Evidence for the Formation of Acylated or Phosphorylated Monoperoxyphthalates in the Catalytic Esterolytic Reactions in Cationic Surfactant Aggregates

Santanu Bhattacharya* and Karnam Snehalatha

Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India

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Monoperoxyphthalate (MPP) was solubilized in three different aqueous cationic surfactant aggregates composed of (i) a micellar cetyltrimethylammonium chloride (CTACl) solution; (ii) an oil-in-water (O/W) microemulsion (ME) stabilized by CTACl, and a cosurfactant, *tert*-butyl alcohol, and (iii) a vesicular medium composed of dispersions of dihexadecyldimethylammonium chloride (DHDAC). At pH ∼8.5 and 25 °C, each of these formulations was used to cleave *p*-nitrophenyl diphenyl phosphate (PNPDPP). The aggregate and the maximum pseudo-first-order rate constants ([MPP] = 4×10^{-5} M, and [PNPDPP] = 1×10^{-5} M) for the PNPDPP cleavages are the following: buffer alone, 0.00034 s^{-1}; micelle: 0.024 s^{-1}; ME: 0.0048 s^{-1}; and vesicle: 0.025 s^{-1}. Importantly all the catalytic formulations showed "turnover" behavior in the presence of excess substrates. By the combined use of ^{1}H- and ^{31}P-NMR spectrometry and synthesis, it was possible to provide evidence for the formation of acylated or phosphorylated monoperoxypthalates in the catalytic hydrolyses in cationic surfactant aggregates.

Introduction

Reagents derived from hydrogen peroxide (H_2O_2) are of great importance in synthetic as well as in practical organic chemistry.[1] For example, the peracids are effective reagents for several important synthetic transformations.[2] Bunton and others[3] emphasized the reactivity of hydroperoxide and *m*-chloroperbenzoate (MCPBA)[3a] nucleophiles in micelles toward dephosphorylation of nerve gas simulant, *p*-nitrophenyl diphenyl phosphate (PNPDPP). Several related reagents such as peroxycumyl ion,[3b] and a *functional* hydroperoxide surfactant,[3c] were also used for effecting deacylation reactions in aqueous micellar media. The hydroperoxide anion (HO_2^-), an α-effect nucleophile[4] of lower basicity ($pK_a \sim 11.0$) than OH^-, has also been employed to neutralize real nerve agents[5] in strongly basic media.[6] But all of them showed only *stoichiometric* reactivity and by themselves most of these were found to be either shock-sensitive or explosive.[7] Lack of catalytic properties in addition to such hazardous properties of the above cited reagents severely limit their potential for their practical use in the decontamination of toxic and persistent organophosphates.

From the continuing work of several research groups, a number of reagents have now been developed for dephosphorylation.[8−12] However, very few reagents are currently available that are inexpensive, biodegradable, and at the same time show *catalytic* rather than stoichiometric dephosphorylating activities at pH ≤ 9. Notable exceptions include micellar iodosobenzoate (IBA) and related derivatives,[13] a reagent developed by Moss, and metallomicelles developed by Menger.[14] Given the unusual importance[15] of the destruction of phosphorus-based persistent toxins, there is a pressing need for the sustained search of alternative decontamination recipes and examination of their detailed mechanistic rationale.

* Author to whom the correspondence should be addressed, Fax: +-91-080-334-1683; E-mail: sb@orgchem.iisc.ernet.in.

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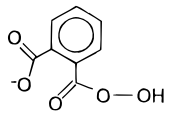
Toward this goal, we considered monoperoxypthalate (MPP)[16] as a reagent which is neither shock-sensitive, nor deflagrating, and is available both in bulk as well as a laboratory reagent. Despite being well known as a reagent, its use in dephosphorylation reactions was not exhaustively examined.[17] Consequently, the key mechanistic details of MPP's hydrolytic functions remains obscure. Due to our interest in the chemistry of aggregates, we have been examining the reactivity and mechanism in several organized assemblies including micelles,[18] hydrophobic ion-pairs,[19] bilayers,[20] microemulsions,[21] and other supramolecular aggregates.[22] In this report, we demonstrate the utility of MPP in cationic aggregates as a potent, truly *catalytic* reagent for both deacylation and dephosphorylation reactions and also provide evidence of the intermediates formed during such reactions by ^{1}H- and ^{31}P-NMR spectroscopy for the first time.



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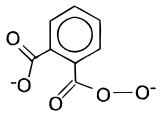

1a

Results and Discussion

Although MPP (magnesium salt of monoperoxyphthalate) finds widespread use in various synthetic transformations,[1,2] the abilities of the MPP toward deacylation or dephosphorylation reactions in water were not explored in detail. This could be due to its high susceptibility to decomposition in aqueous solution at ~50% dissociation of the peroxy acid.[3a,23] The presence of heavy metal ions and borate buffer further accelerates this decomposition process. The commercially available salt of the MPP, **1**, contains a COO^- group and an ortho peroxy acid (C(O)OOH) function. The ability of MPP to act as a nucleophile comes from the deprotonation of the C(O)OO*H* (peracid) moiety. The presence of this electron-withdrawing, ortho substituent -C(O)OOH facilitates ready ionization of the C(O)OH group at pH 5 in the parent monoperoxyphthalic acid. However, the ionization of C(O)OOH in monoanionic **1** in water into a dianion, **1a**, is much less facile due to the lack of resonance stabilization of the resulting anion. We thought that the binding of MPP dianion with a cationic aggregate matrix should mitigate the of pK_a C(O)OOH in MPP. Since the nucleophilic capacity of MPP originates from the ionization of C(O)OOH (α-effect nucleophile) in MPP into the $C(O)OO^-$ form, **1a**, it is important to achieve this ionization at lower pH. It is well known[24] that aromatic solutes interact strongly with micellar and related cationic headgroups, which should further help the binding of MPP anions. We thought that solubilization of MPP in *cationic* micellar or related aggregates would assist ion-pair [$CTA^+ \cdots {}^-OOC$] formation and thereby bring MPP closer to the aggregate headgroup region, and this should also assist in mitigating the pK_a of C(O)OOH in MPP relative to the same in water. Therefore at the start we decided to determine the pK_a values of MPP in water as well as in cationic micelles.

