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A Bifunctional Catalysis of Ester Hydrolysis as Promoted by Cooperation of Functional Macrocycle and Functional Detergent¹)

by

Junzo SUNAMOTO*, Hiroki KONDO*, Hiroshi OKAMOTO**, and Osamu TERADA***

Hydrolytic decomposition of $p$-nitrophenyl hexadecanoate (PNPP) was drastically accelerated by a cooperative action of 10-hydroxy-11-hydroxyimino[20]paracyclophane (Oxime-I) and hexadecyl (imidazol-4-ylmethyl)-dimethylammonium chloride hydroperchlorate (Im-I) at 25°C in the moderately alkaline region. The pH-rate profile for this reaction as well as those for individual catalysts (Oxime-I and Im-I) reveals that the cooperation of anionic imidazole moiety of Im-I and free form of Oxime-I was responsible for the observed rate enhancement. Time-dependent uv-spectral change of the reaction system suggests that the initial acylation of imidazole by the substrate is followed by the deacylation with Oxime-I. The overall reaction is, hence, an acyl group transfer from the substrate to Oxime-I via Im-I. Through detailed kinetic studies, the high efficiency of this bifunctional catalysis was attributed to a nucleophilic-general acid catalysis by anionic imidazole group of Im-I and free hydroxyimino moiety of Oxime-I, which occurs in the ternary complex consisting of the substrate, Im-I, and Oxime-I.

Multifunctional catalysis, as typically seen in the charge-relay system of serine proteases such as $\alpha$-chymotrypsin, appears to be mainly responsible for the high efficiency of enzymic reactions. A number of hydrolytic enzyme models, most of which is monofunctional, has been developed to mimic some aspects of enzymic catalysis with modest success. Improvement of catalytic efficiency in model systems may be achieved by placing two or more catalytic groups on a catalyst molecule so that they can work cooperatively toward a given substrate. However, well-defined examples of concerted action of two or more catalytic groups are rather scarce in nonenzymatic systems. Because, it is difficult to arrange more than two functional groups in a geometrically specified orientation by which they...
can work simultaneously. This discussion holds as well for the macrocyclic enzyme models extensively developed by Murakami and his collaborators.\;[7,8] [20] Paracyclophanes bearing a hydroxyimino or amino moiety are potent catalysts in the hydrolytic decomposition of carboxylate or carbonate esters with a long alkyl chain.\;[9-11] Introduction of a trimethylammonium group to the benzene ring of 10-hydroxyimino-[20]paracyclophane brought about a further rate acceleration by two orders of magnitude in the hydrolysis of \( p \)-nitrophenyl carboxylates compared to the case of the monofunctional paracyclophane.\;[12] Hence, it is clear that an efficient catalysis is expected once a mutually favorable arrangement of two catalytic groups is achieved. Unfortunately, however, approach along this line is often hampered because of the difficulty involved in the synthesis of such catalysts.

Another defect of current simple enzyme models is extremely small turnover number in catalysis. For example, hydrolysis of carboxylate esters with the catalyst having a nucleophile such as hydroxy, sulphydro, amino, or imidazole moiety proceeds, in most cases, via an acyl group transfer from the substrate to either of these nucleophilic sites of the catalyst (Scheme I). The acyl-intermediate then breaks down by the action of another nucleophile present on the catalyst itself or in solution, regenerating an original active catalyst. Unless the rate of deacylation exceeds that of acylation \((k_2 > k_1)\), the acyl-intermediate is accumulated and the catalyst loses activity. This is true for most of the nucleophiles whose nucleophilicity is high, since a good nucleophile is in general a poor leaving group.\;[4]

In order to overcome these difficulties we constructed a simple bifunctional catalysis consisting of a macrocycle, 10-hydroxy-11-hydroxyimino [20] paracyclophane (Oxime-I) and a functional detergent, hexadecyl (imidazol-4-ylmethyl)-dimethylammonium chloride hydroperchlorate (Im-I).\;[13] The macrocyclic cavity of Oxime-I should provide a favorable field for incorporation of a substrate.\;[7] The incorporated substrate would then be processed by the action of hydroxyimino moiety of Oxime-I and/or imidazole group of Im-I in the cavity. A largest rate acceleration was, in fact, observed in the ternary reaction system composed of Oxime-I, Im-I, and \( p \)-nitrophenyl dodecanoate or hexadecanoate (substrate). Interestingly, the rate observed for the binary catalyst system was much larger than the sum of the rates obtained for the individual catalysts.\;[13] In the present article we would like to present results of more detailed kinetics on this ternary reaction system to make clear the mechanism and nature of multifunctional catalysis.

