Dephosphorylation in Functional Micelles. The Role of the Imidazole Group

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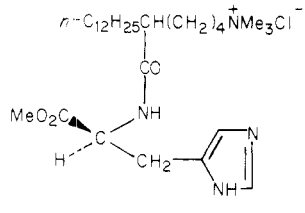
Dephosphorylation of *p*-nitrophenyl diphenyl phosphate (*p*-NPDPP) is effectively catalyzed by micelles of [5-[(α-*N*-L-histidinyl methyl ester)carbonyl]*n*-heptadecyl]trimethylammonium chloride (**1**) and dimethylhexadecyl[(4-imidazolyl)methyl]ammonium chloride (**2**) by factors of 95 and 400, respectively, at pH ca. 8. The kinetic solvent–deuterium isotope effects k_{H_2O}/k_{D_2O} of 2–2.8 are consistent with imidazole moieties of the surfactants acting as general bases and activating a water molecule. They do not, however, promote phosphorylation of added *n*-decanol. There is no build up of phosphorylated intermediate during reaction. An increase of pH markedly speeds reaction in micelles of **2**, presumably by generating a nucleophilic imidazolide anion, but has a smaller effect on reaction in micelles of **1**. The variation of rate with pH in solutions of micellized **2** allows separation of the contributions of reactions involving an undissociated imidazole group and an imidazolide anion. Comparison of the second-order rate constant for dephosphorylation by a nonmicellized catalyst, trimethyl[(4-imidazolyl)-methyl]ammonium chloride (**3**), with the first-order rate constant in micellized **2** at pH 7–8 shows that the rate enhancement in the functional micelle is caused almost wholly by increased concentration of reactive groups at the micellar surface. Micelles of **1** also speed dephosphorylation of diethyl and di-*n*-hexyl *p*-nitrophenyl phosphate, but they are relatively ineffective catalysts of the spontaneous hydrolysis of the 2,4-dinitrophenyl phosphate dianion. Anionic resins immobilize **1**, providing a reusable catalytic system for dephosphorylation.

There are many examples of rate enhancements by micelles of functional surfactants in water.[3-7] Deacylation is the most studied reaction, and imidazole, oximate, hydroxamate, amino, thiol, and hydroxyl groups have been used as nucleophiles in functional micelles or comicelles. Of especial importance is the work of Moss[4] and Tonellato[5] and their collaborators which shows that deacylation in micelles or comicelles containing both imidazole and hydroxyl groups gives *O*-acyl intermediates but that these intermediates are themselves formed from *N*-acyl intermediates, showing that the imidazole group is acting as a nucleophile rather than as a general base, and in most systems the functional group of the surfactant acts as a nucleophile.

The chiral surfactant **1**, derived from L-histidine and isolated as a single diastereomer, is an effective deacylating agent and gives stereoselective deacylations of chiral esters.[8] Similar stereoselective deacylations by mixed micelles have also been observed.[9]



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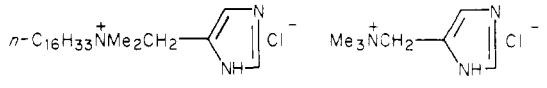
Dephosphorylation by functional micelles has not been widely studied, but micelles or comicelles derived from choline[6,10] or oxime[11] derivatives are effective reagents at pHs high enough for deprotonation of the functional group to give highly nucleophilic alkoxide or oximate moieties.

Micelles of **1** speed the hydrolysis of *p*-nitrophenyl diphenyl phosphate (*p*-NPDPP) at pH 8–9, and the deuterium isotope effects are consistent with the imidazole group acting as a general base and activating a water molecule.[12] We have extended this preliminary study by examining other dephosphorylations, and we have also used the functional surfactant **2** and the nonmicellar model compound **3**.



2 **3**

The other substrates were diethyl and di-*n*-hexyl *p*-nitrophenyl phosphate (**4** and **5**) and the 2,4-dinitrophenyl phosphate dianion (**6**) which was used because its hydrolysis involved spontaneous elimination of metaphosphate ion rather than nucleophilic attack on phosphorus.

A few experiments were run with comicelles of **1** or **2** with either hexadecyltrimethylammonium bromide (CTABr) or Brij (polyoxyethylene oxide 20-hexadecyl ether) or the choline derivative **7**, which at high pH gives the reactive zwitterion **8**.[6]

$$n\text{-}C_{16}H_{33}N^+Me_2CH_2CH_2OHBr^- \underset{}{\overset{OH^-}{\rightleftharpoons}} n\text{-}C_{16}H_{33}N^+Me_2CH_2CH_2O^-$$

7 **8**

Experimental Section

Materials. The preparation or purification of some of the surfactants has been described.[6,8] The imidazole surfactant, **2**, was prepared as the dichloride, dimethylhexadecyl[(4-imidazolium)methyl]ammonium dichloride, by the method of Tagaki and co-workers from *N*,*N*-dimethylhexadecylamine (0.01 mol) and

