A Macrocyclic Enzyme Model System. Substrate Specificity for the Inclusion into Paracyclophane Oxime and the Subsequent Acyl Transfer Reaction

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In order to characterize the intrinsic nature of 10-hydroxy-11-hydroxyimino[20]paracyclophane (Oxime-I) as being an enzyme model, the acylation reactions of Oxime-I with *p*-nitrophenyl carboxylates have been investigated in an alkaline aqueous acetone. The usual saturation-type kinetics has been observed for the reactions of *p*-nitrophenyl decanoate (PNPD), laurate (PNPL), and palmitate (PNPP) with Oxime-I. The acyl transfer reaction was consistent with the following two sequences: (1a) pre-equilibrium formation of the inclusion complex between Oxime-I and a substrate, (1b) pre-equilibrium acid dissociation of the inclusion complex, and (1c) rate-determining acyl transfer from a substrate to Oximate-I in the inclusion state; and (2a) pre-equilibrium acid dissociation of Oxime-I, (2b) pre-equilibrium formation of the inclusion complex between Oximate-I and a substrate, and (2c) rate-determining acyl transfer from a substrate to Oximate-I in the inclusion state. On the basis of the compensation correlation between enthalpy and entropy for binding process, the inclusion of a long chain carboxylate into the cavity of Oxime-I is controlled by entropy. It is interesting to note that the binding constants for the present inclusion complexes are considerably larger ($K \simeq 10^4 - 10^5\ M^{-1}$) than those for the complexes formed between phenyl esters or azo dyes and cycloamyloses ($K \simeq 10^2 - 10^3\ M^{-1}$). Thus, the paracyclophane skeleton provides an effective binding site due to the hydrophobic cavity. Since the most stable inclusion complex was formed with PNPD, the inclusion of the present esters may be not exclusively due to the hydrophobicity provided by an apolar hydrocarbon tail, but significantly due to the molecular geometry or bulkiness characterized by the folded ester molecule. This effect approves the presence of substrate specificity due to the molecular geometry for Oxime-I. In reference to the activation parameters, the acyl transfer process is controlled by entropy in a manner similar to the alkaline hydrolysis in the bulk phase and the complex formation as well. PNPP is least stabilized in the binding process due to the most unfavorable entropy effect, whereas most favorably deacylated in the acyl transfer due to the entropy factor. Consequently, the net change in the free energy is largely reflected on the entropy term. The deceleration effect observed in the reaction of PNPD with Oxime-I at a higher acetone content and the kinetic behavior of alkaline hydrolysis of the three carboxylate esters have also been discussed.

Micellar surfactants,[1] synthetic water-soluble polymers carrying various functional groups,[2] and macrocyclic compounds[3] are the three major types of enzyme models recently developed. Of these three model systems, macrocyclic compounds may exhibit several unique characters which can never be expected for other two systems: 1) a steady binding site provided by a characteristic ring conformation of hydrophobic nature; 2) a high substrate specificity brought about by the geometric requirements for binding of substrates into these macrocyclic cavities as well as by spatial geometries of substrate molecules incorporated into these cavities; and 3) a high possibility of introducing a charge relay system or a polyfunctional catalytic one, which is provided by the spatial arrangement of appropriate functional groups. Consequently, a well-designed macrocyclic compound may exhibit a catalytic ability more favorably than other types of enzyme models, providing one binding site and one catalytic function for each substrate.

From these view points, we have previously prepared two macrocyclic oximes, 10-hydroxy-11-hydroxyimino-[20]paracyclophane (Oxime-I) and 2-hydroxycyclodecanone oxime (sebacoin oxime, Oxime-II), and investigated their catalytic efficiencies for the deacylation reaction of *p*-nitrophenyl carboxylates.[4,5] The sizable cyclic cavity of Oxime-I provided an appropriate binding site as expected owing to the restricted ring conformation as well as to the hydrophobicity of paracyclophane skeleton. Oxime-I was, thus, acylated by *p*-nitrophenyl laurate (PNPL) and decanoate (PNPD), which were suitably incorporated into the Oxime-I cavity because of their relatively large molecular volume in a folded form. On the contrary, the oxime did not show any catalytic activity toward smaller esters such as *p*-nitrophenyl acetate (PNPA) or hexanoate (PNPH). Oxime-II did not undergo any reaction with all of these esters under the same conditions. In order to characterize the intrinsic nature of Oxime-I as being an enzyme model, we have employed an additional substrate having a longer alkyl chain, *p*-nitrophenyl palmitate (PNPP). On the basis of thorough thermodynamic and kinetic analyses for binding and acyl transfer processes, respectively, the substrate specificity has been elucidated for the inclusion into Oxime-I and for the subsequent acyl transfer reaction.

Experimental

Materials. Cyclic oximes, Oxime-I and -II, and two *p*-nitrophenyl carboxylates, PNPD and PNPL, were the same as those used in our previous investigations.[5] *p*-Nitrophenyl palmitate (PNPP) was commercially obtained from E. Merck, Darmstadt and used after the inspection of its purity (Found: C, 70.06; H, 9.08; N, 3.90%; mp 64.0 °C).

