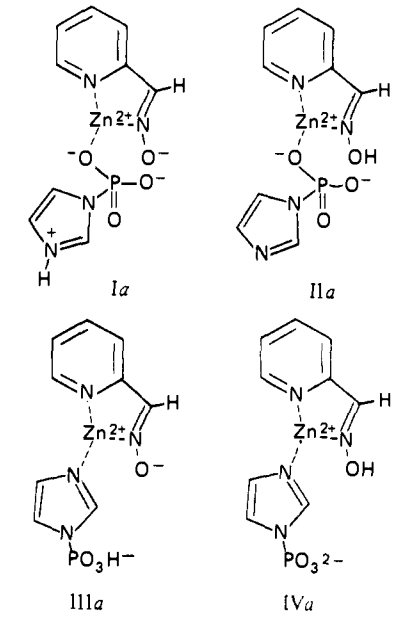

Figure 7. Plausible isomers for the neutral form of the ternary complex PIm-Zn^{2+}-PCA.

transfer is unexpected on the basis of the hydrolysis rate data. The neutral ternary complex, which we have denoted HX in Scheme I, has four plausible isomeric forms I–IV (Figure 6). Although all four can assume the appropriate geometry for phosphoryl transfer, I would be expected to be the most reactive, since phosphoryl-transfer reactions show strong dependence on the conjugate acidity of the leaving group, which should fall in the order $RN^+H_3 > RNH_2$–$Zn^{2+} > RNH_2$, and in addition, in I the attacking nucleophile is the oxime anion, which should be more potent than the protonated oxime, as in II or IV. Thus, the explanation of the low k value for PA could be that the fraction of HX in the isomeric form I derived from PA is very much lower than the fraction of HX derived from the three other phosphoramidates. Support for this hypothesis comes from the result that K_A is higher for PA than it is for the other phosphoramidates (Table IV) and by the a priori consideration that the fraction of XH present in the isomeric forms III or IV should increase with decreasing basicity and increasing ligand strength of the amine moiety of the phosphoramidate. Of the four aliphatic phosphoramidates, the amine moiety of PA is not only the least basic (Table IV), but also would be expected to be the strongest amine ligand for Zn^{2+}-PCA, since the amine moieties of the other phosphoramidates have bulky substituents, which should considerably reduce their affinities for Zn^{2+}-PCA.

A problem in assessing the significance of the observed β value is that unreactive isomers of XH may also be stoichiometrically important for N-(n-butyl)PA, N-(phenethyl)PA, and N-(benzyl)PA, so that the rate constants used in obtaining β may only be lower limits for the microscopic rate constants for phosphoryl transfer via isomer I. Based on the K_A values, we would expect the value of k to be lower with respect to the microscopic constant for N-(n-butyl)PA than for N-(benzyl)PA, so that the observed β may be more negative than the β value for phosphoryl transfer via isomer I, although it should be pointed out that the fitted K_A values for these three phosphoramidates all have large error ranges and that furthermore there is no obvious a priori reason, as there is for PA itself, why the isomeric forms III and IV should be more favorable for N-(n-butyl)PA than for N-(benzyl)PA.

The value of k for N-MePIm is a factor of 3.3 as large as that for PIm, yet their rates of monoanion hydrolysis differ by only 15%. As above, we can explain these results on the basis of the greater stoichiometric importance of unreactive neutral ternary complexes for PIm than for N-MePIm, and this is a particularly attractive hypothesis in this case, since of the four plausible isomers of XH for PIm (Ia–IVa, Figure 7) only the reactive one (Ia) can also be formed from N-MePIm and K_A for PIm is much larger than it is for N-MePIm (Table IV). It should be pointed out that this hypothesis contradicts our earlier suggestion[2a] that PIm bound Zn^{2+}-PCA primarily through the phosphate, which was based primarily on the result that the apparent association constants (K_{app}) (determined graphically) of PIm to Zn^{2+}-PCA were similar at pH 4.8 (53 M^{-1}) and 6.4 (62 M^{-1}). Substituting the parameter values listed in Tables I and IV into eq 11 gives K_{app} values of 34 M^{-1} at pH 4.8 and 112 M^{-1} at pH 6.4. Thus the basis for our earlier suggestion was apparently incorrect.

