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A BIFUNCTIONAL ENZYME MODEL. PROXIMITY EFFECT OF A FUNCTIONAL DETERGENT IN THE DEACYLATION OF p-NITROPHENYL CARBOXYLATES INCORPORATED INTO THE MACROCYCLIC CAVITY

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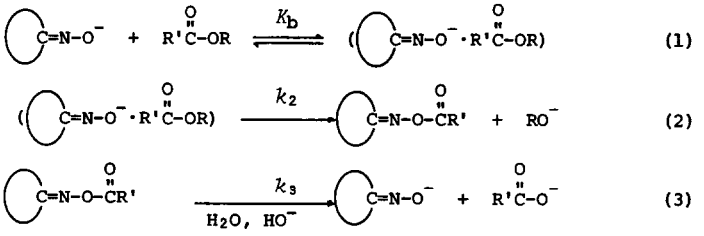
Recently, studies of enzyme-model catalysis using functional macrocycles have been extensively developed.[1] We have previously synthesized a functional macrocyclic compound, Oxime-I (10-hydroxy-11-hydroxyimino[20]paracyclophane) which was designed as a simple enzyme model.[2] Oxime-I has an oxime group as the nucleophilic catalysis site and provides a sizable interior cavity which acts favorably to incorporate relatively bulky substrates. As a group transferase model, Oxime-I has shown significant catalytic effects on the acyl transfer reactions of *p*-nitrophenyl carboxylates[3] and L-leucine-*p*-nitroanilide,[4] the sulfate transfer of aryl sulfates,[5] and the phosphate transfer of bis-*p*-nitrophenyl phosphate.[4] These effects seem to be ascribed to the considerable ability of Oxime-I to bind substrates (K_b=10^4∿10^7 M^{-1}),[3] and to the large rate acceleration of the second stage [reaction 2](for the acyl transfer,[3] k_2=10^{-3}∿10^{-1} sec^{-1}) as well as the substrate specificity caused by geometrical requirement of the macrocyclic cavity.



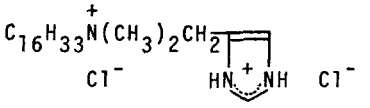
(1)

(2)

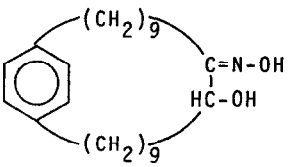
(3)

The hydrolytic catalysis is to be observed if k_3 exceeds the rate constant for the corresponding alkaline hydrolysis (k_{hyd}) in the bulk solution, and then the turnover behavior of Oxime-I will be attained. In order to utilize Oxime-I, therefore, as a catalyst for ester hydrolysis, another functional group which promotes the deacylation of acyloxime [reaction 3] must be introduced to the Oxime-I system. *N*,*N*-Dimethyl-*N*-hexadecyl-*N*-(4-imidazolium)methylammonium

dichloride (Im-I) was prepared to utilize as a cofactor in the Oxime-I system according to the method by Tagaki and his co-workers[6] with some modification and the cooperative effect due to the presence of Im-I at concentrations lower than its cmc[6] was examined in the Oxime-I-catalyzed acyl transfer.



Im-I



Oxime-I

The liberation of *p*-nitrophenol was followed spectrophotometrically at 400 nm. Significant rate enhancement was observed by the addition of Im-I to the Oxime-I-catalyzed reaction system (system C): rate acceleration by 300-fold over the simple alkaline hydrolysis, 22-fold over the Oxime-I catalysis (system A), and by 39-fold over the Im-I catalysis (system B), respectively, for the reaction using PNPP (*p*-nitrophenyl palmitate) as the substrate. The effectiveness of Oxime-I catalysis was, thus, raised at least 20-fold upon addition of the cofactor. The enhancement effect was also observed for reactions of other substrates, PNPD (*p*-nitrophenyl decanoate) and PNPL (*p*-nitrophenyl laurate), as summarized in Table 1. If we define the second-order rate constant for the cooperative catalysis as listed in Table 1, the present system displays a significant rate acceleration greater than the acyl-transfer activity of α-chymotrypsin for *p*-nitrophenyl acetate (k_2=563 sec^{-1} M^{-1}).[7]

Table 1. Apparent first-order rate constants for *p*-nitrophenol release from *p*-nitrophenyl carboxylates in the presence of Oxime-I and/or Im-I at 20.3°C, μ=0.10 (KCl), and pH 10 in 10.9%(v/v) aqueous acetone[a)]

10^5[Substrate] M	Spontaneous hydrolysis $10^4 k_{hyd}$, sec^{-1}	System A[b)] $10^4 k^A_{obs}$, sec^{-1}	System B[c)] $10^4 k^B_{obs}$, sec^{-1}	System C[d)] $10^4 k^C_{obs}$, sec^{-1}	k_{cat}[e)] sec^{-1} M^{-1}
PNPD: 0.992	0.85	4.5	13.5	153	3130
PNPL: 1.033	0.18	3.00	28.9	70.5	1420
PNPP: 1.168	0.14[f)]	1.90	1.08	41.6	835

a) pH was adjusted with 0.001 M borate-0.004 M carbonate buffer.
b) 1.978 x 10^{-5} M of Oxime-I was added.
c) 0.475 x 10^{-5} M of Im-I was added. k_{obs}'s were obtained on the later stage of reaction.
d) Oxime-I and Im-I were added, each concentration being maintained at the same one as used in systems A and B, respectively. k_{obs}'s were evaluated on the later stage of reaction.
e) Second-order rate constant for the cooperative catalysis as calculated from the observed pseudo first-order rate constant according to $(k^C_{obs} - k^A_{obs})/[\text{Im-I}]$.
f) Since the rate was too slow to determine under the same reaction condition as those used for others, the rate constant was estimated using the activation energy.