Kinetic Measurements. The rate of liberation of *p*-nitrophenoxide ion was measured at 400 nm either on a Hitachi 124 recording spectrometer or on a Shimadzu-Bausch & Lomb Spectronic 88 equipped with a Riken Denshi SP-G3 recorder. Reactions were initiated by the addition of a substrate dis-

solved in acetone to a reaction solution which was pre-incubated at an appropriate temperature in the thermostatted cell set in the spectrometer. The preparation of stock and reaction solutions, and the measurement procedures were essentially the same as those described previously.[5] The rate constants were checked by duplicate runs and observed to retain an accuracy within ±1%.

pH Measurements. pH-Values of the reaction solutions, which were prepared with 10.9% (v/v) aqueous acetone as the solvent, were measured and calibrated by using two separate buffer solutions in the same solvent, the pH values of which were referred to those measured in the aqueous system. The measurements were carried out either with a TOA HM-9A recording pH meter or a TOA HM-5A pH meter equipped with an attachment for expandomatic reading. Both pH meters were connected with a Metrohm EA-125 combined electrode or a set of Metrome EA-107H glass electrode and Beckman 39402 saturated calomel electrode.

Results and Discussion

As shown in Scheme 1, the present reaction system may be roughly classified into two competitive reactions: the alkaline hydrolysis of a free substrate in the bulk solution and the acyl transfer reaction occurring in the inclusion complex formed between Oxime-I and a substrate.[5] For the reactions of PNPD, PNPL,[5] and PNPP with Oxime-I, the usual saturation-type kinetics has been observed. The rate constant of alkaline hydrolysis k_{hyd} can be separately obtained from the rate measurements in the absence of Oxime-I. Kinetic results obtained for PNPD and PNPP are summarized in Tables 1 and 2, respectively.

$$\mathrm{S} + \text{Oxime-I} \overset{K}{\rightleftharpoons} (\text{S—Oxime-I})$$
$$\downarrow k_{hyd} \qquad\qquad \downarrow k_{acyl}$$
$$\mathrm{P_1} \qquad\qquad \mathrm{P_2}$$

Scheme 1.

Alkaline Hydrolysis of p-*Nitrophenyl Carboxylates.* In the absence of cyclic oximes, *p*-nitrophenyl carboxylates undergo usual hydrolytic decomposition through the hydroxide ion catalysis. The rate of alkaline hydrolysis of carboxylates having a long hydrocarbon chain was largely inhibited with increasing the chain length as previously indicated by Menger and Portnoy:[6] $k_{hyd}=4.47\times10^{-3}\,s^{-1}$ at pH 10.71 for PNPD, $1.41\times10^{-4}\,s^{-1}$ at pH 10.31 for PNPL,[5] and $7.24\times10^{-5}\,s^{-1}$ at pH 10.71 for PNPP (at 43.1 °C), respectively, among which the values for PNPD and PNPP were estimated by the aid of activation energies. A large decrease in the hydrolysis rate along with the extension of hydrocarbon chain is readily explained by the self-aggregation effect due to hydrophobic interaction. The ester carbonyl group, as being the reaction center, is presumably shielded from the attack of a nucleophile by the hydrophobic aggregation. The hydrolysis rate of these esters was retarded also with increasing the initial concentration[6] due to the similar reason. In this work, therefore, we have maintained the initial ester concentration at about 1.0×10^{-5} M, where a small change in the ester concentration gives off a

Table 1. First-order rate constants for the *p*-nitrophenol release from *p*-nitrophenyl palmitate in the presence of Oxime-I in 10.9% (v/v) aqueous acetone at $\mu=0.10$ (KCl)

Temp. °C	[Oxime-I] $\times10^6$ M	$k_{obs}\times10^4$ s^{-1}
$[PNPP]_0=9.65\times10^{-6}$ M and pH 10.71[a]		
49.8±0.1	20.1	52.9
	11.2	40.2
	10.1	35.8
	4.02	20.3
	2.01	10.3
	1.31	7.91
	1.01	6.75
	None	0.82
39.9±0.1	20.9	24.2
	10.4	16.5
	4.17	9.17
	2.07	4.96
	1.04	3.00
	None	0.62
30.1±0.1	20.9	9.64
	13.6	9.11
	10.4	8.17
	4.17	4.30
	2.07	3.13
	1.36	1.95
	None	0.35
20.2±0.1	20.1	3.08
	11.2	3.03
	10.1	2.68
	4.02	2.10
	2.01	1.33
	1.01	0.82
	None	0.18[c]
$[PNPP]_0=9.65\times10^{-6}$ M and pH 11.78[b]		
49.8±0.1	20.1	257
	10.4	178
	4.02	75.2
	2.01	29.2
	1.31	22.4
	1.01	17.3
	None	2.24
39.9±0.1	20.9	101
	13.6	86.7
	4.17	40.8
	2.07	18.8
	1.36	13.0
	None	1.29
20.2±0.1	20.1	22.3
	13.1	18.9
	4.02	11.7
	2.01	7.85
	1.31	6.00
	1.01	3.17
	None	0.28

a) Buffer, 9.64×10^{-3} M Na_2CO_3—0.03×10^{-3} M $Na_2B_4O_7$. b) Buffer, 1.15×10^{-2} M NaOH. c) Estimated from the data obtained at other temperatures using the activation energy, because of the extremely slow rate under the present kinetic conditions.