Discussion

Previously[4] we suggested that protonation of the imidazole ring was necessary for phosphoryl transfer in the ternary

Table V. Phosphoramidate Hydrolysis vs. Intracomplex Transfer Rate Constants

Phosphoramidate	Hydrolysis rate constants, M^{-1} min^{-1} [a]	Intracomplex transfer rate constant, min^{-1}	Intracomplex hydrolysis, M
N-(*n*-butyl)PA	3.53×10^{-7}	0.26[b]	7.4×10^5
N-(phenethyl)PA	2.43×10^{-6}	1.75[c]	7.2×10^5
N-(benzyl)PA	5.11×10^{-6}	3.4[c]	6.7×10^5

[a] Reference 6a, 20 °C. [b] 20 °C, interpolated from results at 10 and 29.2 °C (Table IV). [c] Constant at 10 °C multiplied by $k_{20°}/k_{10°}$ (equal to 3.5) for *N*-(*n*-butyl)PA.

complex PIm-Zn^{2+}-PCA, based on the known unreactivity of the PIm dianion toward hydrolysis, and a slight downturn in rate above pH 6.1. Since our rate data were only reliable up to pH 6.5 due to precipitation problems, our suggestion, though reasonable, was not clearly demonstrated. Transfer via the PIm-Ni^{2+}-PCA complex has a much lower pH optimum and thus, as seen in Figure 1, the downturn in rate at high pH, as well as the absence of a downturn for *N*-MePIm, is much more clearly demonstrated, confirming our earlier suggestion.

For all of the phosphoramidates examined, the rate data for phosphoryl transfer within the ternary complex are adequately accounted for assuming that only PCA anion is reactive as a nucleophile and that PCAH, in which the oxime oxygen is protonated, is not. This does not prove that PCAH is inert, but it must be at least 20–50 times less reactive than PCA anion. Furthermore, PAH, which can presumably form a ternary complex analogous to that formed by PCA, but with a nitrogen in place of the oxime oxygen, is also very unreactive as a nucleophile. This order of nucleophilicity in the ternary complex, oxyanion much greater than either neutral hydroxy group or weak base amine, contrasts sharply with that found in a previous study[12] of the reactivity of various nucleophiles toward *N*-phosphoryl-4-methylpyridinium ion, in which the relative second-order rate constants fell in the order: weak base amine (acethydrazide, 11) > oxyanion (OH^-, 1.3) ≈ neutral hydroxy group (H_2O, 1.0). The very low reactivity of phosphoryl dianions toward anion attack, exemplified by the above result, has been ascribed to a strong charge repulsion between the two reactive centers,[13] so that the high relative reactivity of PCA anion in the ternary complex can reasonably be attributed to a charge shielding effect of Zn^{2+}.

This catalytic effect of Zn^{2+} would be in addition to its obvious role in acting as a template by bringing together both nucleophile and phosphoryl dianion within the ternary complex and thus converting a second-order reaction into a first-order one. A similar conclusion can be reached from more quantitative considerations. Table V compares the rate constants for phosphoryl transfer to Zn^{2+}-PCA with those obtained for phosphoramidate hydrolysis. Assuming that, as above, the hydroxide rate is a factor of 1.3 faster than the H_2O rate leads to a ratio of 5×10^5 M for the rate constant for intracomplex PCA attack divided by the second-order rate constant for hydroxide ion attack. Hydroxide ion is some 10 pH units more basic than the Zn^{2+}-PCA anion and the Bronsted nucleophilic β for oxyanion attack on phosphodiester monoanions is 0.3.[13] Even assuming a much lower β of 0.1 for oxyanion attack on phosphoramidate monoanion zwitterions gives a rate factor for intracomplex transfer vs. second-order nucleophilic attack of Zn^{2+}-PCA anion on phosphoramidate of 5×10^6 M. This is an extremely large value for a template effect[2d,14] and suggests an additional role for Zn^{2+}, which we are attributing to charge shielding.

