Esterolysis in Cyclodextrin–Polyelectrolyte Systems

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The hydrolyses of *m*-nitrophenyl acetate (MNPA) and *p*-nitrophenyl indol-3-ylacetate (PNPIA) in the presence of α- or β-cyclodextrin and polyelectrolytes or surfactans have been carried out. The acceleration and deceleration effects of the added electrolytes on the acylation and deacylation reaction have been investigated. The polyelectrolytes examined were poly-(4-vinyl-*N*-ethylpyridinium bromide), poly-(4-vinyl-*N*-n-butylpyridinium bromide), poly-(4-vinyl-*N*-benzylpyridinium chloride) (BzPVP), the copolymer of 4-vinyl-*N*-hexadecylpyridinium bromide (5%) and 4-vinyl-*N*-benzylpyridinium chloride (95%) (C_{16}BzPVP), diallyldiethylammonium chloride–sulphur dioxide copolymer, poly-L-lysine, sodium polyethylenesulphonate and sodium polystyrenesulphonate. The surfactants were hexadecyltrimethyl-ammonium bromide, sodium dodecyl sulphate, and poly(oxyethylene) dodecyl ether. The acylation step of MNPA with cyclodextrin was inhibited by cationic and hydrophobic polyelectrolytes such as C_{16}BzPVP, and surfactants; this was attributed to the adduct formation between the additives and cyclodextrin. (The dissociation constants were estimated from kinetic data.) In contrast, the acylation and deacylation steps of PNPIA with cyclodextrin were accelerated by the addition of both cationic and hydrophobic polyelectrolytes (*e.g.* C_{16}BzPVP and BzPVP); this was ascribed to the accumulation effect of PNIPA and cyclodextrin around the polyelectrolyte.

RECENTLY, keen attention has been paid to the 'catalytic' action of polyelectrolytes [1] and surfactants.[2] In these investigations, the important contributions of electrostatic and hydrophobic interactions in a large number of organic reactions have been pointed out.[3] In the present paper, the influence of polyelectrolytes on ester hydrolyses catalysed by cyclodextrin is described. From this kind of experiment we expected, by using a polyelectrolyte as a monitoring probe, to add to our knowledge both of the interactions which occur between a substrate and cyclodextrin and the electrostatic and hydrophobic nature of both species. In this report, we describe our experiments on the acylation step of ester hydrolyses which have been catalysed by cyclodextrin using both a weakly hydrophobic ester (MNPA) and a strongly hydrophobic one (PNPIA); we also describe work on the deacylation step which was examined using PNPIA.

EXPERIMENTAL

Materials.—α-Cyclodextrin(cyclohexa-amylose) and β-cyclodextrin (cyclohepta-amylose) were commercially available highest grade reagents (Nakarai Chemicals Co., Kyoto) and were used without further purification. *m*-Nitrophenyl acetate (MNPA) was prepared from *m*-nitrophenol according to the procedure used by Chattaway.[4] *p*-Nitrophenylindol-3-ylacetate (PNPIA) was prepared according to the method described by Menger and Bender.[5] The details of the preparations of poly-(4-vinyl-*N*-ethylpyridinium bromide) (C_2PVP), poly-(4-vinyl-*N*-n-butylpyridinium bromide) (C_4PVP), poly-(4-vinyl-*N*-benzylpyridinium chloride) (BzPVP), a copolymer of 4-vinyl-*N*-hexadecylpyridinium bromide (5%) and 4-vinyl-*N*-benzylpyridinium chloride (95%) (C_{16}BzPVP), diallyldiethylammonium chloride–sul-

[1] See for example, H. Morawetz, *Accounts Chem. Res.*, 1970, **3**, 354; N. Ise, 'Polyelectrolytes and their Applications,' ed. E. Sélegny and H. Rembaum, Reidal, Dordrecht, Holland, 1975, p. 71.

[2] See for example, E. H. Cordes and R. B. Dunlop, *Accounts Chem. Res.*, 1969, **2**, 329; E. J. Fendler and J. H. Fendler, *Adv. Phys. Org. Chem.*, 1970, **8**, 271; J. H. Fendler and E. J. Fendler, 'Catalysis in Micellar and Macromolecular Systems,' Academic Press, New York, 1975.

[3] See for example, (*a*) T. Okubo and N. Ise, *Proc. Roy. Soc.*, 1972, *A*,**327**, 413; (*b*) T. Okubo and N. Ise, *J. Amer. Chem. Soc.*, 1973, **95**, 2293; (*c*) T. Okubo and N. Ise, *J. Org. Chem.*, 1973, **38**, 3120; (*d*) K. Mita, S. Kunugi, T. Okubo, and N. Ise, *J.C.S., Faraday I*, 1975, 936.