(1) To whom correspondence should be addressed.
(2) The present addresses of the remaining authors may be obtained from the corresponding author.
(3) Tonellato, U. In "Solution Chemistry of Surfactants"; Mittal, K. L., Ed.; Plenum: New York, 1979; Vol. 2, p 541.
(4) Moss, R. A.; Nahas, R. C.; Ramaswami, S. *J. Am. Chem. Soc.* **1977**, *99*, 627. Moss, R. A.; Lee, Y. S.; Lukas, T. J. *Ibid.* **1979**, *101*, 2499.
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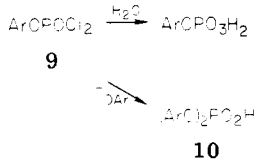
Scheme I



4-(chloromethyl)imidazole (0.05 mol) in refluxing C_6H_6.[13] The initial product was dried and dissolved in MeOH which was saturated with NH_3. The solid was chromatographed on Cellex N-1 with Et_2O. This procedure and saturation with NH_3 was repeated several times, and finally a solution in EtOH was treated with HCl gas. The hygroscopic dichloride was recrystallized from C_6H_6–*n*-C_6H_{12}. Anal. Calcd for $C_{22}H_{45}N_3Cl_2 \cdot {}^1/_2H_2O$: C, 61.3; H, 10.7; N, 9.8; Cl, 16.5. Found: C, 61.1; H, 11.0; N, 9.8; Cl, 16.6.

The imidazole derivative **3** was prepared as the dichloride, trimethyl[(4-imidazolium)methyl]ammonium dichloride, from 4-(chloromethyl)imidazole (0.01 mol) and Me_3N (10 mL) in EtOH. The solution was left for 1 day and then heated under reflux for 8 h. After removal of solvent the residue in H_2O was treated with 6 M HCl, and the solvent was pumped off. The solid was dried in vacuo (P_2O_5) and recrystallized (EtOH); mp 206–207.5 °C dec. Anal. Calcd for $C_7H_{15}N_3Cl_2$: C, 39.6; H, 7.5; N, 19.8; Cl, 33.5. Found: C, 39.2; H, 7.4; N, 20.1; Cl, 34.0.

The trisubstituted phosphates were prepared by standard methods.[14]

A sample of 2,4-dinitrophenyl phosphate was prepared as the lutidinium salt (**6**) by a very simple method which is much easier than existing methods.[15] Phosphate esters are often prepared by hydrolyzing the dichlorides **9** (Scheme I) and are isolated as salts with a tertiary amine. However, this method fails for preparation of **6**, probably because 2,4-dinitrophenoxide ion is formed and attacks the dichloride to give the phosphate **10**.[15] This undesired reaction can be eliminated by working at high dilution in dilute acid. A solution of 50 mg of 2,4-dinitrophenyl phosphoryl dichloride (**9**, Ar = 2,4-dinitrophenyl) in 5 mL of dry Et_2O was slowly added to 200 mL of well-stirred 10^{-2} M HCl and ice. The ether was quickly removed on a rotary evaporator, the solution was concentrated to 50 mL by lyophilization, and any solid was discarded. The remaining solvent was removed, the solid was dissolved in Et_2O and 2,6-lutidine in Et_2O was added dropwise until the solution became slightly yellow. A white precipitate of lutidinium 2,4-dinitrophenyl phosphate then formed and was recrystallized (EtOH): mp 138–139 °C dec (lit.[15a] mp 142 °C); yield 70%. The product gave a quantitative yield of 2,4-dinitrophenoxide ion in hydrolysis catalyzed by alkaline phosphatase. Anal. Calcd: C, 42.1; H, 3.8; N, 11.3; P, 8.35. Found: C, 41.9; H, 3.8; N, 11.7; P, 8.65.

Kinetics. The reactions were followed spectrophotometrically in aqueous solution at 25.0 °C. The phosphates were added in dioxane (5 μL) to 3 mL of reaction mixture, except for 2,4-dinitrophenyl phosphate which was added as an aqueous solution. Substrate concentrations were (1–2) × 10^{-5} M, and the first-order rate constants, k_Ψ, are in reciprocal seconds. The pH of the solutions was measured under the conditions indicated and was controlled, where possible, with borate buffer. Borate ion appears to interact only weakly with cationic micelles. Acetate ion was used at pH <6 in experiments in solutions of **2**. The rate–surfactant profiles and the deuterium solvent isotope effects for reactions in solutions of **1** and in solutions of **2** at pH (pD) 8.0 were measured in borate buffer. The value of pD was calculated by using the equation of Glasoe and Long.[16] A few measurements were made without buffer, with measurement of the pH before and after the reaction. The pH changed little during hydrolysis with a substrate concentration of (1–1.5) × 10^{-5} M, and good first-order rate constants were obtained from the first part of the run. These rate constants for the reaction of *p*-NPDPP in 2 × 10^{-3} M **1** were obtained at pH 5.45, 6.40, and 7.0 and were in good agreement with those from experiments in buffered solutions, suggesting that our rate constants are not strongly affected by buffer effects.

Table I. Effect of Buffer Composition on Hydrolysis of *p*-NPDPP in CTABr[a]

10^3[CTABr], M	10^4k_Ψ, s^{-1}	10^3[CTABr], M	10^4k_Ψ, s^{-1}
	0.097	1.0	4.46 (1.01)
0.25	0.89	2.0	3.52 (0.90)
0.50	4.49 (0.96)	5.0	2.27 (0.66)
0.75	4.49		

[a] At 25.0 °C and pH 8.0 with 0.02 M sodium borate plus boric acid; the values in parentheses are for 0.015 M sodium borate plus HCl.