Although Benkovic and Sampson[6a] reported a leaving-group β of −1.0 for the hydrolysis of phosphoramidate anions predominately in the zwitterionic form, a plot of their data for just the three phosphoramidates used to obtain a β value in this study (Figure 5) gives a value of −0.83. Thus for these three phosphoramidates the β value for intracomplex phosphoryl transfer is essentially identical with that obtained for hydrolysis, so that, as judged solely by this criterion, the presence of Zn^{2+} has little distorting effect on the structure of the transition state in phosphoryl transfer. It is true, as pointed out above (see Results), that our observed β value may reflect substitution effects on both rate and isomerization equilibrium constants, and therefore that the β value for the rate constant may be less negative than that observed, but unless the difference were large, i.e., more than 0.2 log unit, which is unlikely, the conclusion that Zn^{2+} doesn't have a distorting effect on the transition state would remain unchanged, since one would expect a less negative β for the more powerful nucleophile Zn^{2+}-PCA anion than for water.[12,15]

In a related study on phosphate diester monoanions, Steffens et al.[2d] have measured a Bronsted leaving-group β of −0.7 for the Zn^{2+}-catalyzed hydrolysis of substituted phenyl esters of lactic acid phosphate, which is considerably lower than the value of −1.2 measured for hydrolysis in the absence of Zn^{2+}. Unfortunately, the rate data for the Zn^{2+}-catalyzed reaction were obtained at a Zn^{2+} concentration much lower than that required for saturation, so that the value of −0.7 reflects substituent effects on both the hydrolysis rate constant and on the binding constant of Zn^{2+} to lactic acid *O*-phenyl phosphate. Nevertheless, it is unlikely that differences in Zn^{2+} binding would be sufficient to alter the qualitative conclusion that the leaving-group β is significantly less negative in the presence of Zn^{2+} than in its absence.

Some general conclusions which have emerged from studies on nucleophilic reactions of phosphorus esters are that nucleophilic attack on phosphate monoester dianions or phosphoramidate monoanion zwitterions proceeds either via an SN1(P) "metaphosphate" mechanism or something very close to it characterized by highly negative β leaving-group values, that nucleophilic attack on phosphate triesters have essentially SN2(P) character, characterized by much less negative β leaving-group values, and that nucleophilic attack on phosphate diester monoanions is somewhat intermediate between these two extremes, although perhaps more SN2(P)-like.[1,16] One possible explanation for the apparent difference seen in the Zn^{2+} effect in the Steffens et al.[2d] study and ours is that the partial charge neutralization which would be expected to be a consequence of Zn^{2+} binding to the phosphoryl moiety is sufficient to change a transition state of intermediate character to one which is more SN2(P)-like, whereas it is insufficient to significantly change the character of a transition state which is strongly SN1(P)-like. Further discussion on the nature of the transition state for the phosphoryl-transfer reaction may be found in the accompanying paper.[2c]

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Aromatic Nucleophilic Substitution in Nucleophilic Surfactants. Comparison with Alkoxide Reactions

Clifford A. Bunton* and Simon Diaz[1]

Contribution from the Department of Chemistry, University of California, Santa Barbara, California 93106. *Received November 3, 1975*