pK_a Determination in Cationic Micelles. The determination of the systemic pK_a value of the C(O)OO*H* group in MPP was initiated by measuring the rate constants of the cleavages of *p*-nitrophenyl diphenyl phosphate (PNPDPP) over a pH range of 6−9.5. The apparent pK_a of the *micellar* CTACl-bound MPP was determined from the rate-constant *vs* pH plots for the cleavages of 1×10^{-5} M PNPDPP by 4×10^{-5} M MPP in 1×10^{-3} M micellar CTACl, 0.02 phosphate buffer, at 25 ± 1 °C. The buffer solution also contained 0.33 vol % CH_3CN as residuals from PNPDPP introduction. A plot of log k_ψ *vs* pH (Figure 1a) revealed a discontinuity at pH ~8.1 which was taken as the systemic pK_a for the conversion of -C(O)OOH to the nucleophilic, anionic $C(O)OO^-$ form of MPP under the micellar reaction conditions. To further ensure this result we also obtained the pK_a value of MPP in CTACl micelles by potentiometric titrimetry *cf.* Experimental Section. This also gave similar pK_a value of MPP (~8.0) under micellar conditions.

On the basis of the known analogies of the kinetic effects on the surfaces of various association colloids as long as the surface charge is similar (cationic),[24] we assume that the values obtained in micellar media would be similar in other cationic aggregates examined herein. On the basis of the titrimetric measurements, we found the pK_a of this peracid group of MPP in water to be ~8.5 which is considerably higher than that in related *m*-chloroperbenzoic acid (mCPBA) which has a pK_a of ~7.6 for the C(O)OOH function. The higher pK_a of **1**, relative to mCPBA, should be at least partly due to the fact that **1a** must ionize from a monoanion to a dianion, while mCPBA ionizes from a neutral acid to a monoanion.

Kinetic Studies of Hydrolysis of PNPDPP with Excess Catalyst. Micelles. At the start, a micellar solution of CTACl in 0.02 M phosphate buffer, pH = 8.5, was chosen as the medium for the cleavages of PNPDPP by an excess of MPP. The dephosphorylation properties of monoperoxyphthalate were assessed from a complete rate constant *vs* [CTACl] profile for the cleavages of 1×10^{-5} M PNPDPP by 4×10^{-5} M MPP at 25 ± 1 °C under

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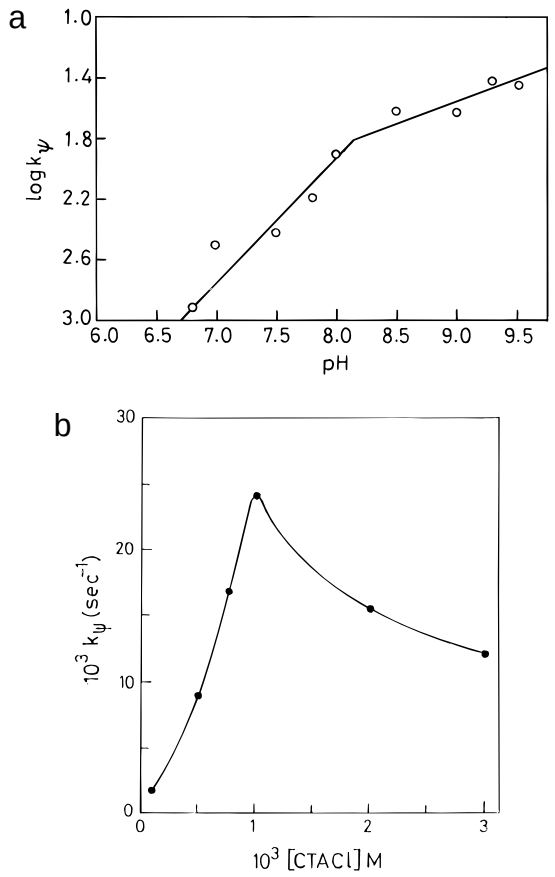

Figure 1. (a) The pH-rate constant profile for cleavage of 1 × 10^{-5} M PNPDPP by 4 × 10^{-5} M MPP in phosphate buffer. See text for a detailed description of kinetic methods and reaction conditions. (b) Pseudo-first-order rate constants for the cleavage of 1 × 10^{-5} M PNPDPP by 4 × 10^{-5} M MPP as a function of [CTACl] at pH = 8.5.