[4] F. D. Chattaway, *J. Chem. Soc.*, 1931, 2495.

[5] F. M. Menger and M. L. Bender, *J. Amer. Chem. Soc.*, 1966, **88**, 131.

phur dioxide copolymer (DECS), poly-L-lysine (PLL), sodium polyethylenesulphonate (NaPES), and sodium polystyrenesulphonate (NaPSt) have been described elsewhere.[3b,6] Hexadecyltrimethylammonium bromide (CTABr), sodium dodecyl sulphate (NaLS) and a nonionic surfactant, poly(oxyethylene)dodecyl ether (Brij-35) were commercially available.

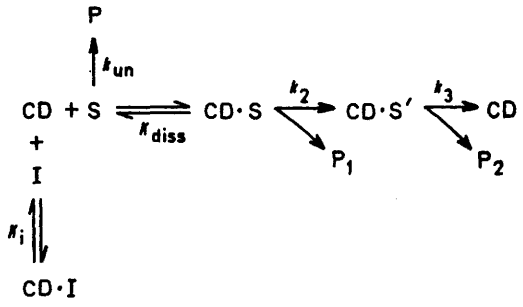
Kinetic Measurements.—Fast reactions were followed with a stopped-flow spectrophotometer (Model RA1100, Union Giken, Hirakata, Osaka, Japan) with a dead time smaller than 1 ms. For slower processes, a high sensitivity spectrophotometer (full scale, 0.01 O.D.) (Union SM401) was used. In these spectrophotometers the temperature of the thermostatted water circulated through a jacket holding the observation cell was such as to maintain the reacting solution at 25 ± 0.2 °C. The pH of the solution was determined by using a pH-meter of Hiranuma Rat 101 (Hiranuma, Mito, Ibaraki, Japan). In all measurements, the pseudo-first-order condition was maintained by using a large excess of cyclodextrin. The acylation reaction of NMPA was followed using changes in the absorbance at 390 nm, and the acylation and deacylation steps of PNPIA were followed using changes in the absorbances at 400 and 272 nm, respectively.

Determination of Dissociation Constants, K_{diss}, of the Cyclodextrin–Ester Complex from the Kinetic Method.[7]—The observed first-order rate constants for hydrolyses of phenyl esters (as measured by phenol release, acylation step) in the absence (k_{un}) and in the presence (k_{obs}) of cyclodextrin were determined. By plotting $1/(k_{obs} - k_{un})$ against 1/[cyclodextrin], a straight line was obtained having a slope of K_{diss}/k_2 and a y intercept equal to $1/k_2$, where K_{diss} is the dissociation constant of the cyclodextrin–ester complex with a 1 : 1 stoicheiometry, and k_2 is the rate constant for the reaction of the complexed ester ($= k_{obs} - k_{un}$, at infinite cyclodextrin concentration).

Determination of the Inhibition Constant K_i.—The dissociation constant of the cyclodextrin–inhibitor (polyelectrolytes and surfactants) complexes, K_i, was determined by measuring the rate of the acylation step of MNPA hydrolysis in the presence of a fixed amount of cyclodextrin and varying concentrations of added inhibitor by the method of Bender *et al.*[8] [see Scheme 1, equation (1)]. By plotting the inhibitor

$$[I] = \left(\frac{k_2 - k_{obs}}{k_{obs} - k_{un}}\right) \cdot \frac{[CD]K_i}{K_{diss}} - K_i \qquad (1)$$

concentration against $(k_2 - k_{obs})/(k_{obs} - k_{un})$ an approximately straight line is obtained with a y intercept of equal to $-K_i$ and a slope of [cyclodextrin]K_i/K_{diss}. In many hydrolyses of phenyl esters by cyclodextrin, the rate conconstants of the acylation step were much larger than those of the deacylation step.[9] For this reason the deacylation step was neglected in deriving equation (1).



SCHEME

RESULTS AND DISCUSSION

Hydrolyses of MNPA and PNPIA in the Presence of Cyclodextrin.—First, the hydrolyses of *m*-nitrophenylacetate (MNPA) and *p*-nitrophenylindol-3-ylacetate (PNPIA) in the presence of α- and β-cyclodextrins were carried out; the relevant parameters are given in Table 1.

