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Ester Hydrolysis by Poly(Allylamine)s Having Hydrophobic Groups: Catalytic Activity and Substrate Specificity

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ESTER HYDROLYSIS BY POLY(ALLYLAMINE)S HAVING HYDROPHOBIC GROUPS: CATALYTIC ACTIVITY AND SUBSTRATE SPECIFICITY

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ABSTRACT

The hydrolysis of phenyl esters having anionic, hydrophobic, and stereoisomeric groups by poly(allylamine)s (**I**) with various hydrophobic groups was investigated. The derivatives of **I** with dodecyl and benzyl groups (**IIc** and **III**) form a hydrophobic microdomain near the catalytic site. For **I**, the hydrolysis rate of 4-acetoxy-3-nitro-benzoic acid is extremely high, indicating a significant contribution of the electrostatic effect. **IIc** (degree of substitution, DS $\geqq$ 0.12) and **III** (DS $\geqq$ 0.48) have

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a considerable large catalytic activity for the more hydrophobic substrates than *p*-nitrophenyl acetate. The reaction mechanism was found to be of the Michaelis–Menten type. According to kinetic and thermodynamic analysis, it was found that the lower polarity ($E_T \approx 55$) and compact structure lead to effective hydrophobic interaction and high substrate binding, resulting in enhancement of the catalytic activity. In the case of **III** (DS = 0.75), it was suggested that the substrate should be bound and fixed in the hydrophobic void consisting of the face conformation. In conclusion, the substrate specificity of the catalytic polymer is caused by a microdomain which has a hydrophobic steric structure.

INTRODUCTION

It is well known that an enzyme is a kind of protein containing polypeptide chains which consists of about 20 different kinds of amino acids and has a high reactivity and substrate specificity.

Fischer proposed an enzyme model which has substrate-binding and catalytic active sites [1]. The substrate specificity of an enzyme is caused by the steric structure of active sites which have a hydrophobic environment. The substrates are caught by the active site, and substrate binding with an enzyme is due by ionic interaction, hydrogen bond interaction, and especially hydrophobic interaction. The bound substrate is converted by an intramolecular first-order reaction mechanism with the catalytic functional group. The kinetics is analyzed by a Michaelis–Menten type reaction [2].

The model system of such an enzyme has been widely investigated using a spherical micelle and bimolecular vesicle having a hydrophobic reaction failed to elucidate the stereospecific hydrolysis reaction [3–5]. As a result, the design of the size, form, and microenvironment of the hydrophobic molecular assembly yields enhanced stereospecific recognition of the molecules [6, 7]. On the other hand, synthetic polyelectrolytes were investigated as a model of synthetic enzyme [8–10] for the characteristics of their electrostatic and hydrophobic interaction in relation to the specific organized environment. For example, an amphiphilic polycation, dodecylated poly(4-vinylpyridine), is known to form an intramolecular micelle which provides a globular protein model [11–13]. The hydrophobic moiety has a more rigid structure than a surfactant micelle [14], and accelerates the alkaline hydrolysis and decarboxylation reaction of the included substrate [15, 16].

Klotz et al. reported a high catalytic activity and asymmetric selective reactivity of flexible branched poly(ethyleneimine) (*b*-PEI) which has a long alkyl chain group and an imidazol group [17–26]. Sisido et al. showed that those polymers form a domain consisting of two phases, a cluster of low mobility and a surrounding region of relatively high mobility. The domain structure concentrates hydrophobic molecules more effectively than low molecular micelles [27]. On the other hand, Pshezhetskii et al. demonstrated increased activity of hydrolysis with the substitution of an alkyl or benzyl group in linear poly(ethyleneimine) (*l*-PEI) by the Michaelis–Menten type reaction. They explained this behavior by the similarity of structure to α-chimotrypsin [28–30]. Smets et al. indicated that the high catalytic activity of *b*-PEI is due to the branched primary amino group [31].

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Poly(allylamine) (**I**), a relatively new commercially available material, is a novel polymer with only the primary amino group as the side chain. As a starting material for a functional polymer through a variety of chemical modifications, as well as a water-soluble cationic polymer, **I** is a promising material [32, 33]. We reported that **I** has a higher activity of hydrolysis than *b*-PEI, and that **I** covalently bound with β-cyclodextrin (CD) shows an inclusion ability of substrate and controlled catalytic activity as the result of the cooperative effect of the amino group and the CD ring [34, 35].

