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The effect of lecithin dispersed by surfactants on the hydrolysis of *p*-nitrophenyl acetate

TOSHIHISA YOTSUYANAGI*, JUN MIZUTANI, KEN IKEDA, *Faculty of Pharmaceutical Sciences, Nagoya City University, Nagoya 467, Japan*

Surfactant aggregates dispersed in the aqueous phase provide complex environments for various organic and inorganic reactions where reaction rates are often retarded or enhanced (Fendler & Fendler 1975a). We examined the hydrolysis of procaine and 2-diethylaminoethyl *p*-nitrobenzoate in liposomal suspension (Yotsuyanagi et al 1979a, b) and found that the hydrolysis of procaine was retarded by liposomes but with 2-diethylaminoethyl *p*-nitrobenozate both retardation and enhancement were observed, depending on the pH of the dispersed medium. These ester degradations followed first order kinetics in the liposomal suspension as well as in the corresponding aqueous bulk solution.

Later, we reported that the hydrolysis of *p*-nitrophenyl acetate (*p*-NPA) was enhanced in liposomal suspension, hydrolysis being accelerated more in the presence of unilamellar liposomes than in the presence of multilamellar liposomes (Yotsuyanagi & Ikeda 1980). Furthermore, the enhancement of hydrolysis observed in the early stage of reaction was gradually exhausted and was followed by a slow linear phase comparable to the spontaneous hydrolysis process. The results generated problems about the rate enhancement mechanism and the exhaustion of lecithin action with time. One possibility was that the exhaustion could be due to acetylation of the phosphate group of the lecithin molecule, which prevented further orientation of the substrate on the vesicles from being favourable for attack by hydroxide ion. If so, the initial rapid degradation might be due to the acetylation rather than the favourable orientation of the substrate for hydroxide ion attack.

We now describe the effect of lecithin solubilized by cetyltrimethylammonium bromide (CTAB) and polyoxyethylene lauryl ether (Brij 35), i.e. lecithin-surfactant mixed micelles, on the rate of *p*-NPA hydrolysis. When solubilized by surfactants, lecithin molecules are less restricted than in liposomes and thus may provide more information for the mechanistic understanding of whether such a highly arranged structure as exists within liposomes is essential for the rapid degradation and the subsequent exhaustion of the lecithin action. Quaternary ammonium surfactants such as CTAB and dodecylammonium bromide have often been used as dispersing agents for functional surfactants (Gitler & Ochoa-Solano 1968; Kunitake et al 1976). Upon mixed micelle formation with Brij 35, the lecithin molecule appears to be intercalated into the Brij 35 micelle in such a manner as to locate its hydrophobic groups in the apolar core and its polar head groups into exterior palisade layer, as described in the mixed micelle of Triton X-100 and dimyristoyl phosphatidylcholine (Ribeiro & Dennis 1975).

The lecithin-surfactant mixed micelle was prepared in Tris buffer solution (0·05 M, pH = 8·0, μ = 0·1) throughout. The concentration of the surfactants, CTAB and Brij 35, was maintained at 1×10^{-2} M while the lecithin content was varied. The reaction rate was followed in terms of the appearance of *p*-nitrophenol. In the absence of lecithin, the hydrolysis rate of *p*-NPA was only slightly enhanced or retarded by increasing either CTAB or Brij 35 concentration up to 1×10^{-2} M in the conditions used.