TABLE 1

Parameters of hydrolyses of MNPA and PNPIA at pH 10.9 both in the absence and in the presence of cyclodextrin at 25 °C [a]

Ester	Cyclodextrin	[CD]/M	Hydroxide ion rate, $k_{un} \times 10^3/s^{-1}$	$k_{obs} \times 10^2/s^{-1}$	k_{obs}/k_{un}	k_2/s^{-1}	k_2/k_{un}	$K_{diss} \times 10^2$/M
MNPA [b]	α-CD	0.010	7.2	75	104	2.04	284	1.8 ± 0.3
	β-CD	0.005	7.2	27.2	37.8	0.87	121	1.2 ± 0.4
PNPIA [c]	β-CD	0.003 75	20	15.6	7.8	0.218	10.9	0.20 ± 0.03

[a] All experiments were done at pH = 10.9, using sodium carbonate buffer in 0.5 (v/v) % acetonitrile–water. [b] [MNPA] = 0.1 mmol l^{-1}. [c] [PNPIA] = 23.4 μmol l^{-1}.

The values of k_{un}, k_{obs}, and k_2 for MNPA obtained by us are slightly different from those of Bender *et al.* This may be mainly due to differences in the experimental conditions such as the pH and ionic strengths of the solutions. The values of k_{obs}/k_{un}, k_2/k_{un}, and K_{diss} of MNPA were, however, the same within experimental error.

Polyelectrolyte Effects on the Acylation Step.—The hydrolysis of MNPA in the presence of cyclodextrin and polyelectrolyte was carried out. The addition of both a simple- and a poly-electrolyte decreased the reaction rate as is clearly shown in Figure 1. Thus all of the simple- and the poly-electrolytes including the nonionic surfactant, Brij-35, examined in the present experiments are inhibitors upon the cyclodextrin-catalysed hydrolysis of the ester. The competitive inhibition constant, K_i, was determined in each case and the results are given in Table 2. The treatment of data is exemplified in Figure 2. From these data it is apparent that surfactants such as CTABr, NaLS, and Brij-35, are extremely strong inhibitors. Recently, the strong binding of ionic surfactants with cyclodextrin was discussed upon the basis

[6] S. Harada and K. Arai, *Makromol. Chem.*, 1967, **107**, 78.
[7] H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.*, 1934, **56**, 658.
[8] R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Amer. Chem. Soc.*, 1967, **89**, 3242.
[9] R. L. VanEtten, G. A. Clowes, J. F. Sebastian, and M. L.

of conductimetric measurements.[10] Among the polyelectrolytes, the most hydrophobic one, C_{16}BzPVP is the strongest inhibitor. The binding strength of cationic polyelectrolyte, *i.e.*, PLL, BzPVP, C_4PVP, and C_{16}BzPVP is similar to those of various benzoate derivatives found by Bender *et al.*[8] It should be noted here that the binding interactions of MNPA with cationic polyelectrolytes are assumed to be very weak, since the alkaline hydrolysis of *p*-nitrophenyl acetate was *not* strongly accelerated by cationic polymers such as C_4PVP, BzPVP, and C_{16}BzPVP.[3c,11] Thus, it is concluded that inhibitory action of polyelectrolyte on the acylation reaction of MNPA is attributed to the strong interactions between polyelectrolyte and cyclodextrin by the hydrophobic forces. The hydrophobic attractive interactions between MNPA and surfactants such as CTABr, NaLS, and Brij-35 are considered to be ineffective,[3c] because the experiments were carried out with surfactant concentrations lower than the critical micelle concentration.

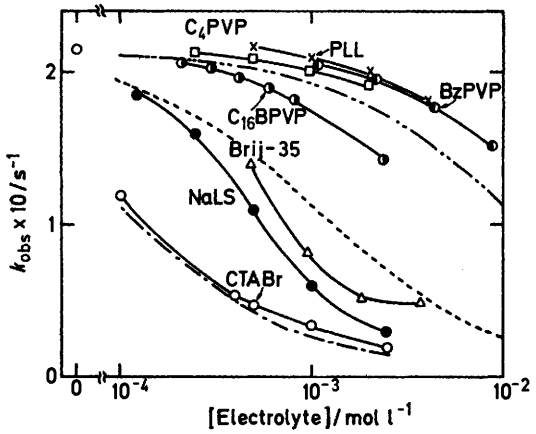


FIGURE 1 Electrolyte effects on the acylation step of the hydrolysis of MNPA catalysed with α-CD at 25 °C. [MNPA] = 0.1 mmol l^{-1}, [α-CD] = 2 mmol l^{-1}, pH = 10.9, sodium carbonate buffer in 0.5 (v/v) % $CH_3CN \sim H_2O$. ——— : calc. from equation (1), $K_i = 10^{-2}$ mol l^{-1}; – – – – : $K_i = 10^{-3}$ mol l^{-1}; – – – : $K_i = 10^{-4}$ mol l^{-1}