Table 2. First-order rate constants for the *p*-nitrophenol release from *p*-nitrophenyl decanoate in the presence of Oxime-I in 10.9% (v/v) aqueous acetone at μ=0.10 (KCl)

Temp. °C	[Oxime-I]×10^6 M	$k_{obs}\times10^4$ s^{-1}
[PNPD]$_0$=10.3×10^{-6} M and pH 10.12[a]		
35.4±0.1	20.5	19.6
	10.3	16.0
	6.18	13.1
	4.10	9.92
	1.03	6.92
	None	4.13
25.5±.01	20.5	8.00
	10.3	6.67
	6.18	4.60
	4.10	3.93
	2.05	3.03
	None	1.55
15.5±0.1	20.5	2.65
	10.3	2.23
	6.18	1.83
	4.10	1.47
	None	0.50
[PNPD]$_0$=10.3×10^{-6} M and pH 10.71[b]		
35.6±0.1	20.5	34.3
	10.3	33.9
	4.10	28.8
	2.05	24.2
	1.03	22.0
	None	20.6
24.8±0.1	20.5	12.3
	10.3	11.1
	4.10	9.33
	2.05	7.53
	None	7.03
14.3±0.1	20.5	3.05
	10.3	2.80
	4.10	2.37
	2.05	2.13
	None	1.83

a) Buffer, 7.53×10^{-3} M Na_2CO_3–2.46×10^{-3} M $Na_2B_4O_7$
b) Buffer, 9.64×10^{-3} M Na_2CO_3–0.03×10^{-3} M $Na_2B_4O_7$

relatively minor effect on the hydrolysis rate[6] and the linearity of first-order plot is good at least for the first half-life (rate data given in Table 3).

The base-catalyzed hydrolysis rate is in general subjected to the second-order kinetics. In the present cases, however, the overall rate was not observed to depend on hydroxide ion concentration linearly as seen in Fig. 1. The rate becomes less dependent on hydroxide concentration either by decreasing the reaction temperature or by increasing the length of hydrocarbon chain of substrates. Though convincing evidence is not available at present, the self-aggregation would be favored with decreasing temperature and increasing hydrocarbon chain length, and subsequently the reaction center would be more completely encased and protected from the attack of nucleophile. As a result, the simple bimolecular mechanism with participation of hydroxide ion may be no longer observed. These effects must be reflected on the entropy of activation in terms of solvation and molecular mobility. As is clear from the apparent activation parameters listed in Table 4 for the hydrolysis of PNPD and PNPP obtained at pH 10.71, the reaction is more effectively controlled by the entropy term along with the extension

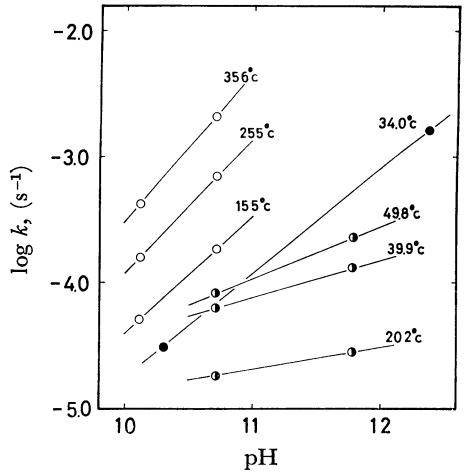


Fig. 1. pH-rate correlations for alkaline hydrolysis of PNPD (○), PNPL (●), and PNPP (◑) at various temperatures: solvent, 10.9% (v/v) aqueous acetone; μ=0.10 (KCl) (refer to Tables 1 and 2).

Table 3. Kinetic parameters for the reactions of *p*-nitrophenyl decanoate, laurate, and palmitate with Oxime-I at μ=0.10 (KCl)

Substrate	pH	Temp. °C	$10^4\times k_{hyd}$ s^{-1}	$10^4\cdot1/\gamma\cdot k'_{acyl}$ s^{-1}	$10^{-4}\times K_b$[c] M^{-1}
PNPD[a]	10.12	35.4	4.13	32.7	41.5
	10.12	25.5	1.55	11.6	100
	10.12	15.1	0.50	4.17	(167)
	10.71	35.6	20.6	46.9	40.0
	10.71	24.8	7.03	16.1	93.5
	10.71	14.3	1.83	6.61	(167)
PNPL[b]	10.31	43.1	1.4	12.0	20.2
	10.31	33.9	0.3	6.28	43.7
	12.37	42.1	37.1	162	10.6
	12.37	34.1	16.1	71.6	18.1
	12.37	24.5	5.1	52.7	38.5
	12.37	15.2	2.1	19.1	(105)
PNPP[a]	10.71	49.8	0.82	112	5.82
	10.71	39.9	0.62	44.1	15.0
	10.71	30.1	0.35	11.5	38.5
	10.71	20.2	0.18	3.55	(940)
	11.78	49.8	2.24	627	3.59
	11.78	39.9	1.29	251	8.16
	11.78	20.2	0.28	29.7	51.3

a) Parameters were calculated from the data listed in Table 1 or 2 by the aid of Eq. (1). b) Data were quoted from Ref. 5. pH-Values have been reevaluated according to the present procedure. c) Values in parentheses may involve a sizable error due to computational means.