the conditions described above. The concentration of CTACl was gradually increased from 1 × 10^{-4} M to 3 × 10^{-3} M, and reactions were followed with nucleophile in excess over substrate by monitoring the release of *p*-nitrophenoxide at 400 nm. The reproducibility of k_ψ was within ±2% in duplicate runs. The k_ψ *vs* [CTACl] profile (Figure 1b) shows that the reactivity increases initially with increasing [CTACl] reaching a maximum at [CTACl] = 1 × 10^{-3} M, and then the k_ψ slows down probably because of the dilution effect, *i.e.* the number of available MPP anions per micelle decreases with increase in [CTACl]. This rate constant *vs* [surfactant] correlation resembles other known examples of micellar catalysis.[9a] A catalytic rate constant of 846 M^{-1} s^{-1} was observed at pH ~8.5, [MPP] = 4 × 10^{-5} M, [CTACl] = 1 × 10^{-3} M, [PNPDPP] = 1 × 10^{-5} M. In contrast MPP in buffer (pH ~8.5) alone in the absence of any CTACl showed insignificant activity toward PNPDPP (k_{cat} = 0.085 M^{-1} s^{-1}). Thus the reactivity of MPP was enhanced in cationic micelles by *ca.* 4-orders of magnitude relative to MPP in buffer alone. We have attributed the binding of the substrate to the micellar CTA/MPP aggregates as the key reason for the observed kinetic potentiation of MPP in micellar CTACl.

Microemulsions. For comparison, the ability of MPP to act as a nucleophile for the cleavages of PNPDPP was also tested in a *cationic* microemulsion (ME) system under comparable conditions. In oil-in-water microemulsion (O/W ME)[25] comprised of CTACl (5 wt %), *t*-BuOH (5 wt %), cyclohexane (2.5 wt %), and phosphate buffer, pH ~8.5 (87.5 wt-%), MPP (4 × 10^{-4} M) showed much less reactivity than in micelles. On a second-order basis, the catalytic rate constant for PNPDPP cleavage in this ME system was 17.0 M^{-1} s^{-1} after correction for 100% ionization of the C(O)OOH group in MPP assuming a similar pK_a of MPP in CTACl stabilized O/W ME comparable to that in CTACl micelles. Significantly reduced reactivity in ME may originate from the inaccessibility of the hydrophobic PNPDPP to the highly water-soluble MPP in O/W ME. This is expected as their mutual inclusion sites could be profoundly different.[26] It is also important to note that in this recipe, the [CTACl]/[MPP] ratio is 16-fold higher than that in the previously described micellar system. Thus in this situation, the number of MPP ions per ME droplet is also much lower than that in the micelle, making the MPP in ME formulation kinetically less competent.

Table 1. Comparison of Kinetic Data for MPP-Catalyzed Hydrolyses of *p*-Nitrophenyl Diphenyl Phosphate in Different Cationic Aggregates[a]

reaction media	10^3[surf.], M	10^5[MPP], M	$10^3 k_\psi^{max}$, s^{-1}	k_{cat},[b] M^{-1} s^{-1}	k_{cat}^{rel} (k_{cat}/k_0)
water	–	400.0	0.34	0.085	1.0
$ME_{o/w}$[c]	160.0	40.0	4.8	16.78	197.4
micelle	1.0	4.0	24.2	846.04	9953.4
vesicle[d]	0.5	4.0	25.3	884.49	10405.8

[a] Conditions: 0.02 M phosphate buffer, pH 8.5, μ = 0.1 (KCl), 25 ± 0.1 °C, [PNPDPP] = 1 × 10^{-5} M. [b] $k_{cat} = k_\psi^{max}$/[MPP], corrected for 100% ionization of the catalytic system. See text for discussion of the apparent pK_a of MPP in cationic aggregates. [c] Microemulsion (O/W) was prepared by mixing CTACl (5 wt %), *t*-BuOH (5 wt %), 0.02 M phosphate buffer, (87.5 wt %), and cyclohexane (2.5 wt %). [d] [DHDACl] = 5 × 10^{-4} M, see text for the preparation of vesicles.

Vesicles. Cleavage of PNPDPP by MPP was also studied in *cationic* vesicles composed of dihexadecyldimethylammonium chloride (DHDAC), which were prepared by sonication of 5 × 10^{-4} M of DHDAC in phosphate buffer, pH ~8.5, using an immersion probe [108 mm × 3 mm (diameter)] ultrasonic processor (Heat Systems) for 6 min at 25 W. PNPDPP (1 × 10^{-5} M) was reacted with MPP (4 × 10^{-4} M) in this vesicular medium under pseudo-first-order conditions as given in Table 1. The catalytic rate constant for PNPDPP in this vesicular system was determined and found to be ~885 M^{-1} s^{-1} after correction to full ionization of the nucleophilic C(O)-OOH → C(O)OO^- ion in vesicle-bound MPP. We assume herein that in *cationic* DHDAC vesicles the pK_a of MPP is comparable to that in *cationic* CTACl micelles. The slightly greater kinetic potency of *ca.* 1.05-fold observed in the MPP mediated PNPDPP cleavage in DHDAC vesicles than in CTACl micelles could result from tighter and more hydrophobic aggregate organization in the vesicles.[27]

Kinetic Studies with Excess Substrate. Next the peroxy acid MPP was examined for its ability to act as a true catalyst (turnover) in the dephosphorylation as well as in the deacylation reactions. With an excess of