Fig. 1 indicates that the hydrolysis followed first order kinetics in the absence of the mixed micelle and was clearly accelerated in the presence of lecithin dispersed by CTAB. Furthermore, as with liposomal suspensions, the initial rapid phase was followed by a slow linear phase comparable to the spontaneous hydrolysis process. When the effect of lecithin on the ratio of the pseudo-first order rate constant (obtained by extrapolating the initial stage of the reaction to the rate constant of the spontaneous hydrolysis), was plotted, a straight line was obtained, indicating that the degree of enhancement of the hydrolysis rate was directly proportional to the lecithin content (Fig. 2). This 'en-

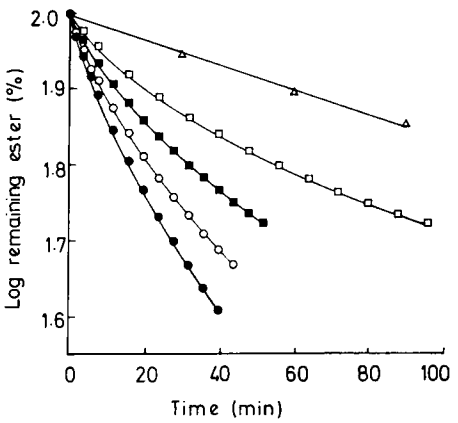

FIG. 1. First-order plots for hydrolysis of *p*-nitrophenyl acetate (*p*-NPA) in the lecithin-cetyltrimethylammonium bromide (CTAB) mixed micelle system at 25 °C. Tris-(hydroxymethyl)-aminomethane buffer at pH = 8·0 (μ = 0·1) was used. Initial *p*-NPA concentration; $1 \cdot 0 \times 10^{-4}$ M. CTAB concentration; $1 \cdot 0 \times 10^{-3}$ M. Lecithin concentrations; (△) 0·0 M, (□) $1 \cdot 0 \times 10^{-3}$ M, (■) $2 \cdot 0 \times 10^{-3}$ M, (○) $3 \cdot 0 \times 10^{-3}$ M and (●) $4 \cdot 0 \times 10^{-3}$ M.

* Correspondence.

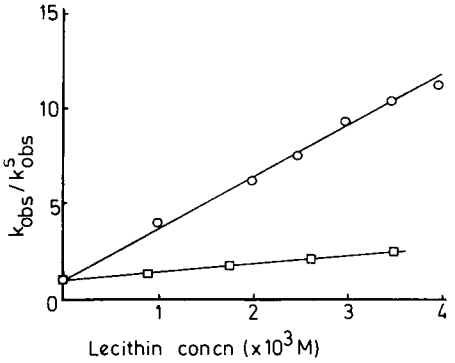

FIG. 2. The effect of lecithin on the pseudo-first order rate constant, k_{obs}, for hydrolysis of *p*-NPA in the presence of $1{\cdot}0 \times 10^{-2}$ M CTAB (○) and Brij 35 (□). k^{s}_{obs} is the rate constant in the absence of lecithin ($2{\cdot}6 \times 10^{-3}$ min^{-1}).

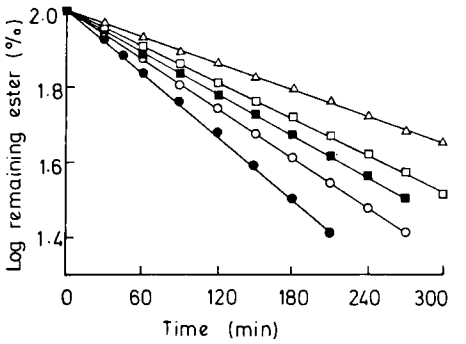

FIG. 3. First-order plots for hydrolysis of *p*-nitrophenyl acetate (*p*-NPA) in the lecithin-Brij 35 mixed micelle system at 25 °C. Tris(hydroxymethyl)-aminomethane buffer at pH = 8·0 (μ = 0·1) was used. Initial *p*-NPA concentration; $1{\cdot}0 \times 10^{-4}$ M. Brij 35 concentration; $1{\cdot}0 \times 10^{-2}$ M. Lecithin concentration; (△) 0·0 M, (□) $0{\cdot}88 \times 10^{-3}$ M, (■) $1{\cdot}76 \times 10^{-3}$ M, (○) $2{\cdot}64 \times 10^{-3}$ M and (●) $3{\cdot}52 \times 10^{-3}$ M.

hancement ratio' per mole of lecithin dispersed by CTAB was 2700, which is roughly five times greater for the hydrolysis of *p*-NPA in comparison with the enhancement ratio of lecithin as unilamellar liposomes (ca 570).

