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INFLUENCE OF THE FLUIDITY OF DIALKYLAMMONIUM BILAYER MEMBRANES ON THE CATALYTIC HYDROLYSIS OF p-NITROPHENYL PALMITATE

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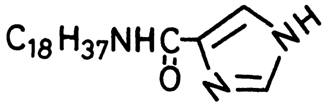
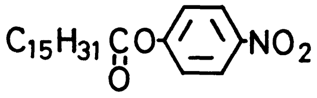
In the hydrolysis of p-nitrophenyl palmitate catalyzed by a long-chain imidazole in the presence of the aqueous bilayer membrane of double-chain ammonium surfactants, the Arrhenius plots showed inflection points which were apparently related to the phase transition of the bilayers.

Quaternary ammonium salts with two normal alkyl chains(C_{10} - C_{20}) spontaneously produce the bilayer structure when dispersed in water.1-5) The synthetic bilayer membrane is in many respects similar to that of biomembranes1,4,6) and, therefore, its highly oriented structure would affect the membrane-associated reactions in many specific ways. We reported already that the inter-vesicle reaction was very much slower than the intra-vesicle counterpart due to rate-limiting substrate transfer in the catalytic hydrolysis of a phenyl ester in the presence of ammonium bilayer vesicles.7)

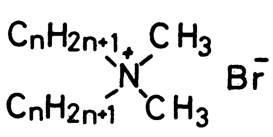
In this communication, we would like to report that the crystalline-liquid crystalline phase transition of the ammonium bilayer influences the rates of the hydrolysis of p-nitrophenyl palmitate(PNPP) catalyzed by imidazole-carbo-N-octadecylamide(C_{18}ImAm). The structures of these compounds are shown below together with those of dialkylammonium salts.



C_{18}ImAm



PNPP



n = 12 $2C_{12}\overset{+}{N}2C_1\overset{-}{Br}$
14 $2C_{14}\overset{+}{N}2C_1\overset{-}{Br}$
16 $2C_{16}\overset{+}{N}2C_1\overset{-}{Br}$
18 $2C_{18}\overset{+}{N}2C_1\overset{-}{Br}$

The catalyst, C_{18}ImAm was prepared from 4-imidazolecarbonyl chloride and octadecylamine according to the procedure used for the dodecyl derivative[8]; mp 115 - 116°C. Found: C, 72.02; H, 11.21; N, 11.30 %. Calcd($C_{22}H_{41}N_{3}O$); C, 72.62; H, 11.36; N, 11.55 %. The preparation of PNPP, mp 63 - 65°C(lit.[9] 62 - 64°C) and dialkylammonium bromides[1,2] has been described before.

The phase transition temperature T_c of aqueous dialkylammonium bilayers(2.5 wt%, 30 - 50 mM)(1 M = 1 mol dm^{-3}) was estimated from the endothermic peak in differential scanning calorimetry(Rigaku-denki, Model Unix).[10] The results are given in Table 1, the data for a series of the phosphatidylcholine bilayer being included for the purpose of comparison. These bilayers possess fundamentally the same structure and the the nature of the phase transition(crystalline — liquid crystalline) was supported for $2C_{18}N^{+}2C_{1}Br^{-}$ by the X-ray diffraction data.[11] The phase transition temperature increased with increasing chain lengths in both series. The T_c increment due to addition of the ethylene unit was approximately 20°C in the case of phosphatidylcholine, but was 11 - 13°C in the series of dialkylammonium bromides.

Table 1. Phase Transition Temperature of Bilayers

Alkyl(acyl) chain length	$2C_{n}N^{+}2C_{1}Br^{-}$ °C	Phosphatidylcholine[12] °C
12	11-16	0
14	23-29	23
16	37-39	41
18	50-52	58

The hydrolysis of PNPP was carried out as follows. The stock solutions were prepared by dissolving the substrate, the catalyst and the dialkylammonium surfactant in dilute hydrochloric acid(pH 2 - 3) by sonication(Bransonic Sonifier 185, power level 30 - 40). A necessary amount of the stock solution was added to a buffer(0.01 M borate) solution in a uv cell which had been maintained at a given reaction temperature, and the p-nitrophenolate anion formed was followed at 401 nm with a Hitachi 200 uv-visible spectrophotometer. The hydrolysis was conducted at pH 9.3 ± 0.1 with ionic strength adjusted to 0.01 by KCl: $[C_{18}\text{ImAm}] = 1.01 \times 10^{-6}$ M, $[\text{PNPP}] = 1.4 \times 10^{-5}$ M, $[2C_{n}N^{+}2C_{1}Br^{-}] = 1.00 \times 10^{-3}$ M. The p-nitrophenol release obeyed the pseudo first order rate law. This indicates that the catalytic reaction proceed without accumulation of an acyl imidazole intermediate. The catalytic rate constant, $k_{1,obsd}$ was obtained by correcting the first-order rate constant of spontaneous hydrolysis.