**Scheme I**

\[
\begin{align*}
R-C-O-\bigcirc-NO_2 + \text{Catalyst} - XH & \xrightarrow{k_1} \text{Catalyst} - X-C-R + HO-\bigcirc-NO_2 \\
\text{Catalyst} - X-C-R + YH & \xrightarrow{k_2} R-C-Y + \text{Catalyst} - XH
\end{align*}
\]
Fig. 1 pH-Rate profiles for the hydrolytic decomposition of PNPP as promoted by several catalyst systems in aqueous media containing 10.9% (v/v) ethanol at 25.0°C and μ = 0.10 (KCl): spontaneous (▲), Oxime-I (△), Im-I (●), and Oxime-I+Im-I (○). [PNPP] = 0.990×10⁻⁵ M. [Im-I] = 4.98×10⁻⁴ M. [Oxime-I] = 1.98×10⁻⁵ M.

Results and Discussion
Kinetics of the hydrolytic decomposition of a carboxylate ester, p-nitrophenyl hexadecanoate (PNPP) have been investigated at 25.0°C in aqueous media containing 10.9% (v/v) ethanol. Catalytic efficiency of pertinent catalyst systems in the hydrolysis of PNPP is compared at pH 9.70, where the spontaneous (alkaline) hydrolysis of substrate is virtually negligible (Table I). The correlations between catalysis rates for each catalyst system and pH are shown in Fig. 1.

Individual Catalytic Efficiencies of Im-I and Oxime-I. Im-I is effective at a concentration as low as 5×10⁻⁴ M, which is below its reported cmc of 1.05×10⁻⁴ M. This rate enhancement stems solely from the structural characteristic of Im-I to have a hydrophobic alkyl chain, positive charge, and imidazole moiety in a molecule, since at the same concentration imidazole itself shows only an indiscernible rate enhancement (Table I). As the substrate also has a long alkyl chain, Im-I and the substrate can associate in solution even below their own cmc’s, leading to an increased chance for the substrate to transfer an acyl moiety to the catalyst. In the Im-I catalysis logkobs-value increases linearly with pH over the range of 8-11. This means that the anionic form of imidazole moiety of Im-I is catalytically active, since the second pK_a of Im-I falls at about pH 9. The anionic imidazole moiety of Im-I is first acylated by the substrate, as evidenced by the appearance of absorption band at 245 nm which is
Table I. Comparison of catalytic efficiency of various catalyst systems in the hydrolysis of PNPP in an aqueous medium containing 10.9% (v/v) ethanol at pH 9.70, 25.0 °C, and μ=0.10 (KCl) a)

<table>
<thead>
<tr>
<th>Catalystb) : Concentration, M</th>
<th>k_{obs}, s^{-1}</th>
<th>Relative rate</th>
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<tbody>
<tr>
<td>None</td>
<td>6.53×10^{-6}</td>
<td>1</td>
</tr>
<tr>
<td>Imidazole 4.93×10^{-6}</td>
<td>1.79×10^{-5}</td>
<td>2.7</td>
</tr>
<tr>
<td>Im-I 4.98×10^{-5}</td>
<td>6.66×10^{-4}</td>
<td>102</td>
</tr>
<tr>
<td>Oxime-I 1.98×10^{-5}</td>
<td>2.22×10^{-4}</td>
<td>34</td>
</tr>
<tr>
<td>Oxime-I 1.98×10^{-5} + Imidazole 4.93×10^{-6}</td>
<td>3.34×10^{-4}</td>
<td>51</td>
</tr>
<tr>
<td>Oxime-I 1.98×10^{-5} + Im-I 4.98×10^{-6}</td>
<td>1.98×10^{-3}</td>
<td>303</td>
</tr>
<tr>
<td>P* 1.98×10^{-5}</td>
<td>9.67×10^{-6}</td>
<td>1.5</td>
</tr>
<tr>
<td>P* 1.98×10^{-5} + Im-I 4.98×10^{-6}</td>
<td>1.07×10^{-5}</td>
<td>164</td>
</tr>
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a) [PNPP]=9.90×10^{-6}M.
b) For abbreviations of catalysts see text.

Scheme II

characteristic of an acyl-imidazole (Fig 2). 13,17) The time course of this absorption band indicates that the intermediate collapses rather quickly due presumably to the attack by the hydroxide ion present near the cationic center of acyl-Im-I (Scheme II). 17,18)

In the case of Oxime-I, 2×10^{-5} M catalyst accelerated 34-fold the hydrolytic decomposition of PNPP in mild alkaline media. It should be noted that at pH 9.70 the hydroxyimino group of Oxime-I is predominantly in its un-ionized form. This suggests that the free oxime as well as the oximate is catalytically active, as will be discussed later in more detail. In the Oxime-I catalysis the pH-rate profile is composed of two linear lines with an intersection at pH 11.5. This reflects an acid dissociation of hydroxyimino moiety of the macrocycle and indicates that the anionic species, the oximate, is much more reactive. 7,9,11) It has already been established that the reaction of anionic Oxime-I with carboxylate esters involves the nucleophilic attack of the oximate group on the ester carbonyl carbon. 7,9) It should be emphasized, however, that
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the neutral species of Oxime-I is catalytically active, too. A similar behavior of neutral hydroxyimino moiety has been reported by Murakami and his coworkers in the hydrolysis of carboxylate esters bearing a long alkyl chain.\(^{19}\) They concluded from the product analysis that the nucleophilic attack of free hydroxyimino moiety on the ester carbonyl takes place. The special environment provided by hydrophobic domain of Oxime-I may be responsible for this unusual nucleophilicity of the neutral species.\(^{19}\)