Table II. Micellar Effects upon pK_A'[a]

	surfactant	
10^4[D], M	1	2
0.2		7.02 ± 0.03
1.0	7.53 ± 0.16	6.62 ± 0.08
2.0	7.38 ± 0.05	
5.0	6.70 ± 0.04	
8.0	6.60 ± 0.18	
10.0		6.23 ± 0.10

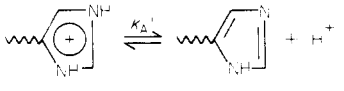
[a] Values of pK_A'; D designates surfactant.

The buffer can cause major problems in studies of micellar catalysis, especially if it contains counterions which interact strongly with the micelle (cf. Table I). Two buffers were used, one made up in the normal way from borate ion and boric acid and the other from borate ion and HCl. Chloride ion inhibits bimolecular reactions of anionic nucleophiles or bases in cationic micelles[17] so that the reaction is slower when the buffer is made up with HCl (Table I). A buffer mixture of sodium borate and boric acid was used for all our kinetic measurements except for some of our earlier work and where specified.

Reaction Products. Repetitive scans were made on a reaction mixture containing 3 × 10^{-5} M *p*-NPDPP and 2 × 10^{-3} M **2** at pH 7.6 (0.02 M phosphate) and 25.0 °C. There was a well-defined isosbestic point at 303 nm, and the rate constants calculated from absorbances at 270 and 360 nm were 5 × 10^{-3} s^{-1}, which is within experimental error and agrees with those obtained by following the reaction at 410 nm. The spectrum at the end of the reaction was that expected for a mixture of *p*-nitrophenoxide ion and diphenyl phosphate and had the expected pH dependence.

A few reactions of *p*-NPDPP were carried out with *n*-decanol or *p*-cresol added to solutions of **1** or **2**. At the end of the reaction the surfactant was precipitated with $NaClO_4$, and the solution and precipitate were extracted with Et_2O or $CHCl_3$. Small amounts of *p*-cresyl or *n*-decyl diphenyl phosphate were detected by TLC, and the R_f values coincided with those of authentic materials. In no cases were these esters other than minor products.

Micellar Effects on Deprotonation. Micellization increases the extent of deprotonation of the imidazolium moiety (eq 1).



(1)

The values of the apparent acid dissociation constants, pK_A', for the first deprotonation of the dichlorides of the functional surfactants **1** and **2** were measured spectrophotometrically at 190–220 and 200 nm, respectively, in unbuffered solutions. The values of pK_A' for the nonmicellized surfactants are typical of imidazole derivatives and they decrease on micellization (Table II). Each value is the mean of three or four determinations, and the standard deviations are shown.

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(15) (a) Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415. (b) Bunton, C. A.; Fendler, E. J.; Fendler, J. H. *Ibid.* **1967**, *89*, 1221.

(16) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.

(17) Bunton, C. A. In "Reaction Kinetics in Micelles"; Cordes, E. H., Ed.; Plenum: New York, 1973; p 73.

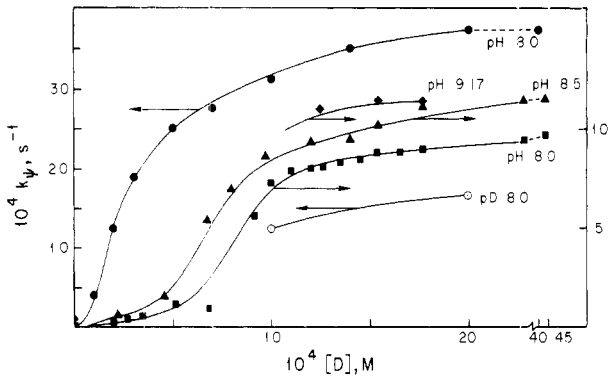

Figure 1. Dephosphorylation of *p*-nitrophenyl diphenyl phosphate in functional micelles: reaction in **1**, solid boxes, triangles, and diamonds; reaction in **2**, solid circles and (in D_2O) open circles.

Table III. Effect of Added Solutes on Reaction of *p*-NPDPP with 1[a]

10^4[1], M	10^4[*p*-NPDPP], M	[solute], mM (solute)	$10^4 k_\psi$, s^{-1}
17.8	0.14		11.2
17.8	0.14	1.8 ($C_{10}H_{21}OH$)	7.45 (3.13)[b]
17.8	0.14	4.4 ($C_{10}H_{21}OH$)	5.60
3.0	3.0	10 (Brij)	0.22
3.0	6.0	10 (Brij)	0.22
6.0	6.0	10 (Brij)	0.42

[a] At 25.0 °C in 0.015 M borate buffer, pH (pD) 8.5. [b] The value in parentheses is in D_2O.