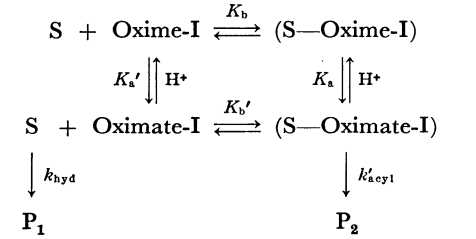
Table 4. Apparent activation parameters for alkaline hydrolysis of *p*-nitrophenyl carboxylates[a)]

Substrate	pH	$\Delta H^{\ddagger}_{hyd}$ kcal/mol	$\Delta S^{\ddagger}_{hyd}$ e.u.	$\Delta G^{\ddagger}_{hyd}$ (303 K) kcal/mol
PNPD	10.12	18.0	−14.9	22.5
	10.71	21.2	−1.7	21.7
PNPP	10.71	10.6	−44.0	24.0
	11.78	11.5	−39.7	23.6

a) Solvent, 10.9% (v/v) aqueous acetone; μ=0.10 (KCl). These apparent activation parameters were evaluated directly from the overall rate constants listed in Tables 1 and 2. The true second-order rate constants were not evaluated due to some difficulty in estimating hydroxide-ion effect (see Fig. 1).

of alkyl chain. The loss of free energy of activation ($\Delta\Delta G^{\ddagger}_{hyd}$) per methylene unit (see Fig. 3) is estimated approximately as 370 cal mole^{-1}. This value, which is due to the contribution from hydrophobic interaction,[7)] is very close to that observed for the aminolysis of phthalic anhydride with amines of long alkyl chain,[8)] where the rate enhancement was observed with increasing hydrophobic character of nucleophiles. Thus, it is clear that the hydrophobicity of an apolar hydrocarbon tail of esters gives out a large effect on their hydrolytic reactivities.

Formation of Inclusion Complex and Subsequent Acyl Transfer Reaction. In the presence of Oxime-I, the inclusion of a *p*-nitrophenyl ester of carboxylate with a long alkyl chain into the cyclic cavity takes place prior to the acyl transfer from the carboxylate to Oxime-I[5)] as represented in Scheme 1. The driving force for the inclusion of a substrate was primarily attributed to the hydrophobic interaction between the alkyl chain of a substrate and the paracyclophane skeleton of Oxime-I, which has been verified by the effects of organic solvent and added urea on the kinetic behavior.[5)]



P_1: carboxylate and *p*-nitrophenolate ions
P_2: acylated Oxime-I and *p*-nitrophenolate ion

Scheme 2.

As pointed out in our previous paper,[5)] the acyl transferring rate is dependent on pH of the medium. Thus, it seems reasonable to establish the whole reaction sequences in more critical manner as shown in Scheme 2. This means that the real nucleophile is not the neutral oxime but the oximate group of Oxime-I in the inclusion complex. As a consequence, the equilibrium constants and the specific rate constant for acyl transfer defined in Scheme 2 can be evaluated by the aid of Eq. (1), the derivation of which is given in Appendix.

$$\frac{1}{k_{obs}-k_{hyd}} = \frac{\left(\frac{1+\beta}{1+\alpha}\right)+K_b[S]_T}{\left(\frac{1}{\gamma}k'_{acyl}-k_{hyd}\right)K_b[C]_T} + \frac{1}{\left(\frac{1}{\gamma}k'_{acyl}-k_{hyd}\right)} \quad (1)$$

and $\alpha = \frac{K_a}{[H^+]}$, $\beta = \frac{K_a'}{[H^+]}$, and $\gamma = \frac{[H^+]}{K_a} + 1$,

where k_{obs} is the observed pseudo-first-order rate constant, and $[S]_T$ and $[C]_T$ stand for the total concentrations of substrate and Oxime-I, respectively. Thus, a good linear relationship must be obtained by plotting the left-hand side of Eq. (1) against the reciprocal initial concentration of Oxime-I, when the initial concentration of substrate and pH of the reaction medium are maintained constant. Since $(1/\gamma)k'_{acyl}$ is obtained as a constant value from Eq. (1), K_a and k'_{acyl} can be evaluated by Eq. (2).

$$k = \frac{\gamma}{k'_{acyl}} = \frac{[H^+]}{K_a k'_{acyl}} + \frac{1}{k'_{acyl}} \quad (2)$$

In fact, all the kinetic data were satisfactorily analyzed by the aid of Eqs. (1) and (2). This seems to validate the reaction sequences given in Scheme 2.

Table 5. Thermodynamic parameters for binding of *p*-nitrophenyl carboxylates with Oxime-I[a)]

Substrate	pH	$\Delta G^{\circ}_{303\,K}$ kcal/mol	ΔH° kcal/mol	ΔS° e.u.
PNPD	10.12	−8.01	−11.97	−13.06
	10.71	−7.96	−12.07	−13.55
PNPL	12.37	−7.50	−14.4	−22.8
PNPP	10.71	−7.65	−16.2	−28.2
	11.78	−7.32	−16.1	−28.9

a) Solvent, 10.9% (v/v) aqueous acetone; μ=0.10 (KCl).

Binding constants and the corresponding thermodynamic parameters for the combination of Oxime-I either with *p*-nitrophenyl decanoate or palmitate are listed in Tables 3 and 5, respectively, along with those for the combination of the oxime with *p*-nitrophenyl laurate previously investigated[5)] upon re-evaluation by Eqs. (1) and (2). The binding constants for these inclusion complexes are considerably larger ($K \simeq 10^4$—10^5 M^{-1}) than those for the complexes formed between phenyl esters[9-11)] or azo dyes[12)] and cycloamyloses ($K \simeq 10^2$—10^3 M^{-1}). The difference is also reflected on the respective reaction conditions: only equimolar or twice molar amount of Oxime-I relative to the substrate was used to gain a significant catalytic effect, while the cycloamyloses were required in a large excess (about 10^3 fold).[9,10)] The free energy change due to binding is in a range of −3——5 kcal mol^{-1} for the cyaloamylose system,[9,11)] while −7——8 kcal mol^{-1} for the Oxime-I system. This means that the paracyclophane skeleton provides a more favorable binding site due to the hydrophobic cavity.