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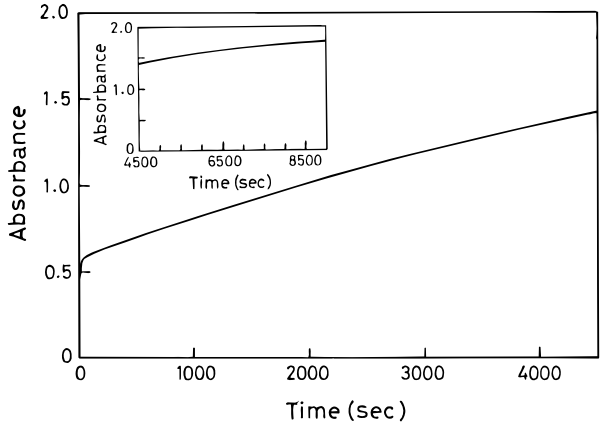
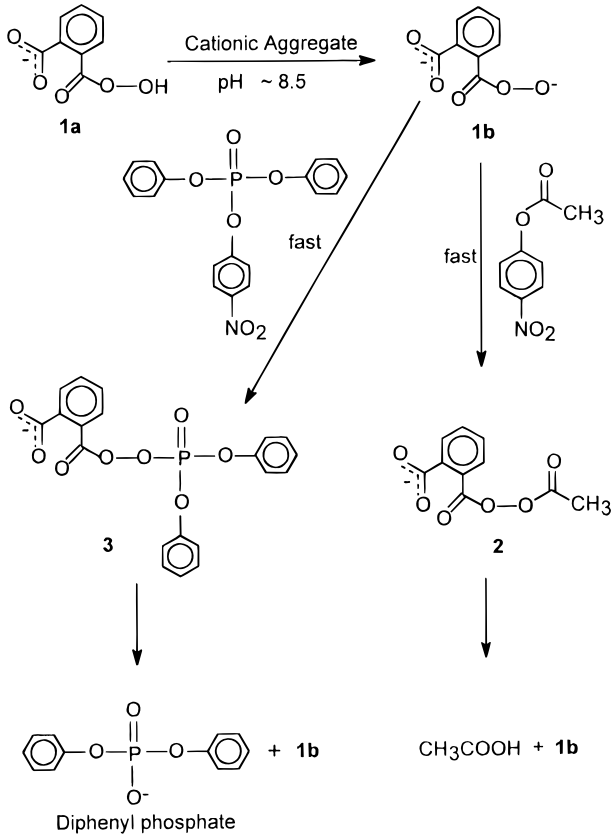


Figure 2. Kinetics under excess substrate conditions. Plot of absorbance *vs* time (s) for excess substrate cleavage. Reaction conditions at pH 8.5, 0.02 M phosphate buffer, $\mu = 0.08$ (KCl), 25 ± 0.1 °C, [CTACl] = 1×10^{-3} M, [MPP] = 4×10^{-5} M, [PNPDPP] = 1.0×10^{-4} M. Inset shows the "plateau" of the absorbance change with the time base extended to 4500−9000 s region.

PNPDPP over MPP in CTACl (1×10^{-3} M) in phosphate buffer at pH ~8.5, the reaction was found to be quite fast in the initial stages, but later the reaction slowed down considerably and it took 2 h for *quantitative* esterolysis of *excess* PNPDPP (Figure 2). Thus, rapid phosphorylation of the available MPP in CTACl by excess PNPDPP in 0.02 M, pH 8.5 buffer led to a rapid release ("burst")[28] of *p*-nitrophenoxide ion followed by a slower, nearly linear (with time) liberation of *p*-nitrophenoxide ion. When an excess of substrate is cleaved, an apparent plateau region is reached toward the end of the esterolysis presumably because of the released $(PhO)_2P(O)O^-$ ions which might absorb strongly to cationic micellar surfaces altering the effectiveness of the catalytic surface. The linear segment of the kinetic profile most probably represents the OH^--mediated, rate-determining scission of phosphorylated monoperoxyphthalate. The regenerated micellar CTA^+-bound $C(O)OO^-$ (MPP) is then rapidly rephosphorylated by the excess PNPDPP present in the reaction mixture (see below). In this situation, the intercept on the *y*-axis of the linear segment gives the absorbance of the *p*-nitrophenoxide ion that is stoichiometrically almost equivalent to the MPP that was phosphorylated in the initial burst phase. The ratio of the slope of the linear segment/[MPP] gives the rate constant of turnover (k_{turn}). The k_{turn} for the cleavage of 1×10^{-4} M PNPDPP by 3.3×10^{-5} M MPP (CTACl = 1×10^{-3} M, pH ~8.5) was found to be ~2×10^{-4} s^{-1}. The corresponding turnover rate constant, k_{turn}, for the cleavage of 1×10^{-4} M PNPA by 2.5×10^{-5} M MPP in micelles ([CTACl] = 1×10^{-3} M, pH ~8.5) was also determined and was found to be ~1.9×10^{-4} s^{-1}. A similar kinetic profile with slow turnover was also observed for the cleavage reactions of excess PNPDPP in CTACl ME and in DHDAC vesicles (figure not shown). These findings establish the utilities of MPP in different organized media toward dephosphorylation reactions.