Results

Reactions in the Histidine Surfactant 1. Hydrolysis of *p*-Nitrophenyl Diphenyl Phosphate. The rate–surfactant profiles (Figure 1) are typical of micellar-catalyzed reactions in that the surfactant has little effect at very low concentrations, but as micelles form the rate increases to a constant value, which corresponds to complete substrate incorporation. An increase in pH slightly increases k_ψ (Figure 1). Cationic micelles speed the attack of OH^- on *p*-NPDPP,[14a] but this effect is probably unimportant here, because of the low concentration of OH^- at pH 9. However, micelles of **1** behave like other cationic micelles in mildly catalyzing attack by nucleophilic anions, because addition of 10^{-2} M F^- to 1.37×10^{-3} M **1** at pH 8.0 increases the rate approximately fourfold to 2.8×10^{-3} s^{-1} (cf. Figure 1).[18]

Effects of Added Surfactant or Solute. In an attempt to observe the buildup of an intermediate phosphorylated histidine, we carried out the reaction using the substrate in excess over **1**, in the presence of a large amount of Brij (polyoxyethylene oxide 20-hexadecyl ether) so that the substrate should be fully micellar bound. The reaction was cleanly first order with no "burst" of *p*-nitrophenoxide ion (cf. ref 3–5). The first-order rate constants, k_ψ, did not depend upon the substrate concentration, but they increased with increasing content of the reactive surfactant (**1**) in the comicelle (Table III).

Nonionic solutes typically reduce micellar catalysis,[19] and *n*-decanol and Brij dilute the reactive imidazole moieties in the micelle. Small amounts of *n*-decyl diphenyl phosphate are formed with added *n*-decanol (Experimental Section), but the small contribution of this reaction does not offset the deactivation of the micelle.

Table IV. Solvent Deuterium Isotope Effects on the Reaction of *p*-NPDPP in Micellized 1[a]

10^4[1], M	pH (pD)	$10^4 k_\psi$,[b] s^{-1}	k_{H_2O}/k_{D_2O}
17.8	8.0	8.92 (3.27)	2.7
19.9	8.0	9.20 (3.15)	2.9
17.8	8.5	11.2 (4.43)	2.5
19.9	8.5	11.3 (4.46)	2.5
17.8	9.0	11.4 (5.36)	2.1
19.9	9.0	11.5 (5.77)	2.0

[a] At 25.0 °C in 0.015 M borate buffer. [b] The values in parentheses are in D_2O.

Table V. Dephosphorylation in Micelles of the Histidine Surfactant 1[a]

10^4[1], M	substrate: 2,4-$(NO_2)_2$-$C_6H_3OPO_3^{2-}$	substrate: $(EtO)_2POO$-$C_6H_4NO_2$[b]	substrate: $(HexO)_2$-$POOC_6H_4NO_2$
	83	2.1	~2
3.8		3.8	
4.6			70.7
6.8			105
9.1	319	5.2	113
11.4	464		121
13.7		6.7	
17.1	569	10.7	120[c]
22.8	603		123
28.5	625		

[a] Values of $10^7 k_\psi$ (s^{-1}) at 25.0 °C in 0.015 M borate buffer (pH 8.5) unless specified. [b] pH 8.0. [c] In D_2O at pD 8.5, $10^7 k_\psi = 36.5$ s^{-1}, and at pH (pD) 9.3, $10^7 k_\psi = 417$ and 141 s^{-1} in H_2O and D_2O, respectively.

These observations suggest that there is no buildup of a covalent intermediate during reaction, but they do not exclude its transient formation.

Deuterium Solvent Isotope Effects. The values of the deuterium solvent isotope effect are larger than expected for nucleophilic attack by the histidine moiety on the phosphoryl group (Table IV). They are, however, in the range considered typical of general-base catalysis of attack by water.[20] A problem in analyzing these data is that if an imidazolide anion were the reagent, there would be a large normal isotope effect upon deprotonation of the imidazole group of the histidine residue of **1**. However, this explanation would require the reaction to be very sensitive to pH, because the pK_A'' for deprotonation of the imidazole group is probably greater than 13.[21] The solvent isotope effect is unaffected by addition of *n*-decanol (Tables III and IV).

Hydrolysis of Other Phosphate Esters. Micelles of **1** speed the dephosphorylation of other trisubstituted phosphate esters (Table V). The rate–surfactant profiles for dephosphorylation of di-*n*-hexyl *p*-nitrophenyl phosphate (Table V) are similar to those shown in Figure 1 for *p*-NPDPP, and the rate is increased by a factor of approximately 60 (in water $10^7 k \approx 2$ s^{-1}, at pH 8.5[14b]). The kinetic solvent isotope effects, k_{H_2O}/k_{D_2O}, for the di-*n*-hexyl phosphate are very similar to those for *p*-NPDPP (Tables IV and V). The increase of rate of dephosphorylation of

(18) In micelles of cetyltrimethylammonium bromide, OH^- and F^- give similar rates of dephosphorylation of *p*-NPDPP and it is safe to assume that direct attack of OH^- is unimportant at pH <9.
(19) Cordes, E. H.; Gitler, C. *Prog. Bioorg. Chem.* **1973**, *2*, 1.
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(21) The pK_A'' for deprotonation of benzimidazole has been reported as 12.3[22a] and 11.26[22b] from measurements in buffered solution. The lower value appears to be incorrect, because in unbuffered solution $pK_A'' = 12.8$.[22c]
(22) (a) Yatsimirski, A. K.; Osipov, A. P.; Martinek, K.; Berezin, I. V. *Colloid J. USSR (Engl. Transl.)* **1975**, *37*, 470; (b) Hisano, T.; Ichikawa, M. *Chem. Pharm. Bull.* **1974**, *22*, 1974. (c) Romsted, L. S., unpublished results.