The $k_{1,obsd}$ values were very large: eg., ca. 0.05 s^{-1} at 30°C in the presence of $2C_{12}N^{+}2C_{1}Br^{-}$ aggregates. The aggregate weight of the dialkylammonium salt was estimated to be in the range of 10^{6} - 10^{7} daltons.[1,13] Both the catalyst and PNPP are insoluble in water and must be completely bound to these aggregates. On average, one vesicle would contain 1 - 20 catalyst molecules and 30 - 300 substrate molecules. Therefore, PNPP must be hydrolyzed predominantly by the catalyst molecules existing in the same vesicle.[14]

Figures 1a - 1d are the Arrhenius plots for the catalytic and spontaneous hydrolyses. The spontaneous hydrolysis was hydroxide-catalyzed as shown by the linear pH-rate profile with the slope of + 1. Its Arrhenius plots are linear with the correlation coefficient of 0.993 to 0.998. The spontaneous hydrolysis is not influenced by the physical state of the bilayer membrane, probably because hydroxide ion is at the surface of the ammonium bilayer.

On the contrary, the Arrhenius plots are not linear for the catalytic hydrolysis that was performed in the presence of $2C_{12}N^+2C_1$, $2C_{14}N^+2C_1$ and $2C_{16}N^+2C_1$ bilayers. The inflection range appears to be related to T_c of the bilayer matrix. Thus, the inflection range was 16 - 22°C for the Arrhenius plot derived from the $2C_{12}N^+2C_1$ bilayer system. This range is just above T_c(11 - 16°C) of this bilayer. In the $2C_{14}N^+2C_1$ system, the inflection range corresponds approximately to T_c: 25 - 30°C vs. 23 - 29°C. However, in the $2C_{16}N^+2C_1$ system, the inflection range(20 - 33°C) is lower than T_c(37 - 39°C). It is also noted that the inflection range is narrow with the $2C_{12}N^+2C_1$ bilayer, but is broader with the other two systems. Inflection in the Arrhenius plot is not seen in the case of the $2C_{18}N^+2C_1$ bilayer, obviously because T_c is higher than the reaction temperature.

These results indicate that the bilayer fluidity affects the rate of the catalytic reaction which proceeds in the membrane matrix. In the same vein, $k_{1,obsd}$ in the lower temperature region was larger in the presence of the $2C_{12}N^+2C_1$ and $2C_{14}N^+2C_1$ bilayers than in the presence of less fluid $2C_{16}N^+2C_1$ and $2C_{18}N^+2C_1$ bilayers. The order of these $k_{1,obsd}$ values should be reversed if the fluidity does not affect the reaction, since the reaction is accelerated with increasing hydrophobicity in the case of the fluid ammonium aggregates.8)

The influence of the crystalline-liquid crystalline transition on the organic reaction has been studied extensively, and recently, the activation parameters were used to discuss the isomerization mechanism of azobenzene.16) To our knowledge, however, similar studies have never been performed in the aqueous bilayer membrane, because convenient membrane systems were not available.

We are obliged to Prof. M. Takayanagi for the use of a differential scanning calorimeter.

References and Notes

1) T. Kunitake and Y. Okahata, J. Am. Chem. Soc., 99, 3860 (1977).
2) T. Kunitake, Y. Okahata, K. Tamaki, F. Kumamaru, and M. Takayanagi, Chem. Lett., 387 (1977).
3) T. Kunitake and Y. Okahata, Chem. Lett., 1337 (1977).
4) C. D. Tran, P. L. Klahn, A. Romero, and J. H. Fendler, J. Am. Chem. Soc., 100, 1622 (1978).
5) K. Deguchi and J. Mino, J. Colloid Interface Sci., 65, 155 (1978).
6) T. Nagamura, S. Mihara, Y. Okahata, T. Kunitake and T. Matsuo, Ber. Bunsenges. Phys. Chem., 82, 1093 (1978).
7) T. Kunitake and T. Sakamoto, J. Am. Chem. Soc., 100, 4615 (1978).
8) Y. Okahata, R. Ando, and R. Kunitake, J. Am. Chem. Soc., 99, 3067 (1977).
9) H. Zahn and F. Shade, Chem. Ber., 96, 1747 (1963).

10) Dialkylammonium salts(50 mg) were dissolved by sonication in 2 ml of deionized water or of 0.01 M borate buffer. A few μl of the solution were placed in measuring pans, sealed and cooled by liquid nitrogen in the sample chamber. The temperature was raised at the rate of 5 K/min. A calibration curve was obtained by using mercury, gallium and naphthalene as reference compounds.

11) T. Kajiyama, A. Kumano, M. Takayanagi, Y. Okahata, and T. Kunitake, Chem. Lett., 645 (1979).

12) D. Chapman, "Form and Function of Phospholipids", Elsevier, Amsterdam, p.117, 1973.

13) The aggregate weight(1 - 10 mM range): $2C_{12}N^+2C_1$, 1×10^6; $2C_{14}N^+2C_1$, 5×10^6; $2C_{18}N^+2C_1$, 1×10^7 daltons (Y. Okahata, unpublished results in these laboratories).