NMR Experiments. As discussed earlier, the origin of the catalytic efficiency of the cationic aggregate-bound MPP ions must lie in their ability to exert their influence as powerful *O*-nucleophiles. The most consistent mechanism to explain the above observations is shown in Scheme 1. Cogent evidence for this two-step mechanism is available from the isolation of the acetate **2** or the diphenyl phosphate intermediate **3** in the reaction of MPP with PNPA or PNPDPP in micellar CTACl conditions and also in the observation of the burst kinetics in the reactions of MPP with both the PNPA and PNPDPP. In order to convincingly demonstrate the intervention of the proposed intermediates, we carried out careful NMR experiments as described in the following.

Scheme 1



^{1}H-NMR Spectrometry. In pH ~8.5 aqueous micellar CTACl (1×10^{-3} M) solution, 4×10^{-5} M MPP, **1**, cleaves PNPA ([PNPA] = 1×10^{-5} M) with k_ψ ~0.054 s^{-1}. The *O*-acetyl monoperoxyphthalate **2** appears to be the most likely intermediate in the above reaction scheme in which MPP when bound to a cationic aggregate surface at pH ~8.5, turns over to hydrolyze excess substrate, PNPA (Scheme 1). Thus if the reaction between **1** (0.05 M) with a 2-fold molar excess of PNPA is carried out in an unbuffered 0.01 M CTACl micellar D_2O/CD_3CN (2:1) medium in the presence of DO^- (pD ~8.85), monitoring by ^{1}H-NMR, we observe the formation of **2**, [$\delta(CH_3)$ ~2.19] and acetate ion $\delta(CH_3COO^-)$ ~2.1], coincident with a concomitant depletion in the signal intensity due to $CH_3COOC_6H_5NO_2$ (δ ~2.3) also detectable from the decrease in its NMR integration (Figure 3). This shows that the intermediate **2** accumulates considerably under conditions of pH ~8.5.

In order to unambiguously demonstrate the intervention of the intermediate **2** proposed in the above reaction (Scheme 1), we also synthesized *O*-acetyl monoperoxyphthalate **2** from the reaction (reflux, 15 min) of monoperoxyphthalate in the presence of a 2-fold excess of acetic anhydride. After the reaction, the excess, unreacted Ac_2O was pumped off to leave an off-white residue. ^{1}H-NMR of this material in a mixture of $CDCl_3$/DMSO-d_6 (9:1)

(28) Bender, M. L.; Kezdy, F. J.; Wedler, F. C. *J. Chem. Educ.* **1967**, *44*, 84.

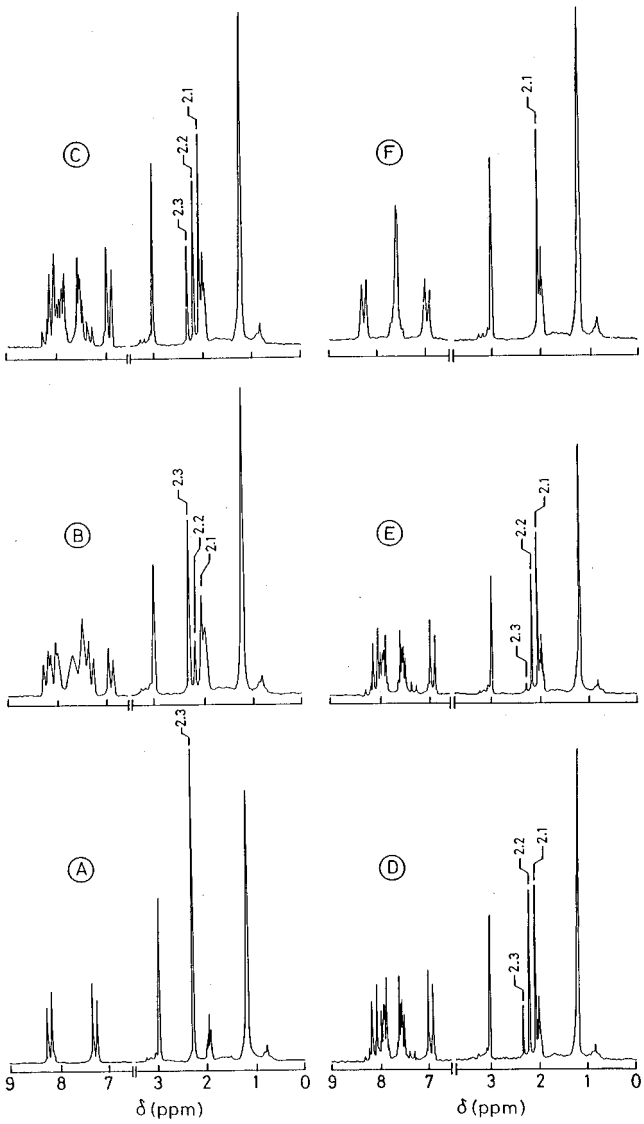


Figure 3. ^{1}H-NMR spectra for the hydrolysis of *p*-nitrophenyl acetate (see text for conditions). (A) *p*-Nitrophenyl acetate in the absence of MPP; (B) after the addition of MPP; (C−E) at intervals of 15 min; (F) after ~6 h.