Table VI. Dephosphorylation of *p*-Nitrophenyl Diphenyl Phosphate in Comicelles of Brij and 2[a]

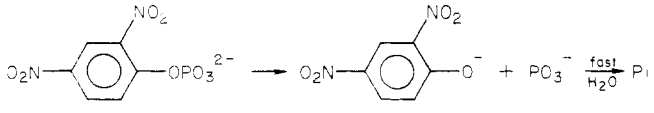
10^5[2], M	10^5[*p*-NPDPP], M	10^5k_Ψ, s^{-1}	10^5[2], M	10^5[*p*-NPDPP], M	10^5k_Ψ, s^{-1}
1.50	1.50	1.15	3.00	1.50	1.59
1.50	3.00	0.99	3.00	3.00	1.42

[a] At 25.0 °C in 10^{-3} M Brij and 0.015 borate buffer (pH 8.0).

di-*n*-hexyl derivative **5** in going from pH 8.5 to 9.3 (Table V) shows that there is an incursion of reaction with imidazolide anion at the higher pH, and the value of k_{H_2O}/k_{D_2O} then depends in part upon the isotope effect on deprotonation of the imidazole moiety.

Micelles of **1** also speed the dephosphorylation of diethyl *p*-nitrophenyl phosphate (Table V), but we did not use enough of the surfactant to fully bind the substrate and did not reach a constant rate for this reaction.

Cationic micelles speed the spontaneous hydrolysis of dinitrophenyl phosphate dianions,[23] and we observe this behavior with 2,4-dinitrophenyl phosphate at pH 8.5 (Table V). The approximately sevenfold rate enhancement is smaller than that with micellized CTABr,[23] and the functional histidine moiety plays no role as a nucleophile or general base in this spontaneous reaction of the dianion. The rate enhancement is due solely to the medium effect of the micelle on this unimolecular reaction. However, micellar-bound aliphatic primary amines attack the aryl group of the 2,4-dinitrophenyl phosphate dianion.[24]



Reactions in the Imidazole Surfactant (2). The rate–surfactant profiles for dephosphorylation of *p*-NPDPP at pH 8.0 are very similar to those in the presence of the histidine surfactant (**1**) (Figure 1), although micelles of the imidazole surfactant (**2**) are the more effective catalysts.

There is no buildup of a phosphorylated imidazole in the course of the reaction because we obtained clean first-order kinetics with no "burst" of *p*-nitrophenoxide when the reaction was carried out with comicelles of Brij and **2** and with [substrate] > [**2**] (Table VI).

The kinetic solvent isotope effects are similar to those found for reactions in the histidine surfactant **1**, and at pH (pD) 8.0 (0.015 M borate), k_{H_2O}/k_{D_2O} = 2.3 for reactions in 1×10^{-3} and 2×10^{-3} M **2** (Figure 1).

Effect of pH on Dephosphorylation in Micelles of 1 and 2. Although the rate–surfactant profiles for dephosphorylation are similar for micelles of the two functional surfactants (Figure 1), there is a difference in the effect of pH, because with a fully bound substrate the rate increases sharply for reaction in the presence of the imidazole surfactant (**2**) (Figure 2). These results suggest that the imidazole of micellized **2** is deprotonated at high pH and that the anionic moiety **2a** is a more effective dephosphorylating agent than the undissociated imidazole (Scheme II).

The value of the apparent pK_A' of micellized **2** is 6.23 (Table II), and we assume that it can be used under our kinetic conditions, i.e., in the presence of 0.02 M buffer.[25]

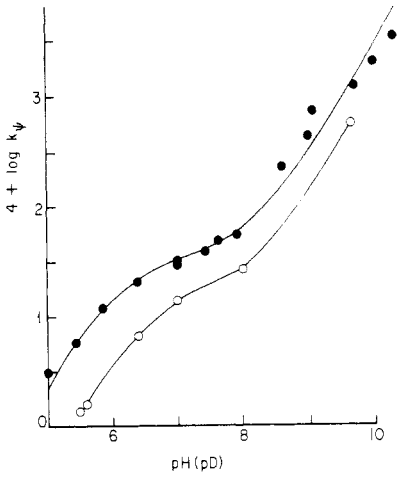
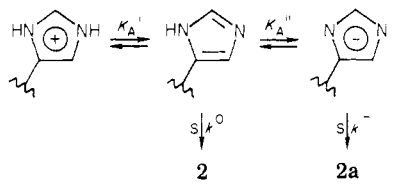


Figure 2. Dephosphorylation of *p*-nitrophenyl diphenyl phosphate in 2–2.4 mM **2**: solid circles in H_2O, open circles in D_2O.