The addition of Im-I at about 2:1 molar ratio of substrate to catalyst species caused complete decomposition of each substrate, indicating that Im-I exhibits the turnover behavior. Spectral changes for the reaction of PNPP in 10.9% aqueous ethanol over the 220-360 nm range are illustrated in Fig. 1 for each of systems A, B, and C, where absorption at 245 nm is due to the acylimidazole intermediate[8] and another at 226.5 nm to the acylated oxime.[9] Tagaki

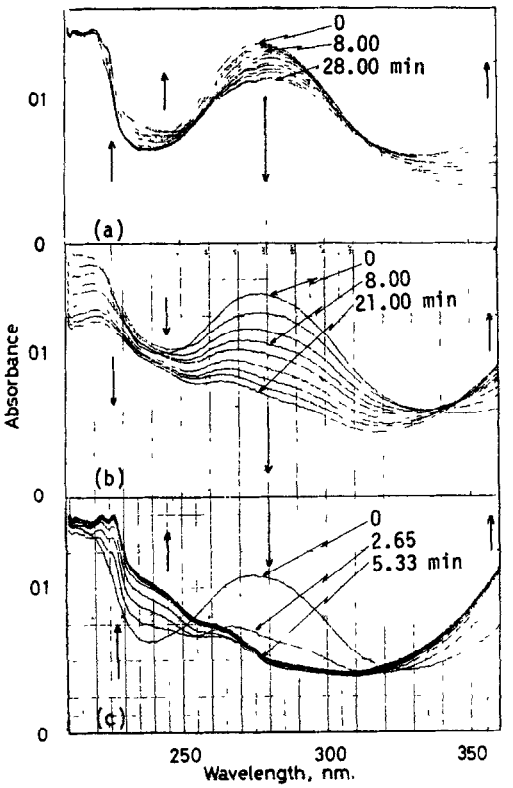
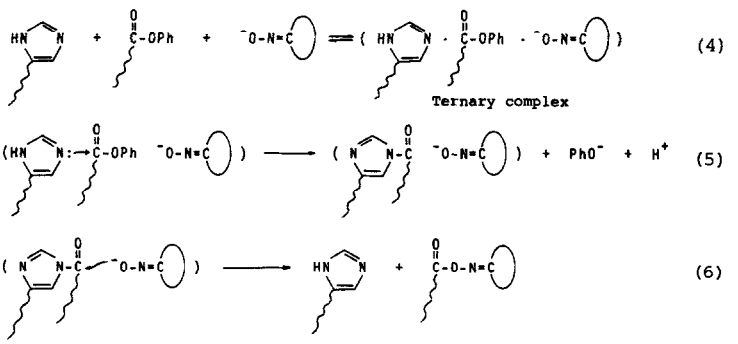


Fig. 1 Spectral changes in the 220-360 nm range during the course of reaction of PNPP in systems A(a), B(b), and C(c) at μ=0.10(KCl), pH 10(with 0.001 M borate and 0.004 M carbonate), and 20.0°C in 10.9% aqueous ethanol; $[PNPP]_0$= 0.959×10^{-5} M in all the systems; 1.94×10^{-5} M of Oxime-I in system A; 0.476×10^{-5} M of Im-I in system B; and a mixture of Oxime-I and Im-I of the same concentrations as those used in systems A and B, respectively, in system C. Vertical arrows in figures indicate the direction of change in intensity during the course of reaction.

and his co-workers pointed out that the acylimidazole intermediate is formed at the rate comparable to that of its decomposition in the hydrolysis of *p*-nitrophenylacetate as catalyzed by the micellar *N*,*N*-dimethyl-*N*-octadecyl-*N*-(4-imidazolium)methylammonium dichloride, whereas the rate-determining step is the deacylation of the acylimidazole in the simple imidazole catalysis. Clearly from Figs. 1-b and -c, a broad absorption band is observed at around 235-245 nm for systems B and C which involve Im-I species. This band is not seen for system A (Fig. 1-a). The absorption intensity at 245 nm decreased along the progress of reaction noticeably for system B. This indicates that the formation rate of the acylimidazole intermediate is very fast and the decomposition process of the intermediate becomes the rate-determining step. On the other hand, the rate of formation of the acyloxime apparently increased by the addition of Im-I as seen in system C (Fig. 1-c). Absorption spectra for system C can not be synthesized

from the corresponding spectral data for systems A and B. Judging from these results, it is most reasonable to assume that the cooperation of Oxime-I with Im-I in the acyl transfer takes place effectively and Im-I may act as a preceding acyl acceptor in the reaction process [reactions 4 and 5]. There are two affirmative evidences for the cooperation of Oxime-I with Im-I: (i) the utilization of CTAB, instead of Im-I, at concentrations below its cmc in the Oxime-I-catalyzed reaction resulted in little rate enhancement, and (ii) the addition of 10-hydroxy-11-oxo[20]paracyclophane in place of Oxime-I to the Im-I-catalyzed reaction did not give out any cooperative catalytic effect on the acyl transfer. Upon surveying the above results, it is most likely that both reactions 4 and 5 occur *via* formation of the *ternary complex* among substrate and catalyst species as illustrated in Scheme 1. The Oxime-I-Im-I system in the present reactions



SCHEME 1

apparently undergoes a bifunctional catalysis which is often observed or speculated in many enzymatic reactions.

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