COMMUNICATIONS TO THE EDITOR

Kinetic Study on the Hydrolysis of *p*-Nitrophenyl Palmitate in Reconstituted BR-DPPC Vesicle

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Chemical reactions in vesicular system can show two different kinetic process: one takes palce on the vesicular surface (exovesicular) and the other occurs at interior site (endovesicular). Moss and Schreck observed biphasic kinetics in the cleavage of Ellman's reagent by dithionite in quaternayr ammonium salt vesicles. This was attributed to exovesicular reaction (fast) and reaction on subsurface (slow), not endovesicular[1]. Later, the same group reported similar biphasic kinetics in hydrolysis of *p*-nitrophenyl esters with long alkyl chain in functionalized thiol surfactant vesicle. But they interpreted that the slow kinetics was artifact and arised from osmometric changes in the vesicles[2]. Thus, differentiation of outside and inside vesicular reactions is not yet demonstrated.

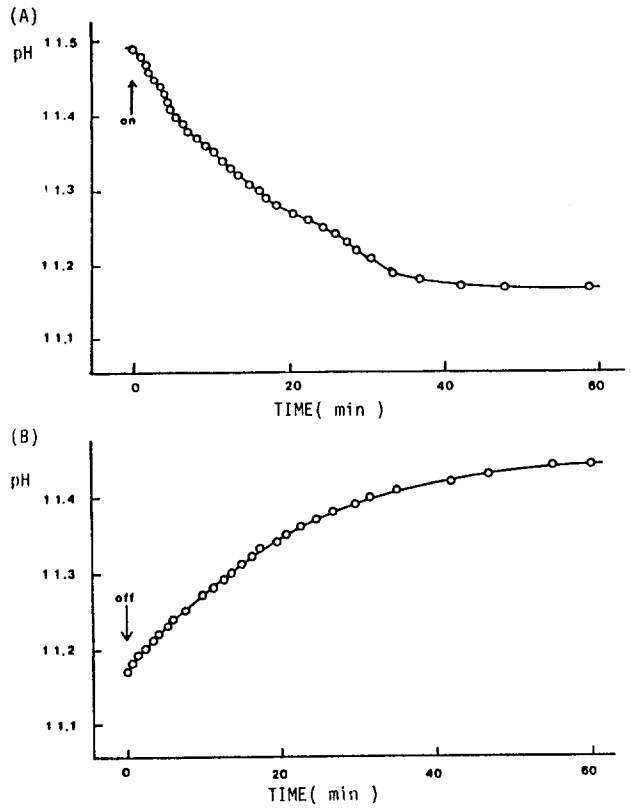

Figure 1. Time dependence of pH in the reconstituted BR-DPPC vesicle at alkaline medium; (A): light illumination, (B): illumination off.

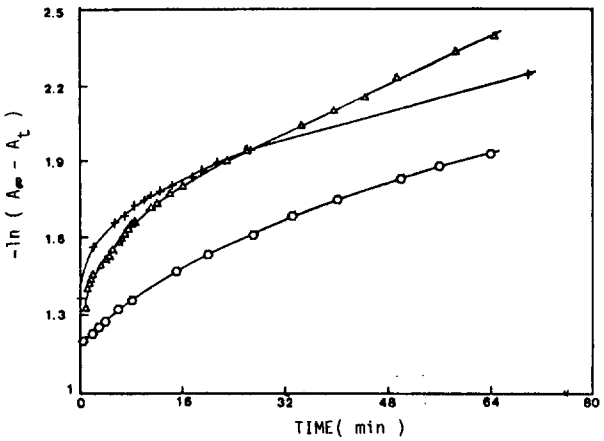

Figure 2. Time dependence on the BR-DPPC vesicular catalyzed hydrolysis of PNPP; +: light on, : light off, : DPPC vesicle only.