Scheme II



The rate constants k^0 and k^- are for dephosphorylation of fully micellar bound substrate catalyzed by the nonionic and anionic imidazole moieties, respectively. Reaction in the aqueous pseudophase can be neglected, and if we neglect reaction in the micelles with hydroxide ion or water, the first-order rate constant, k_Ψ, is given by eq 2.

$$k_\Psi = \frac{K_A'}{a_H^+ + K_A'}\left(k^0 + \frac{k^- K_A''}{a_H^+}\right) \qquad (2)$$

The rate data for reaction in micelles of **2** can be fitted to the parameters $k^0 = 3.6 \times 10^{-3}$ s^{-1} and $k^- K_A'' = 3 \times 10^{-11}$ M s^{-1}, and the curve, calculated by using these parameters and taking a_H^+ = –antilog pH, is shown in Figure 2. The agreement is satisfactory in view of the assumptions in the treatment, especially as regards micellar effects upon buffer equilibria.

The value of $k^0 = 3.6 \times 10^{-3}$ s^{-1} is very close to that of $k = 3.7 \times 10^{-3}$ s^{-1} for the reaction of fully micellar-bound substrate shown in Figure 1, showing that there is little contribution from the imidazolide ion moiety in the reaction in micellized **2** at pH 8.0. We do not know the value of K_A'', but it is probably less than 10^{-13} (cf. ref 21) so that k^- should be greater than 300 s^{-1}. The imidazolide ion would then be more reactive than the imidazole moiety by a factor of greater than 10^5. (We note that the imidazole moiety appears to act as a general base, whereas its anion is probably a good nucleophile in dephosphorylation.)

We did not attempt to analyze the relation between k_Ψ and pD because of isotope effects upon the acid-dissociation constants K_A' and K_A''.

Effects of Added Solutes on Dephosphorylation in Micelles of 2. Several experiments were carried out on

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(24) Bunton, C. A.; Diaz, S.; Hellyer, J. M.; Ihara, Y.; Ionescu, L. G. *J. Org. Chem.* **1975**, *40*, 2313.
(25) Micellar effects upon acid–base equilibria are complicated and not well understood, and some of the problems are discussed in ref 26.
(26) (a) Romsted, L. S. In "Micellization, Solubilization and Microemulsions"; Mittal, K. L., Ed.; Plenum: New York, 1977; Vol. 2, p 509. (b) Bunton, C. A.; Romsted, L. S. In "The Chemistry of Acid Derivatives"; Patai, S., Ed.; Wiley Interscience: New York, 1979; Suppl. B, Part 2, Chapter 17.

Table VII. Dephosphorylation in Comicelles of the Imidazole Surfactant 2[a]

pH	solute	[2]/[solute]	10^3k_Ψ, s^{-1}
8.0			3.12 (3.72)
8.00	CTABr	1	2.27
8.00	CTABr	0.5	1.47
8.00	8	1	2.17 (1.89)
8.00	Brij	0.5	0.58
8.00	*p*-cresol	1	3.74 (4.06)
9.00[c]			(44.7)
9.00	CTABr	1	19.8
9.00	8	1	15.7
10.0[d]			(203)
10.0	CTABr	1	91.7
10.0	8	1	92.8

[a] At 25.0 °C with 10^{-3} M **2**; the values in parentheses are for 2×10^{-3} M **2**. [b] 0.015 M borate. [c] 0.02 M borate. [d] 0.02 M carbonate.

Table VIII. Dephosphorylation by the Model Compound 3[a]

10^3[3], M	10^5k_Ψ,[b] s^{-1}	10^3[3], M	10^5k_Ψ,[b] s^{-1}
c	0.97 (9.34)	5.0	1.62 (8.71)
1.0	1.12 (9.49)	10.0	1.86
2.0	1.20 (9.46)		

[a] At 25.0 °C in 0.015 M borate buffer (pH 8.0). [b] The values in parentheses are in the presence of 10^{-3} M CTABr. [c] In absence of **3**.

the effects of other surfactants or *p*-cresol on dephosphorylation (Table VII). All the added solutes except *p*-cresol slowed the reaction. These results were not unexpected because even the hydroxyethyl surfactant (**8**) decreases the rates of dephosphorylation when comicellized with the imidazole surfactant (**2**) although at high pH it is an effective nucleophile (cf. ref 4–6). Aryl oxide ions are effective dephosphorylating agents, especially in the presence of cationic micelles, and their small contribution to the reaction rate in micelles of **2** is consistent with their effects in micellized CTABr (Table VII and ref 27).

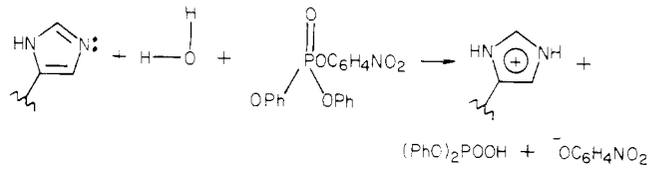
Dephosphorylation in the Presence of the Model Compound 3. In the absence of surfactant the model compound, trimethyl[(4-imidazolyl)methyl]ammonium chloride (**3**) speeds the dephosphorylation of *p*-NPDPP (Table VIII), and the second-order rate constant is ca. 10^{-3} M^{-1} s^{-1}. It slightly decreases the rate in the presence of CTABr, probably because it is too hydrophilic to be bound to the micelles (cf. ref 28), and the added chloride ion is an inhibitor.[17]

Discussion

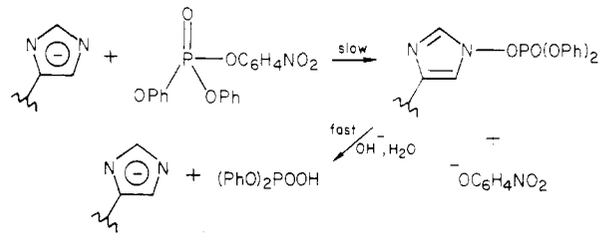
Role of the Imidazole Moiety. The nonionic imidazole group is the main catalytically effective species in the micellar-mediated reactions in the presence of either the histidine surfactant (**1**) or the imidazole surfactant (**2**) at pH <8.5 (Figures 1 and 2). The deuterium solvent isotope effects (Figure 1 and Tables III–V) are larger than expected for nucleophilic catalysis[20] and are consistent with the imidazole acting as a general base and activating a water molecule (Scheme III).