The inclusion of the esters into Oxime-I is considerably dependent on the temperature: the binding constant becomes larger with decreasing the reaction temperature (negative enthalpy change). Such a negative temperature dependency of binding has also

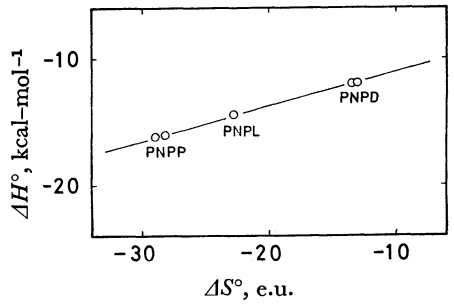


Fig. 2. Isoequilibrium relationship for binding of *p*-nitrophenyl carboxylates with Oxime-I (refer to Table 5).

been noticed for the systems of cycloamyloses[9-12] and of chymotrypsin.[13,14] A different state of affairs has been observed previously:[15,16] the hydrophobic binding increases with increasing temperature. Thus, the positive enthalpy change reported in those studies is not an intrinsic nature of hydrophobic interaction, and the binding behavior is apparently much affected by the nature of host molecules.

There exists a good linear compensation effect between enthalpy and entropy for binding as shown in Table 5 and Fig. 2. Also for the binding process, a considerably low isoequilibrium temperature (β=265 °K) was obtained. This β-value suggests that the inclusion of a long chain carboxylate into the cavity of Oxime-I is certainly controlled by entropy at ordinary temperatures[17] (see Table 5). We can see an analogous compensation effect between enthalpy and entropy in binding process of cycloamyloses with various pseudo-substrates.[18]

We have to notice that the binding stability in terms of free energy change is indicative of the strength of hydrophobic interaction and/or of the structural specificity existing between host and guest molecules. Table 5 indicates that the decanoate ester shows the largest binding stability among three carboxylates (PNPD, PNPL, and PNPP); *i.e.*, the most stable inclusion complex is formed with PNPD. If the binding force is due only to the hydrophobicity, the palmitate ester having the longest apolar tail would be expected to be most strongly bound judging from their reactivities in alkaline hydrolysis. The release of bound water from the catalyst may cause the entropy gain. A substrate molecule, which is more tightly bound with a host molecule, is expected to release more water molecules than those loosely bound, giving off the larger entropy gain. However, this is not the case for the complex formation between PNPD and Oxime-I. The relatively large enthalpy change given out in the binding process seems, therefore, not to be due to the release of bound water in the cavity of Oxime-I. These explanations lead to a conclusion that the inclusion of the present esters may be not exclusively due to the hydrophobicity provided by an apolar hydrocarbon tail, but significantly due to the molecular geometry or bulkiness characterized by the folded ester molecule[5] which is tightly fitted into the cavity. This effect approves the presence of substrate specificity due to the molecular geometry for Oxime-I. Moreover, the enthalpy of binding is evidently insensitive to the pH-value of bulk phase. This fact implies that the intrinsic binding force may be not due to electrostatic interactions such as hydrogen bonding (>C=N–OH··· O=C<) or dipole-ion interaction (>C=N–O$^-$···>C=O).

Investigations dealing with the acyl transfer *via* complex formation between acyl-substrate and nucleophilic catalyst have been recently carried out: *e.g.*, aminolysis of *p*-nitrophenyl decanoate with *n*-decylamine,[16,17] and of phthalic anhydride with various amines having a long alkyl chain.[8] The acyl transfer from a carboxylate ester to cycloamyloses involves the nucleophilic participation of an alkoxide ion derived from the secondary hydroxyl groups of the cycloamyloses.[9-11] A similar mechanistic behavior is considered to take place in the present acyl transfer reaction between a *p*-nitrophenyl carboxylate and an oxime nucleophile. However, in the case of cycloamyloses the subsequent deacylation proceeds at a significantly large rate, while hardly detected in the case of Oxime-I because of the inertness of the acylated oxime to deacylation reaction.[5] Therefore, the present acyl transfer assumes a pseudo-intramolecular mechanism as observed in some aminolysis reactions.[8,16,19]

Kinetic parameters obtained are listed in Table 6 along with their activation parameters calculated from the true rate constants for acyl transfer. Acid dissociation constants for the complexed Oxime-I, which were kinetically estimated (pK_a 10—12), are reasonable in magnitude in reference to that for acetoxime (pK_a 12.4[20]) since an additional substituent effect due to the neighboring hydroxyl group may act in favor

Table 6. Kinetic, thermodynamic, and activation parameters for acyl transfer occurring in the inclusion complexes between Oxime-I and *p*-nitrophenyl carboxylates[a]