Fig. 3 shows that when dispersed by Brij 35, lecithin effected an increase in hydrolysis proportional to its concentration (Fig. 2) but there was no exhaustion of the rate of enhancement as seen with the lecithin CTAB system. The enhancement ratio of 390 is much smaller than the ratios of the lecithin CTAB and unilamellar liposome systems. This suggests that the relatively thick oxyethylene palisade layer of Brij 35 hinders the approach of *p*-NPA molecules to lecithin molecules the head groups of which seem to be the active site and are located in the boundary region between the hydrophilic and hydrophobic portions of the mixed micelle. The results also indicate that the highly arranged structure of the lecithin molecules as liposomes is not a specific requirement resulting in the acceleration of the rate of hydrolysis.

Although reactions in micellar solutions have been explained by conventional kinetic analysis (Fendler & Fendler 1975b), it is difficult to construct the mathematical model of the reaction kinetics covering the present examples, i.e. as the hydrolysis of *p*-NPA proceeded it was accompanied by a diminishing rate of enhancement in the lecithin-CTAB system while it followed straightforward kinetics with the rate enhancement in the lecithin-Brij 35 system.

The initial rapid liberation of *p*-nitrophenol in the liposomal suspension and in the lecithin-CTAB system left open the question whether the behaviour of the lecithin molecule was analogous to the acylation-deacylation mechanism involved in the micellar catalysis of N^{α}-myristoyl-L-histidine and zwitterionic hydroxamate dispersed by CTAB (Gitler & Ochoa-Solano 1968; Kunitake et al 1979). However, the present results perhaps rule out the possibility of the acetylation mechanism because lecithin dispersed by Brij 35 showed no exhaustion and remained active even at 50% degradation of *p*-NPA at which lecithin dispersed by CTAB completely lost its action, when the lecithin concentration was almost comparable in both systems ($\cong 1 \times 10^{-3}$ M).

So far, we have not been concerned with the behaviour of the product (*p*-nitrophenol) of the reaction. *p*-Nitrophenol may compete with *p*-NPA at the active site which seems to be the hydrophilic head group of lecithin. This assumption was thought to explain the exhaustion of lecithin action on the *p*-NPA hydrolysis, but may not account for the discrepancy of the kinetics observed in the lecithin-CTAB and in the lecithin-Brij 35 systems. Accordingly, it is necessary to consider not only the properties of CTAB and Brij 35 solely as dispersing agents but also their contribution to the hydrolytic mechanism in combination with lecithin molecule.

REFERENCES

Fendler, A. H., Fendler, E. J. (1975a) in: Catalysis in Micellar and Macromolecular Systems, Academic Press, New York, pp 104–161

Fendler, A. H., Fendler, E. J. (1975b) in: Ibid. pp 87–88

Gitler, C., Ochoa-Salono, A. (1968) J. Am. Chem. Soc. 90: 5004–5009

Kunitake, T., Shinkai, S., Okahata, Y. (1976) Bull. Chem. Soc. Jpn. 49: 540–545

Kunitake, T., Okahata, Y., Tanamachi, S., Ando, R. (1979) Bull. Chem. Soc. Jpn. 52: 1967–1971

Ribeiro, A. A., Dennis, E. A. (1975) Biochemistry 14: 3746–3755

Yotsuyanagi, T., Hamada, T., Tomida, H., Ikeda, K. (1979a) Acta Pharm. Suec. 16: 271–280

Yotsuyanagi, T., Hamada, T., Tomida, H., Ikeda, K. (1979b) Ibid. 16: 325–332

Yotsuyanagi, T., Ikeda, K. (1980) J. Pharm. Sci. 69: 745–746