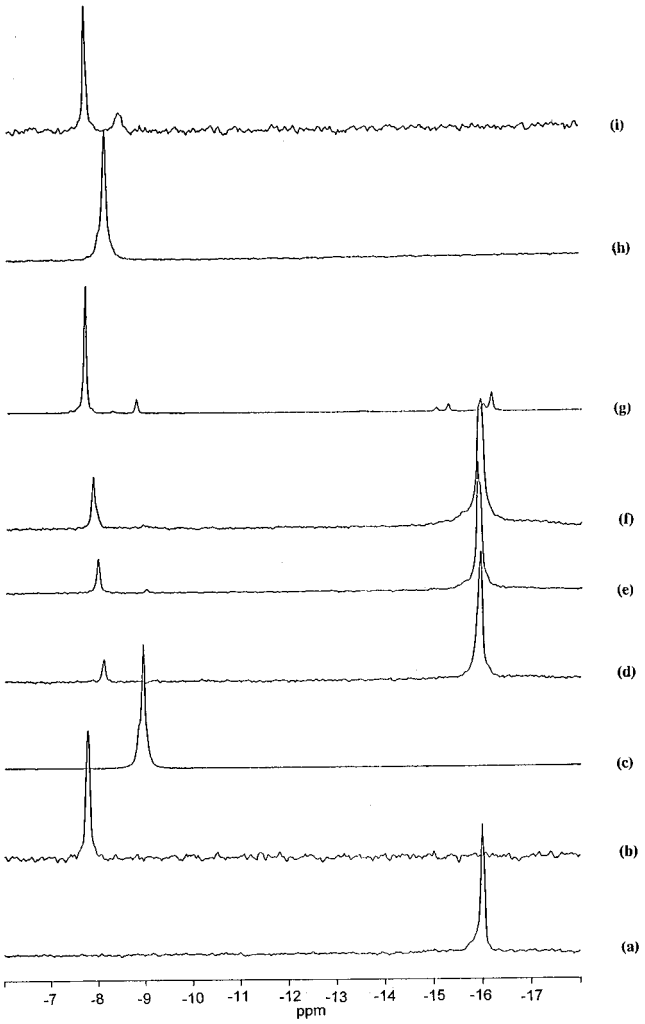


Figure 4. ^{31}P-NMR spectra for the hydrolysis of *p*-nitrophenyl diphenyl phosphate (PNPDPP) in cationic CTACl micelles (see text for conditions); (a) PNPDPP in the absence of MPP; (b) authentic spectrum of diphenyl phosphate under alkaline conditions; (c) spectrum of diphenyl phosphate under acidic conditions; (d−f) after the addition of MPP at consecutive intervals of 15 min; (g) after the addition of alkali to the reaction; (h) authentic spectrum of the intermediate **3**; (i) after the addition of alkali to the sample of the authentic intermediate.

gave a singlet at $\delta(CH_3)$ at ~2.0. Dissolution of the same material in micellar CTACl in D_2O/CD_3CN (2:1) gave the corresponding singlet at δ ~2.2. To further make sure that the above acetylation actually took place on the C(O)OOH function and not on the C(O)OH group in MPP, we also treated *m*-chloroperbenzoic acid (MCPBA) with Ac_2O which led to the formation of a white solid. This solid gave a singlet at $\delta(CH_3)$ at ~2.2 in $CDCl_3$ alone. But since the former was not sufficiently soluble in micellar CTACl in the D_2O/CD_3CN (2:1) medium, the NMR spectrum could not be recorded in the same.

Further evidence of acetylation on -C(O)OOH was available from the IR spectra of the resulting materials. MCPBA gave peaks at 1560, 1695 cm^{-1} with a shoulder at ~1720 cm^{-1}, 1760 cm^{-1} with a shoulder at 1770 cm^{-1} along with a broad peak at ~3200 cm^{-1}. Upon acetylation, the broad peak at ~3200 cm^{-1} disappeared and a new peak at ~1800 cm^{-1} emerged. Similarly, while monoperoxyphthalate gave IR signals at 1550, 1700, and 1738 cm^{-1} respectively, the corresponding product obtained upon acetylation gave IR signals at ~1600, 1740 with a shoulder at 1700 cm^{-1} and importantly a sharp signal at *ca.* 1845 cm^{-1}. The presence of ~1845 cm^{-1} signal implies the formation of a $C(O)OOCOCH_3$ perester.[29] Taken together, the above studies clearly establish the formation of *O*-acetyl monoperoxyphthalate **2** during the micellar reaction.

^{31}P-NMR Spectrometry. MPP-induced reaction on PNPDPP (0.1 M) in 0.01 M CTACl solutions in 2:1 water−acetonitrile, pH 8.5 (unbuffered), were also followed by ^{31}P-NMR spectrometry, with ^{31}P chemical shifts referenced to external 85% H_3PO_4. Under these conditions, authentic PNPDPP gave a ^{31}P signal at ~δ −15.97 in (2:1 H_2O:CH_3CN) (trace a, Figure 4). Pure diphenyl phosphate, $(PhO)_2P(O)O^-$, in this medium gave a signal at ~δ −7.75 (trace b, Figure 4) which upon acidification afforded diphenyl phosphoric acid, $(PhO)_2P(O)OH$, characterized by a signal at ~δ −8.9 (trace c, Figure 4). Trace d in Figure 4 shows the ^{31}P spectrum within ca. 15 min after the addition of 0.05 M MPP to 0.1 M PNPDPP in micellar 0.01 M CTACl media (2:1 H_2O/CH_3CN). As is

(29) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon: New York, 1988.