14) It was confirmed in separate experiments that the rate difference between the inter- and intra-vesicle reactions was much smaller when C_{18}ImAm was used as catalyst in place of the cholesteryl ester of imidazole-carboxylic acid.[15]

15) T. Kunitake and T. Sakamoto, manuscript in preparation.

16) J. M. Nerbonne and R. G. Weiss, J. Am. Chem. Soc., 100, 5953 (1978).

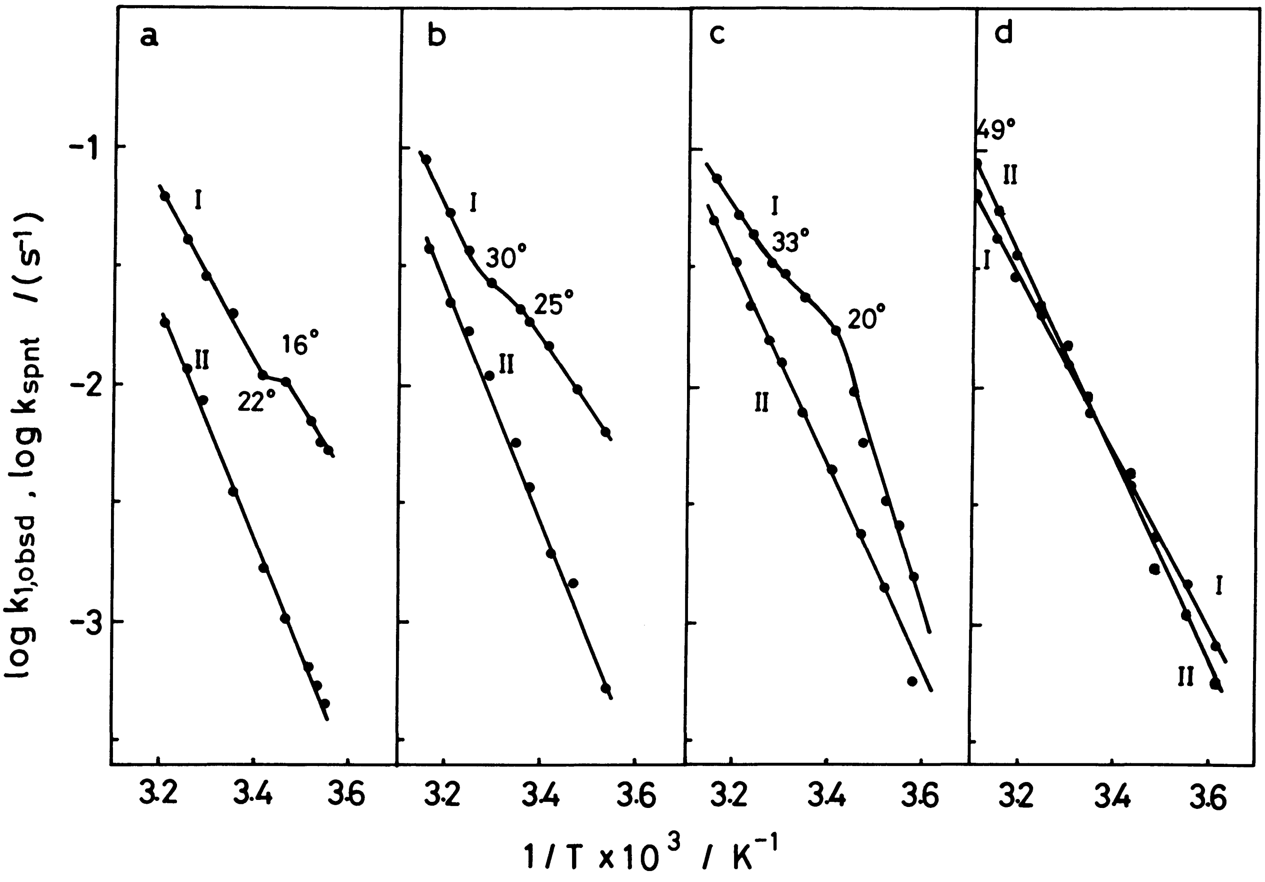


Fig. 1. Arrhenius plots for catalytic(I) and spontaneous(II) hydrolyses in ammonium bilayer membranes.

a) $2C_{12}N^+2C_1Br^-$ bilayer.
b) $2C_{14}N^+2C_1Br^-$ bilayer.
c) $2C_{16}N^+2C_1Br^-$ bilayer.
d) $2C_{18}N^+2C_1Br^-$ bilayer.

Condition: pH 9.3 ± 0.1, $\mu = 0.01$(CKl), $[C_{18}\text{ImAm}] = 1.01 \times 10^{-6}$ M, $[\text{PNPP}] = 1.4 \times 10^{-5}$ M, $[2C_nN^+2C_1Br^-] = 1.00 \times 10^{-3}$ M.

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