	Temp. °C	K_a M^{-1}	pK_a	k'_{acyl} s^{-1}	ΔH^* kcal/mol	ΔS^* e.u.	ΔG^* (303 K) kcal/mol
PNPD	35.6	11.03×10^{-11}	9.99	5.52×10^{-3}			
	25.5	12.57×10^{-11}	9.90	1.86×10^{-3}	17.7	−11.5	21.2
	15.1	7.68×10^{-11}	10.11	0.82×10^{-3}			
PNPL	43	3.46×10^{-12}	11.5	18.2×10^{-3}	17.7	−10.6	20.9
	34	4.29×10^{-12}	11.4	7.80×10^{-3}			
PNPP	49.8	2.25×10^{-12}	11.7	108×10^{-3}			
	39.9	2.14×10^{-12}	11.7	44.5×10^{-3}	17.6	−8.6	20.2
	20.2	7.62×10^{-13}	12.1	9.44×10^{-3}			

a) Kinetic and thermodynamic parameters were obtained from the data listed in Table 3 by the aid of Eq. (2).

of the liberation of oxime proton. The acid dissociation of the PNPD–Oxime-I complex is favored relative to the other, two substrate–Oxime-I system. Since the PNPD–Oxime-I complex is formed by the entropically favored inclusion process (see Table 5), this complex is presumably solvated by water molecules in a more feasible manner for the proton transfer without much destruction of the ice-berg.

On the other hand, their activation parameters show characteristic behavior: the enthalpy of activation is equal to each other among all the substrates while the entropy of activation varies in an decreasing sequence of PNPP, PNPL, and PNPD. These results suggest that the acyl transfer process in the inclusion complexes is controlled by entropy in a manner similar to the alkaline hydrolysis in the bulk phase and the complex formation as well. The palmitate ester, which is least stabilized in the binding process due to the most unfavorable entropy effect, is most favorably deacylated in the acyl transfer process due to the entropy factor.

Catalytic Effect and Substrate Specificity. Clearly from Scheme 1, the catalytic effect of Oxime-I on the acyl transfer reaction can not be simply discussed in terms of the rate ratio k_{obs}/k_{hyd}, because the acyl transfer and the alkaline hydrolysis are rather competitive reactions mutually. Therefore, the reactivity of Oxime-I is compared with that of a smaller cyclic oxime (Oxime-II), which has the same catalysis site as Oxime-I, as previously suggested.[5] The catalytic specificity of Oxime-I for the acyl transfer is defined in terms of $k_{c(Oxime-I)}/k_{c(Oxime-II)}$, where k_c is the apparent catalytic rate constant given by Eq. (3).

$$k_c = \frac{k_{obs} - k_{hyd}}{[\text{Oxime-I or -II}]} \qquad (3)$$

The catalytic specificity obtained under the controlled reaction conditions for the different carboxylate esters of *p*-nitrophenol is shown in Table 7.

Table 7. Catalytic effects of Oxime-I and -II on the *p*-nitrophenyl carboxylates[a]

[Subtrate] $\times 10^5$ M	[Oxime] $\times 10^5$ M	$k_{obs}\times 10^4$ s^{-1}	k_c $s^{-1}M^{-1}$	Rate[b] ratio
PNPA: 1.09	II: 17.5	22.8	0.86	
	II: 1.90	22.6	6.83	1.4
	I: 1.96	23.1	9.20	
PNPD: 1.03	II: 17.5	4.30	0.02	
	II: 1.90	4.30	0.15	420
	I: 1.96	16.8	62.8	
PNPL: 1.00	II: 17.5	0.77	0.05	
	II: 1.90	0.70	0.05	1420
	I: 1.96	15.3	73.2	
PNPP: 1.02	II: 17.5	0.11	0.07	
	II: 1.90	0.11	0.04	1520
	I: 1.96	12.6	62.6	

a) All runs were carried out at 23.5 °C and pH 9.23 (0.03 M $Na_2B_4O_7$) in 10.9% (v/v) aqueous acetone. The rate constants for alkaline hydrolysis of these esters were 21.3×10^{-4}, 4.27×10^{-4}, 0.69×10^{-4}, and 0.10×10^{-4} s^{-1} for PNPA, PNPD, PNPL, and PNPP, respectively, under the present reaction conditions. b) $k_{c(Oxime-I)}/k_{c(Oxime-II)}$, see text.

For a small substrate, *p*-nitrophenyl acetate, both Oxime-I and -II did not show any significant rate enhancement. On the other hand, for larger three esters, PNPD, PNPL, and PNPP, the large rate acceleration in acyl transfer has been observed with Oxime-I, particularly for the latter two esters. These rate accelerations by $1.4—1.5\times 10^3$ fold must be brought about by incorporation of the substrate into the Oxime-I cavity, which results in a pseudo-intramolecular reaction. In other words, the rate enhancement is due neither to a simple hydrophobic entanglement nor to a bimolecular reaction between the substrate and the paracyclophane oxime. Since little effect has been observed on the reaction rate even in the presence of 10 fold excess amount of Oxime-II, there is apparently no significant interaction between the substrates and the smaller cyclic oxime. Consequently, the difference in reactivity between these two cyclic oximes needs to be explained in terms of the correlations between the cavity size of a host molecule and the molecular volume of a substrate in the included state, and of their hydrophobic nature as well. When the folded substrate[5] is effectively incorporated into the cavity, the spatial orientation of the nucleophilic site in the oxime against the carbonyl carbon of the substrate is certainly an important factor to control the subsequent acyl transfer process. This orientation effect seems to be subjected to variation due to the molecular size of an incorporated substrate.