TABLE 2

Dissociation constants of cyclodextrin complexes determined by competitive inhibition of MNPA hydrolysis at 25 °C

Inhibitor	α-CD [a] 10^4K_i/mol l^{-1}	β-CD [b] 10^4K_i/mol l^{-1}
PLL	180 ± 50	330 ± 50
BzPVP	190 ± 30	160 ± 30
C_4PVP	160 ± 50	110 ± 40
C_{16}BzPVP	41 ± 19	50 ± 20
Brij-35	7.0 ± 2.0	9.3 ± 0.4
NaLS	7.1 ± 2.6	2.2 ± 0.4
CTABr	1.1 ± 0.3	2.5 ± 0.5

[a] [MNPA] = 0.1 mmol l^{-1}, pH = 10.9, carbonate buffer in 0.5 (v/v) % acetonitrile–H_2O mixture, [α-CD] = 2 mmol l^{-1}. [b] [β-CD] = 1 mmol l^{-1}.

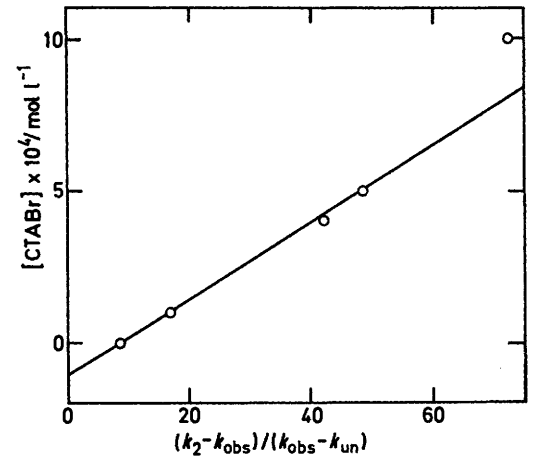


FIGURE 2 Inhibition by CTABr on the hydrolysis of MNPA catalysed with α-CD at 25 °C. [MNPA] = 0.1 mmol l^{-1}, [α-CD] = 2 mmol l^{-1}, pH = 10.9, sodium carbonate buffer in 0.5 (v/v) % CH_3CN–H_2O

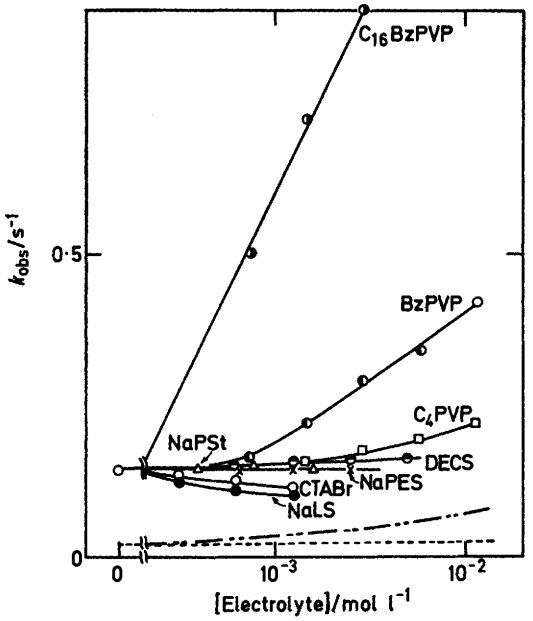


FIGURE 3 Polyelectrolyte effects on the acylation step of the hydrolysis of PNPIA catalysed with β-CD at 25 °C. [PNPIA] = 23.4 μmol l^{-1}, [β-CD] = 2.5 mmol l^{-1}, pH = 10.9, sodium carbonate buffer in 0.5 (v/v) % in CH_3CN–H_2O. ——— : BzPVP in the absence of β-CD; – – – – : C_4PVP in the absence of β-CD

The difference in binding strength between α- and β-cyclodextrin was not observed partly because of the comparatively large uncertainty associated with the K values (the experimental errors were between ±20 and ±30%).

We now discuss the acylation step of the hydrolysis of PNPIA in the presence of simple- and poly-electrolytes and cyclodextrin. In Figure 3, changes of k_{obs} with various electrolytes are shown. Both cationic and hydrophobic polyelectrolytes *accelerated* the acylation reaction, which is in contrast with the results for MNPA. The weakly hydrophobic polycation DECS and anionic

[10] T. Okubo, H. Kitano, and N. Ise, *J. Phys. Chem.*, in the press.