**Ternary Complex Formation and Cooperation of Oxime-I and Im-I.** When both Oxime-I and Im-I are used together, the observed rate enhancement was much larger than the sum of rate constants for individual catalyst system (Table I). This synergism in the rate is characteristic only of the present combination of catalysts. When imidazole was employed instead of Im-I, the observed rate was not very different from that of the monofunctional system by only Oxime-I. The involvement of Oxime-I in this ternary system is manifest, since replacement of Oxime-I by 10-hydroxy-11-oxo[20] paracyclophane (P,\(_0\)) reduced the rate to the level almost comparable to that of the catalysis by Im-I alone. A similar thing happens also in the replacement by imidazole for Im-I during the cooperation with Oxime-I: no drastic rate enhancement was attained. In general, the encounter of three components is unlikely to take place in solution. In the present system, however, all three species, the substrate, Im-I, and Oxime-I are sufficiently hydrophobic to aggregate even at extremely low concentrations, which makes the formation of ternary complex real.

In the presence of a fixed concentration of Im-I (4.98 x 10^-8 M) at pH 9.58, the rate of PNPP hydrolysis saturated with respect to Oxime-I concentration, as have always been observed for the paracyclophane catalysis in the ester hydrolysis.\(^{5,7,8}\) This indicates that the basic feature of substrate incorporation into a macrocyclic cavity is not impaired by Im-I. Judging from the fact that the rate levels off at a lower Oxime-I concentration in the presence of Im-I (data not shown), Im-I rather seems to facilitate the formation of such a ternary complex. In the ternary complex composed of the substrate, Oxime-I, and Im-I, the synergism in rate is especially pronounced below pH 11.5, where the predominant species of the catalysts are un-ionized Oxime-I and ionized Im-I. Time-dependent UV-absorption spectra of the reaction mixture do exhibit an accumulation of acyl-imidazole intermediate at

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**Fig. 2** The time courses of 245 nm absorption band in the hydrolytic decomposition of PNPP as catalyzed by 4.98 x 10^-4 M Im-I (-----) or 4.98 x 10^-4 M Im-I and 1.98 x 10^-3 M Oxime-I (...) in an aqueous medium containing 10% (v/v) ethanol at pH 9.70, 25.0°C, and \(\mu = 0.10\) (KCl). [PNPP] = 0.990 x 10^-4 M.
the very early stage of the reaction, but to a lesser extent than that for the Im-I catalysis (Fig. 2). Instead, a new absorption band centered at 226 nm appears and progresses much faster than that for the Oxime-I catalysis. This band is assignable to an acyl-oxime.\(^{13}\) Hence, the whole reaction sequence may be depicted as follows: the initial attack by the imidazole anion of Im-I on the substrate is followed by the further acyl transfer to the oxime moiety of Oxime-I. The first step must be the rate-determining step, since an extensive accumulation of acylimidazole is not detected spectroscopically. However, the observed synergism of both catalysts in the ternary system can not be ascribed to the quick turnover of the whole reaction sequence. Because, a large rate enhancement is observed even at a very early stage of the reaction. We would like to propose a participation of the free hydroxyimino group of Oxime-I as a general acid catalyst to facilitate the imidazole attack on the ester carbonyl in the first acylation as shown below. Either a neutral or ionized hydroxyimino moiety of Oxime-I will accept the acyl group from the acyl-Im-I at the subsequent step. The acylated Oxime-I appears to be inert to further hydrolysis under these conditions. In any event, the observed bifunctional catalysis leads to a facile group transfer reaction of p-nitrophenyl carboxylates bearing a long alkyl chain under a milder condition than that needed for the Oxime-I catalysis.

**Experimental**

PNPP, Oxime-I, and 10-hydroxy-11-oxo-[20]paracyclophane (P\(_\sigma\)) are the same as those used previously.\(^{7,9}\) The synthesis of Im-I has been reported elsewhere.\(^{14,20}\)

Electronic spectra were taken on a Hitachi 124 recording spectrophotometer. Reaction rates were determined by following the appearance of p-nitrophenolate ion at 400 nm on the same apparatus. A reaction solution was buffered by an appropriate inorganic salt such as sodium borate or carbonate. In high pH regions aqueous sodium hydroxide was adopted instead. A pH value of a reaction solution was read with a Toa HM-5A pH meter connected with a combined electrode GS-135C.

**Acknowledgment**

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**References and Notes**

7. Y. Murakami, J. Sunamoto, and K. Kano,
16. Although a precise $pK_{a1}$ of Im–I has not been obtained because of the instability of Im–I at the alkaline pH, the value of about 9 seems to be a reasonable estimate (Ref. 14). The $pK_{a1}$ and $pK_{a2}$ of imidazole are around 7 and 14, respectively, while the $pK_{a1}$ of Im–I is reported to be 3.5 (Ref. 14).