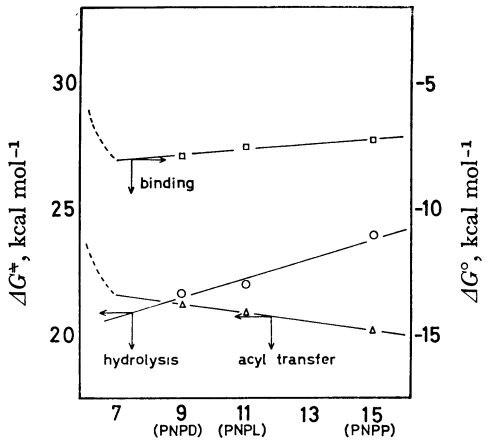


Fig. 3. Correlations between the structure of substrates and free energy changes: binding process of *p*-nitrophenyl carboxylates with Oxime-I (refer to Table 5); acyl transfer process of carboxylates to Oxime-I in the inclusion state on the basis of Scheme 2 (refer to Table 6); alkaline hydrolysis of carboxylates in the bulk solution at pH 10.71 for PNPD and PNPP and 12.37 for PNPL[5] (refer to Table 4).

It is interesting to examine the differences in reactivity in terms of thermodynamic parameters. Figure 3 shows that there are no large differences in free energies of binding among three esters examined, but that the free energy of activation for alkaline hydrolysis increases while that for acyl transfer somewhat decreases with increasing the chain length of acyl residues. A schematic representation for the correlations between relative potential energy due to free energy change and reaction

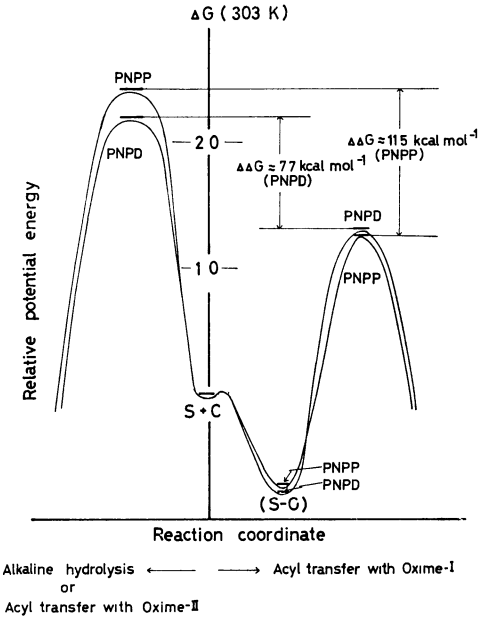


Fig. 4. Reaction coordinate diagram for alkaline hydrolysis and acyl transfer reactions of PNPD and PNPP at 303 K: relative potential energy is represented in free energy change.

coordinate for the two competitive reactions is illustrated in Fig. 4. The activation free energy values for the two carboxylate esters adopted in Fig. 4 in alkaline hydrolysis and acyl transfer reaction are based on Scheme 2 (refer to Tables 4 through 6) so that both reactions can be compared on the equal basis: the difference in $\Delta\Delta G$ between PNPP and PNPD is about 3.8 kcal mol^{-1} at 303 K and pH 10.71.

Deceleration in the Reaction of p-*Nitrophenyl Decanoate with Oxime-I.* Different from the reactions of PNPL and PNPP with Oxime-I, the liberation of *p*-nitrophnoxide ion in the case of PNPD and Oxime-I was somewhat inhibited at a higher acetone content (20.8% (v/v)) with increasing the reaction temperature as illustrated in Fig. 5. An increase in acetone content may cause the decrease in hydrophobicity of the substrate itself, which results in a rate acceleration of the alkaline hydrolysis in the bulk solution.[6] Of course, the decrease in hydrophobicity of the substrate may somehow tend to decrease the binding constant.[5] However, the effect on the binding process is not necessarily large and even some compensation effect comes into play in the subsequent acyl transfer process. As a result, the large rate enhancement for the alkaline hydrolysis in the bulk phase seems to exceed the deacylation in the Oxime-I complex. Therefore, under these circumstances substrate specificity for acyl transfer seems to be more distinctly observed. A similar inhibition phenomenon has been observed in the hydrolysis of ethyl *p*-aminobenzoate in the presence of *β*-cycloamylose.[21]

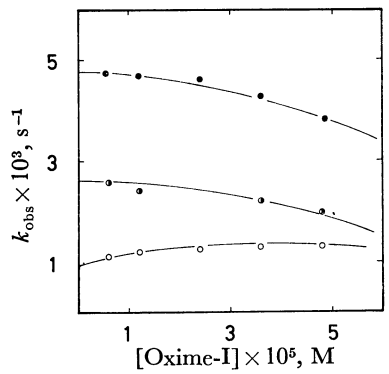


Fig. 5. Correlations between Oxime-I concentration and rate of liberation of *p*-nitrophenoxide ion from PNPD at various temperatures; ● 30.3 °C, ◑ 25.3 °C, and ○ 20.4 °C.

Appendix

Determination of Kinetic and Thermodynamic Parameters for Saturation-type Kinetics. The kinetic and thermodynamic parameters given in Scheme 2 have been evaluated by the aid of Eq. (1). The derivation of this equation is given below.

At the beginning, the abbreviations are made as follows:

S: a substrate species.

C: all the Oxime-I species.

CH: Oxime-I with a undissociated oxime proton.

C$^-$: Oxime-I with an anionic oximate group (Oximate-I in Scheme 2).

S–CH: a substrate–Oxime-I inclusion complex with a undissociated oxime proton (S–Oxime-I in Scheme 2).

S–C$^-$: a substrate–Oxime-I inclusion complex with an anionic oximate group (S–Oximate-I in Scheme 2).