evident from the trace d, a new peak at $\sim\delta$ −8.1 emerges in addition to the signal due to excess unconsumed PNPDPP. The peak at $\sim\delta$ −8.1 accumulates further upon longer incubation of the reaction mixture in the NMR tube as shown in the traces e and f with concomitant depletion in the intensities of the peak at $\sim\delta$ −15.97. Addition of excess OH^- to this reaction mixture resulted in a rapid hydrolysis of the residual PNPDPP as shown by near complete disappearance of its signature at $\sim\delta$ −15.97. Alkaline treatment, however, also results in the replacement of the signal at $\sim\delta$ −8.1 by a major peak at $\sim\delta$ −7.75 and a minor peak at $\sim\delta$ −8.9. In a control experiment, we found that in unbuffered reaction mixtures of PNPDPP and MPP in micellar CTACl (2:1 H_2O/CH_3CN), composition-dependent signals due to the diphenyl phosphate/diphenyl phosphoric acid balance between these limits depending on the concentration of CTA^+ ions, pH, and water.

In order to explain these observations, we propose a plan[30] in which the micellar MPP is first rapidly phosphorylated to afford a diphenylphosphorylated intermediate, which upon alkaline treatment collapses to a mixture of diphenyl phosphate and diphenyl phosphoric acid in the unbuffered media. As expected, the diphenylphosphorylated intermediate **3** (Scheme 1) was found to be sensitive to aqueous media particularly in the presence of cationic surfactant micelles and related association colloids. Thus addition of OH^- to aqueous CTACl solutions of **3** increased the signal intensities due to diphenyl phosphate [−7.75 to −8.9] at the expense of **3** [δ $(PhO)_2P(O)OOC(O)C_6H_4CO_2^-$, −8.11]. To unambiguously demonstrate this point, we also attempted to synthesize an authentic sample of $(PhO)_2P(O)OOC(O)C_6H_4CO_2^-$ by the treatment of MPP (1.0 mmol), **1**, with diphenyl chlorophosphate (0.9 mmol) in the presence of dry pyridine (0.9 mmol). This afforded a material which gave an intense ^{31}P signal at $\delta \sim -8.11$ in micellar CTACl (2:1 H_2O/CH_3CN) media. The material was found to be sensitive to OH^- and the product obtained upon alkali treatment gave a ^{31}P peak which was indistinguishable from that of diphenyl phosphate. Thus from the spectroscopic studies and through successful synthesis of the acetate and diphenyl phosphate of MPP, it becomes clear that the deacylation or dephosphorylation reactions proceed via intermediates **2** and **3** in *cationic* aggregates (Scheme 1).

It should be mentioned herein that the dephosphorylation by monoperoxyphthalate and other peroxide nucleophiles in CTACl micelles were investigated earlier by Bunton and co-workers.[17] The main result of this study shows that the dephosphorylation by MPP dianion in CTACl micelles fits a pseudophase kinetic model. No attempt was made, however, to isolate the intermediate of the process especially in the presence of excess substrates. We have focussed our attention on the mechanistic aspects in the deacylation or dephosphorylation of PNPA or PNPDPP, respectively, and on the kinetic behavior in the presence of excess substrates. On the basis of the present study, we can see why micellar monoperoxyphthalates are such remarkably efficient dephosphorylating or deacylating agents. Under the cationic aggregate conditions, the OH^--mediated scission of the diphenylphosphorylated or acylated monoperoxyphthalate intermediate provides a way for turnover and completion of the catalytic cycle. Therefore the intrinsic reactivity of MPP requires a cationic aggregate environment for its potentiation.

How do the kinetic properties of MPP compare with those of the other micellar reagents for the cleavages of PNPDPP? MPP is certainly superior to phenoxide[31a,b] or benzimidazole[31c] in micellar CTACl media which are only active at pH > 10. However, MPP is characterized by slow rate-limiting turnover in CTACl and is kinetically ∼2.3-fold inferior to iodosobenzoate in CTACl at pH 8.0,[9b] which also turns over rapidly. Nevertheless the availability of a *catalytic* reagent that is available "off-the-shelf" and is nonhazardous and biodegradable should be a welcome addition to the list of effective reagents for decontamination. Examination of the MPP-mediated cleavage chemistry of *O,S*-dialkylphenyl phosphonothioates is currently under examination in our laboratory.

Experimental Section

Materials. All the reagents and solvents were of the highest grade available commercially and used purified, dried, or freshly distilled as required, by literature procedures[32] for the microemulsion preparation. Cyclohexane (Merck) and *tert*-butyl alcohol (SD-fine Chem.) were used at once after distillation. CTACl was obtained from Eastman and recrystallized from acetone. The aqueous phase was prepared using 0.02 M phosphate buffer prepared by mixing disodium hydrogen phosphate and monosodium dihydrogen phosphate with 0.08 M KCl and distilled water. The pH measurements were made with a Schott pH meter CG 825. Freshly prepared solutions were used in all experiments, and the concentration of the peroxy acid was determined by standard methods.[33] PNPDPP was prepared and purified by literature methods.[34]

pK_a Determination. The apparent pK_a of the *micellar* CTACl-bound MPP was determined from the rate-constant *vs.* pH plots for the cleavages of 1×10^{-5} M PNPDPP by 4×10^{-5} M MPP in 1×10^{-3} M micellar CTACl, 0.02 phosphate buffer, at 25 ± 1 °C. The buffer solution also contained 0.33 vol % CH_3CN as residuals from PNPDPP introduction. A plot of logk_ψ *vs* pH gave the systemic pK_a of the *micellar* CTACl bound MPP.