Bacteriorhodopsin (BR) reconstituted vesicles pumped H^+ from inside of vesicles to outside by illumination of light[3] as shown in Figure 1. This can change the reaction rate of hydroxide ion catalyzed hydrolysis of esters incorporated in vesicular system, affording informations on the nature of kinetics in vesicular environment. This communication deals with kinetics of *p*-nitrophenyl palmitate(PNPP) hydrolysis in reconstituted BR-dipalmitoyl phosphatidyl choline (DPPC) vesicle and demonstrate that the biphasic kinetics are indeed due to exovesicular and endovesicular reactions. PNPP and DPPC (1/10 molar ratio) were directiy sonicated in acidic condition for 6-8 min to give clear stock solutions. The incoporation of BR into the vesicle were achieved by using the sonication method of Racker[4]. The reaction was followed by increase in absorbance at 400 nm which is due to a reaction product *p*-nitrophenoxide ion[5,6]. The absorbance change was computer -analyzed by pseudo first-order kinetics and by parametric least squares curve fits; $(A_\infty - At) = R°$(fast) EXP (-k1t) + $R°$ (slow) EXP(-k2t). For the systems with[7] BR-DPPC vesicle, the reaction were carried under steady illumination of light or in dark condition[8]. Spectra of the reaction mixture were taken at various time interval. In Figure 2, $\ln(A_\infty - At)$ was plotted versus t. In the hydrolysis of PNPP in reconstituted BR-DPPC vesicle at steady illumination of light, proton was transferred from inside to outside of the vesicle, and the reaction of PNPP at the outside of vesicle

Table 1. Rate Constants for the Reconstituted BR-DPPC Vesicular Catalyzed Hydrolysis of PNPP at 35 °C, pH 11.75[a]

Condition	k1(min^{-1})	k2(min^{-1})	k1/k2	R °(fast)	R °(slow)
light on	0.242	0.00701	34.6	0.0715	0.179
light off	0.172	0.0114	15.1	0.0715	0.192
none BR	0.0845	0.00772	10.9	0.0707	0.236

[a] R ° is the absorbance of the chemical species at $t=0$. [BR] = 3×10^{-7}M, [DPPC] = 2×10^{-3}M, [PNPP] = 2×10^{-4}M, [borate] = 0.01M.

became decreased. By contrast, at extinction of light, proton was back diffused, and the reaction rate of PNPP at the inside of vesicle became decreased. The change of k1/k2 at the light illuminated condition suggested that the H^+ pumped by light affected the hydrolysis. The difference between the light off condition and none BR[1] condition might be understood as the integral protain's general effect on the fluidity of the bilayer; those effects usually increase the overall reaction rate of the hydrolysis[9], as in our case. The results of Table 1 suggested that fast reaction and slow reaction occured inside and outside of the vesicle, respectively. The present work might support biphasic kinetics of vesicular reaction, and demonstrate how the vesicle can be chemically differentiated at inside and outside of vesicle site.

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Influence of Dealumination and Nonframework Aluminum on the Catalytic Activity of Y Type Zeolite

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The removal of aluminum from the framework of Y type zeolites strongly influences the catalytic activity[1-3]. For Y zeolite prepared by dealumination, interpretation of catalytic data is complicated by the presence of nonframework aluminum species. We have examined the influence of dealumination and nonframework aluminum species on catalytic cracking activity.

Dealuminations were carried out by three preparation methods. A series of dealuminated Y type zeolites without nonframework aluminum was prepared by reaction of NaY with EDTA, according to the procedure described by Kerr[4]. These samples are denoted by a symbol of EDY (Si/Al), where the framework silicon to aluminum ratio is given in parenthesis. A steam-dealuminated Y zeolites (designated SDY) with nonframework aluminum were prepared according to the method of Ward[5]. A $SiCl_4$-dealuminated Y zeolites (designated DY) with nonframework aluminum were prepared according to the procedure described by Beyer *et al.*[6]. The all zeolites thus dealuminated were ion-exchanged three times, 12 hr each, in 1M NH_4NO_3 at 70 °C to remove any remaining Na^+ ions.

The zeolites were tested as catalysts for cumene cracking at 350 °C after pretreatment at 400 °C for 2 hr. Catalyst samples were prepared by mixing the zeolite with silica (Davison 952 grade SiO_2) in order to keep conversions low. Catalytic activities were measured in a pulse microreactor constructed of 1/4 in stainless-steel. The products were analyzed with a Shimadzu 3 BT gas chromatograph using a 3 m Bentone 34 on chromosorb W column at 130 °C.

The initial activity was given in terms of μmole cumene converted perg zeolite during the first pulse reaction. As shown in Figure 1, there is a correlation between cumene cracking activity and the number of framework aluminum ions per unit cell. With EDY samples, the activity increases with the dealumination to a maximum and then decreases. Namely, activity increases progressively according as the number of framework aluminum ions per unit cell changes from 56 to 28. This is related to the increase of acid strength after aluminum removal and the extraction of weak acid site. It has been known that in Y zeolites the location of all the framework aluminum atoms is crystallographically identical, but 35% of these atoms are chemically different[7,8]. Upon fur-

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