Abstract: The reactions of 2,4-dinitrochloro- and fluorobenzene (DNC and DNF) with the alkoxide ions of choline, propargyl alcohol, and 2,2,2-trifluoroethanol give ether intermediates which react readily with hydroxide ion. The nucleophilicities of the alkoxides toward DNC and DNF (in parentheses) are: $Me_3N^+CH_2CH_2O^-$ 19 (27), $HC{\equiv}CCH_2O^-$ 63 (132), $CF_3CH_2O^-$ 11 (10), relative to OH^- in water at 25.0 °C. The second-order rate constants (10^4k^{OH}, l. mol^{-1} s^{-1}) for reaction of DNF, DNC, or the ethers toward OH^- are: DNF, 1200; DNC, 1.42; $-OCH_2CF_3$, 9.1; $-OCH_2C{\equiv}CH$, 3.7; $-OCH_2CH_2N^+Me_3$, 9.1. Micelles of hexadecyl(2-hydroxyethyl)dimethylammonium bromide (n-$C_{16}H_{33}N^+Me_2CH_2CH_2OH$ Br^-, Ia) are effective reagents toward DNC and DNF at high pH and for ionization of the hydroxy group pK_a ~12.3, estimated kinetically for reactions of DNF and DNC in Ia and up to 0.15 M OH^-. In 0.01 M OH^- the reactivity of the 2,4-dinitrophenyl ether of Ia in micelles of Ia is 260 times that of the corresponding ether of choline in water, and the overall rates of nucleophilic attack on DNF and DNC in micelles of Ia are 6000 and 14 000, respectively, relative to reaction in water.

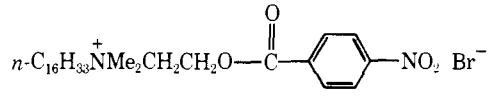
Cationic micelles of the hydroxyethyl surfactants (Ia, b) and related surfactants are effective reagents in reactions of phosphate[2] and carboxylate esters[3,4] and of alkyl halides[5] and carbocations.[6] It was suggested that the alkoxide moiety of the zwitterion (II) acted as a good nucleophile.[2,4,6]

$$RN^+Me_2CH_2CH_2OH \rightleftharpoons RN^+Me_2CH_2CH_2O^- + H^+$$

Ia, R = n-$C_{16}H_{33}$ II
b, R = n-$C_{12}H_{25}$
c, R = Me

The pK_a of choline (Ic) is 13.9,[7] so that II should be generated at high pH, especially if R is sufficiently hydrophobic for micelles to form, because micellization should increase ionization of I. Micelles of Ia, b are crude models for a catalytically active serine residue in an enzyme.[8,9]

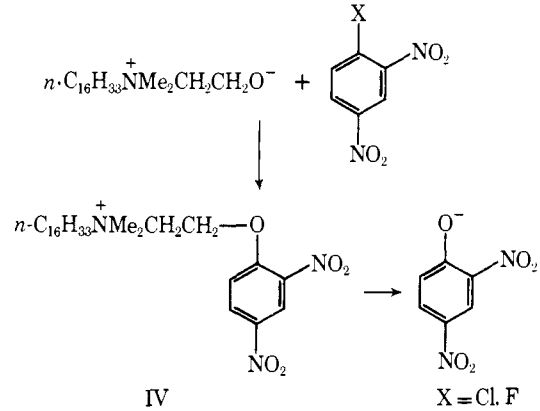
The catalytic effectiveness of micelles of Ia, b could be explained in terms other than nucleophilic attack. For example general acid or base catalysis could be important, and it has been suggested that hydroxide ions at the surface of micellized I could be especially reactive.[5] However the solvent deuterium isotope effects in phosphate ester hydrolyses are those expected for nucleophilic attack by the alkoxide moiety of II,[2c] and micellized Ia is no better a catalyst than the nonfunctional surfactant cetyltrimethylammonium bromide (CTABr) for reaction of hexadecyl(2-hydroxyethyl)dimethylammonium bromide p-nitrobenzoyl ester (III).[13]



III

Berezin and his co-workers have shown that p-nitrophenyl esters will acylate surfactants similar to I,[4] but their experiments were done using an excess of carboxylic ester over the functional surfactant, so that the substrate could markedly perturb the micellar structure.

Our aim was to demonstrate formation of an intermediate formed by nucleophilic attack by micellized II under conditions in which the substrate concentration is much lower than that of the surfactant. The micellar structure should then be little affected by the substrate, and direct comparison can be made with other reactions of micellized I or similar surfactants at high pH.[2-6] The most convenient substrates are 2,4-dinitrochloro- and fluorobenzene (DNC and DNF), because nucleophilic attack upon them should give the ether (IV) which could then be hydrolyzed to 2,4-dinitrophenoxide ion,[14] and perhaps be spectroscopically detectable.



IV X = Cl, F