[11] H. Kitano, M. Tanaka, and T. Okubo, *J.C.S. Perkin II*, 1976, 1074.

polyelectrolytes such as NaPES and NaPSt did not change k_{obs}. The surfactants decreased the acylation reaction. In this case, PNPIA is a hydrophobic substrate and interacts with polyelectrolyte, a conclusion which is supported by the fact that the alkaline hydrolysis rate of PNPIA is accelerated by addition of polyelectrolyte such as C_4PVP and BzPVP (see Figure 3). Hydrophobic effects of polyelectrolytes and micelles on the alkaline hydrolysis of *p*-nitrophenyl esters have been noted by many workers.[3c,11-13] Thus, in the hydrolysis of PNPIA, complex formation of hydrophobic and cationic polyelectrolytes such as C_{16}BzPVP, BzPVP, C_4PVP, may be thought to occur not only with cyclodextrin but also with the substrate. In other words, hydrophobic poly-(4-vinylpyridinium salts) may attract both cyclodextrin and PNPIA in the polymer domain, and the acylation of PNPIA with cyclodextrin may be stimulated by so-called concentration effects. The observed acceleration implies that the rate-enhancing effect of hydrophobic binding between polyelectrolyte and substrate predominates over the inhibitive effect of interaction between polyelectrolyte and cyclodextrin. Because of the extreme difficulty in determining the dissociation constants of polyelectrolyte–substrate and polyelectrolyte–cyclodextrin from the kinetic data they were not carried out.

Polyelectrolyte Effects on the Deacylation Step.—By using PNPIA we could follow the deacyclation step spectrophotometrically since the released acid residue contains an indole ring with a characteristic absorbance peak at 272 nm. Figure 4 shows the polyelectrolyte and surfactant effects on the deacylation step in the hydrolysis of PNPIA with β-CD. Both acceleration and deceleration were observed. Strong basic polymers, *i.e.* poly-(4-vinylpyridine) quarternized with alkyl bromide accelerated the reaction, and the larger alkyl groups, the larger the acceleration (C_4PVP > C_2PVP). This fact may be explained as follows. Both the acylated cyclodextrin–indol-3-ylacetate and hydroxide ions can be accumulated around the polymer catalyst by hydrophobic and electrostatic interactions; this facilitates the deacylation.[9] In contrast the cationic polyelectrolyte DECS is less strongly hydrophobic and thus does not change the deacylation step. Although the complexation of acylated cyclodextrin with polyelectrolyte does give rise to inhibitory effects their strength is considered to be much weaker than the concentration effects for poly(4-vinylpyridinium salts); for DECS they are comparable. The anionic polyelectrolyte, NaPSt, and surfactant, NaLS, are considered to repel the hydroxide ions and the acceleration effects mentioned above are not effective. These anionic species exclusively interact

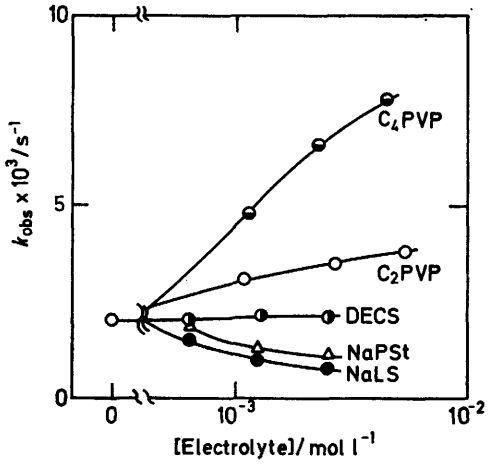


FIGURE 4 Polyelectrolyte effects on the deacylation step of the hydrolysis of PNPIA catalysed with β-CD at 25 °C. [PNPIA] = 23.4 μmol l^{-1}, [β-CD] = 2.5 mmol l^{-1}, pH = 10.9, sodium carbonate buffer in 0.5 (v/v) % CH_3CN–H_2O

with cyclodextrin–indol-3-ylacetate by hydrophobic forces and therefore hinder the deacylation.

From the observed polyelectrolyte effects, it can be concluded that (1) cationic and hydrophobic polyelectrolytes accelerate both acylation and deacylation when the substrate is a hydrophobic one, whereas they decelerate the acylation when the substrate is not hydrophobic. (2) Anionic polyelectrolyte and surfactant slightly decelerate both the acylation and deacylation reaction steps. (3) Many kinds of polyelectrolytes and surfactants bind with cyclodextrin.

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[12] L. R. Romsted and E. H. Cordes, *J. Amer. Chem. Soc.*, 1968, **90**, 4404.

[13] T. Rodulfo, J. A. Hamilton, and E. H. Cordes, *J. Org. Chem.*, 1974, **39**, 2281.