However, the imidazole moiety does not effectively activate the hydroxyl group of an added solute or surfactant (Tables III, V, and VII). The surfaces of normal micelles are highly aqueous, and Menger has suggested that water

Scheme III



Scheme IV



may penetrate deeply into micelles,[29,30] so that water would be the most readily available nucleophile in the vicinity of the phosphoryl group and therefore the most readily activated by the imidazole group.

Although the two functional surfactants behave similarly at pH ca. 8 (Figure 1), there are differences. The rate–surfactant profile rises much more steeply with the imidazole (**2**) than with the histidine (**1**) surfactant probably because of the lower cmc of the former (7.9×10^{-5} M[4] as compared with ca. 2×10^{-4} M for 1[8]). Micelles of the imidazole surfactant also give larger rate enhancements, at pH 7–8, by a factor of ca. 400 as compared with ca. 95 for **1**. (The rate constant in water at pH 8 is ca. 10^{-5} s^{-1}, and CTABr increases the rates of reaction with OH^- or F^- by approximately 1 order of magnitude.[14a])

Reaction at pH >8.5. Imidazole derivatives are very weak acids,[22] but the pH–rate surfactant profiles for several deacylations catalyzed by functional micelles containing the imidazole head group show that imidazolide anions are excellent nucleophiles.[3-5,13,32]

Because of deuterium solvent isotope effects upon deprotonation we cannot obtain kinetic isotope evidence on the catalytic role of the imidazole group at high pH, and there is only limited evidence in the literature to guide us.

Westheimer and his co-workers have shown that heterocyclic bases can act as both nucleophilic and general-base catalysts in dephosphorylation,[33] and it is probable that the reactions at high pH mediated by the imidazole surfactant (**2**) involve nucleophilic attack by the imidazolide anion as shown in Scheme IV.[34] If this explanation is correct, the phosphorylated intermediate must be readily hydrolyzed because we see no evidence for the buildup of an intermediate (Tables III and VI). There is kinetic evidence for the formation of transient intermediates in the imidazole-catalyzed hydrolysis of tetrabenzyl pyrophosphate[33] and in the benzimidazole-catalyzed hydrolysis

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(28) Gitler, C.; Ochoa-Solano, A. *J. Am. Chem. Soc.* **1968**, *90*, 5004.
(29) Menger, F. M. *Acc. Chem. Res.* **1979**, *12*, 111.
(30) Polyoxyethylene head groups of nonionic micelles are also heavily hydrated.[31]
(31) Sepulveda, L.; MacRitchie, F. *J. Colloid Interface Sci.* **1968**, *28*, 19.
(32) (a) Heitmann, P.; Husing-Bublitz, R.; Zunft, H. *J. Tetrahedron* **1974**, *30*, 4137. (b) Martinek, K.; Yatsimirski, A. K.; Levashov, A. V.; Berezin, I., ref 26a, p 489.
(33) Dudek, G. O.; Westheimer, F. H. *J. Am. Chem. Soc.* **1959**, *81*, 2641. Blakeley, R.; Kerst, F.; Westheimer, F. H. *Ibid.* **1966**, *88*, 112.
(34) The value of $k_{H_2O}/k_{D_2O} \approx 2.2$ at pH (pD) = 9.6 (Figure 2) is consistent with the imidazolide anion acting as a nucleophile. The net deuterium solvent isotope effect is due to a normal equilibrium isotope effect on deprotonation of the imidazole group multiplied by an (small) effect on nucleophilic attack. If the imidazolide anion were acting as a general base, we would expect the net isotope effect to be much larger than that observed.

of *p*-nitrophenyl diphenyl phosphate in the presence of CTABr.[35]

The imidazole moiety in the histidine surfactant (**1**) is less reactive than that in the imidazole surfactant (**2**), possibly because it is somewhat buried in micelles of **1**. It is therefore understandable that we see a smaller rate increase from an increase of pH for the reaction in the histidine surfactant (**1**), and such a steric effect should be more pronounced for nucleophilic dephosphorylation than for a general base catalyzed reaction.

The histidine group is believed to play a key role in enzyme-mediated dephosphorylation,[36] and in these systems phosphorylations of histidine and of serine hydroxyl groups have been observed, so that the mechanisms which we postulate have precedence in enzymic systems.

Source of the Rate Enhancements in Functional Micelles. Very large rate enhancements have been observed in the presence of some functional micelles, especially in deacylation.[3-5] Three factors are involved in these rate enhancements as compared to reaction in water: (i) a new reaction path is introduced involving the functional group; (ii) micellization can increase the extent of deprotonation of a functional group; (iii) the functional group and the substrate are concentrated at the micellar surface.