Mass balances in consistent with Scheme 2 are established as follows.

$$[S]_T = [S] + [S\text{–}CH] + [S\text{–}C^-] \quad (4)$$

$$[C]_T = [CH] + [C^-] + [S\text{–}CH] + [S\text{–}C^-] \quad (5)$$

Relevant equilibrium constants are also given.

$$K_a = \frac{[S\text{–}C^-][H^+]}{[S\text{–}CH]} \quad (6); \qquad K_a' = \frac{[C^-][H^+]}{[CH]} \quad (7)$$

$$K_b = \frac{[S\text{–}CH]}{[S][CH]} \quad (8); \qquad K_b' = \frac{[S\text{–}C^-]}{[S][C^-]} \quad (9)$$

Through combination of Eqs. (4), (5), (6), and (8), the following relation is derived.

$$\begin{aligned}[S\text{–}C^-] &= \frac{K_a}{[H^+]}[S\text{–}CH] = \frac{K_a}{[H^+]}K_b[S][CH] \\ &= \frac{K_a}{[H^+]}K_b\{[S]_T - [S\text{–}CH] - [S\text{–}C^-]\} \\ &\quad \times\{[C]_T - [S\text{–}CH] - [S\text{–}C^-] - [C^-]\} \\ &= \frac{K_a}{[H^+]}K_b\left\{[S]_T - \left(\frac{[H^+]}{K_a}+1\right)[S\text{–}C^-]\right\} \\ &\quad \times\left\{[C]_T - \left(\frac{[H^+]}{K_a}+1\right)[S\text{–}C^-] - [C^-]\right\}\end{aligned}$$

Thus, this leads to the following expression.

$$\begin{aligned}[S\text{–}C^-] &= \alpha K_b\{[S]_T - \gamma[S\text{–}C^-]\} \\ &\quad \times\{[C]_T - \gamma[S\text{–}C^-] - [C^-]\}\end{aligned} \quad (10)$$

where $\alpha = K_a/[H^+]$ and $\gamma = ([H^+]/K_a)+1$.

On the other hand, Eqs. (5) and (7) give

$$[C^-] = \frac{K_a'}{[H^+]}[CH] = \frac{K_a'}{[H^+]}\{[C]_T - [S\text{–}CH] - [S\text{–}C^-] - [C^-]\}$$

Thus,

$$[C^-] = \beta\{[C]_T - \gamma[S\text{-}C^-]\} - \beta[C^-]$$

where $\beta = K'_a/[H^+]$.

Rearrangement results in the following relation.

$$[C^-] = \frac{\{[C]_T - \gamma[S\text{-}C^-]\}\beta}{1+\beta} \tag{11}$$

Combining Eqs. (10) and (11),

$$[S\text{-}C^-] = \alpha K_b\{[S]_T - \gamma[S\text{-}C^-]\} \times \left\{[C]_T - \gamma[S\text{-}C^-] - \frac{\beta\{[C]_T - \gamma[S\text{-}C^-]\}}{1+\beta}\right\}$$

Then,

$$(1+\beta)[S\text{-}C^-] = \alpha K_b\{[S]_T[C]_T - \gamma[S\text{-}C^-][S]_T - \gamma[S\text{-}C^-][C]_T + \gamma^2[S\text{-}C^-]^2\} \tag{12}$$

Since $[S]_T[C]_T \gg \gamma^2[S\text{-}C^-]^2$ is valid, under present experimental conditions,[22] Eq. (12) is simplified as

$$(1+\beta)[S\text{-}C^-] = \alpha K_b[S]_T[C]_T - \alpha K_b\gamma\{[S]_T + [C]_T\}[S\text{-}C^-]$$

This gives the relation as follows.

$$[S\text{-}C^-] = \frac{\alpha K_b[S]_T[C]_T}{1+\beta+\alpha\gamma K_b\{[S]_T+[C]_T\}} \tag{13}$$

The overall rate can be expressed as follows.

$$\begin{aligned} k_{obs}[S]_T &= k_{hyd}[S] + k'_{acyl}[S\text{-}C^-] \\ &= k_{hyd}\{[S]_T - \gamma[S\text{-}C^-]\} + k'_{acyl}[S\text{-}C^-] \\ &= k_{hyd}[S]_T - (k_{hyd}\gamma - k'_{acyl})[S\text{-}C^-] \end{aligned}$$

Upon rearrangement after introduction of Eq. (13) into the above equation,

$$\begin{aligned} k_{obs} - k_{hyd} &= (k'_{acyl} - k_{hyd}\gamma)\frac{\alpha K_b[C]_T}{1+\beta+\alpha\gamma K_b\{[S]_T+[C]_T\}} \\ &= \left(\frac{1}{\gamma}k'_{acyl} - k_{hyd}\right)\frac{K_b[C]_T}{\left(\frac{1+\beta}{1+\alpha}\right)+K_b[S]_T+K_b[C]_T} \end{aligned}$$

Thus, the following equation can be derived from the above reletionship.

$$\frac{1}{k_{obs}-k_{hyd}} = \frac{\left(\frac{1+\beta}{1+\alpha}\right)+K_b[S]_T}{\left(\frac{1}{\gamma}k'_{acyl}-k_{hyd}\right)K_b[C]_T} + \frac{1}{\left(\frac{1}{\gamma}k'_{acyl}-k_{hyd}\right)} \tag{14}$$

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