To further ensure this result the respective pK_a values for the MPP in CTACl micelles as well as in pure water (in the absence of any CTACl) were determined by potentiometric pH titration as described in the following. Briefly, solutions of MPP (1×10^{-3} M) in aqueous micellar 1×10^{-3} M CTACl were titrated with aqueous carbonate free 0.1 M NaOH solution under nitrogen. The temperature was maintained at 25 ± 0.1 °C. After the addition of each aliquot of NaOH solution, the pH value was recorded with a Schott pH meter CG 825. The pK_a of MPP in pure water (in the absence of any CTACl) was also determined by titrating the solutions of 1×10^{-3} M MPP in double distilled water with aqueous 0.1 M NaOH as described above. Respective plots of numbers of equivalents of base added *vs* pH gave titration curves, the midpoint of which corresponds to the pK_a values of MPP in pure water or in CTACl micelles.

(30) An intramolecular participation of the ortho-carboxylate function either as a nucleophile or via a general base mechanism appears likely. Similar conclusions have been also extended by Fife and coworkers, see for example, Fife, T. H. In *Adv. Phys. Org. Chem.* **1975**, 11, especially p 73. The likelihood of intramolecular influence by an ortho CO_2^- group in the hydrolysis of monoperoxyphthalic acid has also been suggested by Jones, P. et al. *J. Chem. Soc., Perkin Trans. 2*, **1989**, 443. See also, Fersht, A. R. et al. *J. Am. Chem. Soc.* **1967**, *89*, 4853, 4857.

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(33) Skoog, D. A.; West, D. M. *Analytical Chemistry*, 3rd ed.; Holt, Reinhart and Winston: New York, 1979; p 340.

(34) Gulick, W. M.; Geske, D. H. *J. Am. Chem. Soc.* **1966**, *88*, 2928.

Kinetic Studies. Reactions were carried out in CO_2-free solutions saturated with N_2 and then degassed. Esterolytic reactions were followed on a Shimadzu Model UV-2100 spectrophotometer. Constant temperature baths (TCC-260) maintained reaction temperatures at 25 ± 0.1 °C. All buffers were prepared from double distilled water. Rate constants for the appearance of *p*-nitrophenoxide ion at 400 nm were obtained from computer-generated correlations of $\log(A_\infty - A_t)$ with time. Dephosphorylation or deacylation reactions in different aggregates were followed to >90% completion and showed good first-order kinetics ($r > 0.995$). Values of pseudo-first-order rate constants $k_{\psi max}$ are collected in Table 1. Specific conditions for all the kinetic runs are described under Results and Discussion.

^{1}H- and ^{31}P-NMR Experiments. The isolation of the intermediates involved the hydrolysis of PNPA or PNPDPP with MPP in micellar CTACl. These were studied by the observation of the appearance of new peaks and the disappearance of the peak due to PNPA or PNPDPP, respectively, with time by either ^{1}H-NMR or ^{31}P-NMR spectroscopy. For the ^{1}H-NMR experiment, a 0.3 mL solution of CTACl (1×10^{-2} M) in unbuffered D_2O (pD 8.85) and CD_3CN (2:1) was taken in a NMR tube. An aliquot of PNPA in CD_3CN was injected into NMR tube so that the final concentration of PNPA was 0.12 M. The ^{1}H-NMR of this solution was recorded at ambient temperature before and after the addition of MPP (4×10^{-2} M) in D_2O, and the ^{1}H-NMR of the resulting mixture was also recorded from time to time. ^{1}H-NMR spectra were recorded on a JEOL-FX 90 (90 MHz) spectrometer, and the chemical shifts (δ) are reported relative to tetramethylsilane.

^{31}P-NMR spectroscopic signals were recorded on a Bruker WP-200 NMR spectrometer at 25 ± 1 °C, 81 MHz, using a pulse width of 16 Hz, an acquisition time of 1.016 s, a pulse delay of 2 s, and gated WALTZ-16 decoupling. Synthesis of authentic samples of phosphorylated and acetylated intermediates of MPP are described as follows.

O-Acylated MPP. To 1 equiv of MPP was added excess Ac_2O at ice bath temperature, the reaction mixture was refluxed for ca. 15 min under N_2 atmosphere, and the excess Ac_2O was evaporated under high vacuum. This gave an off-white solid which decomposed on heating at ~240 °C. Due to low stability, this was used without further purification. IR: (Nujol) 1600, 1740 (shoulder at 1700), 1845 cm^{-1}; ^{1}H-NMR (90 MHz): $CDCl_3$/DMSO-d_6 δ 2.0 (s, 3H), δ 8.0 (m, 4H).

O-Phosphorylated MPP. To 1 equiv of MPP in pyridine was added 0.95 equivalent of diphenyl chlorophosphate at 0 °C, and the reaction was stirred at room temperature for ca. 1 h under N_2 atmosphere. The excess pyridine was removed under vacuum. Due to hydrolytic lability of this material, this was used for experiments without further purification. IR: (nujol) 1290, broad peak at 1700 cm^{-1} (with a shoulder at ~1740 cm^{-1}); ^{31}P-NMR (81 MHz): H_2O/CD_3CN (2:1) δ −8.11 (s).

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