The contribution of iii can be estimated on the assumption that rate enhancements of bimolecular reactions are due largely to concentration of reactants in the micellar Stern layer[7,11,27,32b,37] and that one can estimate the volume of this region. Provided that the micellar Stern layers of CTABr and functional micelles, e.g., of **2**, have similar volumes[7,38] (ca. 0.14 L mol^{-1}), the first-order rate constant for a fully micellar bound substrate (k_{max}) should be given by eq 3, where k_w is the second-order rate constant for a

$$k_{max} \approx k_w/0.14 \quad (3)$$

nonmicellized but otherwise similar reactant.[7,39] In the present system the imidazole derivative **3** is a suitable model for the purpose of comparison, and under conditions in which the nonionic imidazole group is the reagent, the second-order rate constant in water is $k_w \approx 10^{-3}$ M^{-1} s^{-1} (Table VII). The predicted maximum rate constant for dephosphorylation of micellar-bound *p*-NPDPP in **2** is therefore ca. 7×10^{-3} s^{-1} whereas the actual value is 3.6×10^{-3} s^{-1} (Figures 1 and 2). This agreement is very good because the model is a very simple one which involves assumptions regarding the volume of the Stern layer in micelles of **2**[39] and the actual rate constants in the micellar pseudophase.

Similar conclusions have been drawn regarding reactions in micelles of the hydroxyethyl surfactant (**8**), but in this case allowance had to be made for incomplete deprotonation of the hydroxyl group under the experimental conditions.[7] However, this simple model fits the rate enhancements of dephosphorylation by comicelles of CTABr and an oximate surfactant.[40]

Table IX. Dephosphorylation Catalyzed by Immobilized **1**[a,b]

	CGC-241 (1:17)	Dowex 50-W (1:6)	C-350 (1:12)
run 1	1.2	2.3	1.6
run 2	1.2	3.0	2.0

[a] Values of $10^4 k_\Psi$ (s^{-1}) at 25.0 °C and pH 8.0. The ratios of **1** to resin, in mequiv, are in parentheses. [b] In the presence of Dowex-50, $k_\Psi \approx 8 \times 10^{-7}$ s^{-1}, and in water $k_\Psi \approx 10^5$ s^{-1}.

Micellar rate enhancements of bimolecular reactions are often described in terms of increased reactant concentrations in the micellar pseudophase.[7,32b,37] They have also been ascribed to favorable effects upon the entropy of activation,[41,42] but these differences in description are more apparent than real, because incorporation of a reactant solute in the micelle involves loss of translational entropy in the initial state and may therefore increase the activation entropy for the overall reaction.

Acknowledgment. Support of this work by the National Science Foundation (Chemical Dynamics) and the Army Office of Research is gratefully acknowledged.

Appendix

Dephosphorylation by Immobilized 1. Immobilized reagents have a number of advantages over their soluble counterparts,[43] and **1**, bound to sulfonated polystyrene ionic exchange resins (Baker analyzed reagent grade), is a reusable catalyst of the dephosphorylation of *p*-NPDPP.

The resin was treated with 2 M NaOH and after a thorough washing with water was dried in vacuo at 50 °C. The resin (6.8×10^{-2} mequiv) was saturated with **1**, and after 24 h the supernatant was removed and its content of **1** determined spectrophotometrically. The resin was washed (H_2O), and 3 mL of 0.015 M borate buffer (pH 8.0) was added followed by 5 μL of 6 mM *p*-NPDPP in dioxane. The formation of *p*-nitrophenoxide ion in the stirred solution was followed spectrophotometrically at 25.0 °C. This formation followed first-order kinetics. The reaction was faster than those in water and in the presence of the resin alone (Table IX).

After complete reaction, the anionic products can be removed with the supernatant, and the resins impregnated with **1** were reusable as catalysts after being washed with water. They were used for several cycles with little loss of activity, and the results for the first two cycles are given in Table IX. The overall rate enhancements are modest, and shortage of **1** prevented our establishing the best conditions for the reactions, but the rate constants can be correlated qualitatively with the ratio of **1** to resin (Table IX).

Registry No. **1**, 55019-70-0; **2**, 57879-45-5; **3**, 57879-42-2; **4**, 311-45-5; **5**, 57016-65-6; **6**, 6186-33-0; **9**, 20056-44-4; *p*-NPDPP, 10359-36-1; 4-(chloromethyl)imidazole, 23785-22-0; Me_3N, 75-50-3.

(35) Quan, C.; Hong, Y.-S., unpublished results.
(36) Hultquist, D. E.; Moyer, R. W.; Boyer, P. D. *Biochemistry* **1966**, *5*, 322. Ostrowsky, W.; Barnard, E. A. *Ibid.* **1973**, *12*, 3893.
(37) Cuccovia, I. M.; Schroter, E. M.; Monteiro, P. M.; Chaimovich, H. *J. Org. Chem.* **1978**, *43*, 2248.
(38) The size of the head group in micellized **2** is such that the volume of the Stern layer is probably larger than that estimated for CTABr,[7,39] and if this is so, our predicted value of k_{max} would be too low.
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