Polymers modified by the introduction of long alkyl or benzyl groups (**II** and **III**) show amphiphilic properties and form a compact hydrophobic domain structure [36, 37]. Through the use of fluorescent probe and spin probe techniques, these polymers were found to form an assembled rigid structure more restricted in their mobilities than "polysoap." It was confirmed that probe molecules are included in the hydrophobic region [37, 38].

We investigated the ester hydrolysis of *p*-nitrophenyl acetate by these polymers and found that the longer the hydrophobic chain group and the higher the degree of substitution, the greater the hydrolysis rate [36]. The results indicated an important role for concentration of the substrate by the hydrophobic reaction field in the enhancement of activity [36, 39].

In this work we further determine the hydrolysis rate of various alkylated and benzylated poly(allylamine)s using five ester substrates of differing ionic natures, hydrophobicities, and stereo isomeric properties. Through use of the data obtained, the rate parameters, and the thermodynamic parameters, we discuss the interrelation among the polarity, polymer configuration of the microenvironment, substrate affinity, and reactivity.

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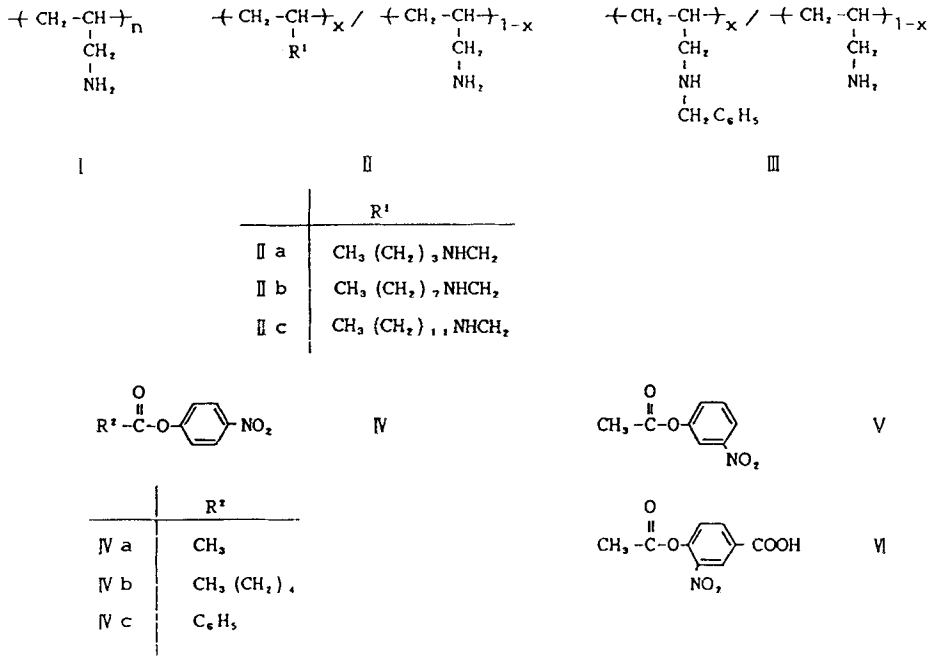
EXPERIMENTAL

Materials

Poly(allylamine)hydrochloride (**I**·HCl) in its scaly form (M_n = 10,000) was kindly supplied by Nitto Boseki Co. It was purified by repeated precipitation from water/methanol. *n*-Alkyl bromide (1st Grade, Wako Pure Chemical Industries), benzyl chloride (G.R., Kanto Chemical Co.), and *n*-propylamine (Special Grade, Kanto Chemical Co.) were used without further purification. Methyl Orange (Indicator, Merck Co.) was recrystallized from water before use. Dansyl acid (G.R., Tokyo Kasei Co.) was recrystallized three times from ethanol. Buffer solution (ionic strength, μ = 0.05 mol·dm^{-3}) was prepared by dissolving 2-amono-2-hydroxymethyl-1,3-propanediol (Analytical Grade, Nakarai Tesque) in aqueous hydrochloric acid.

N-Alkylated and *N*-Benzylated Poly(Allylamine)s (II, III)

The polymers were prepared from **I** and *n*-alkyl or benzyl halide and purified as described in our previous reports [36, 37]. Their structures were confirmed by IR, NMR, and elemental analysis. The structural formulas and the notation of the polymers are shown in Scheme 1, where the symbol x means the degree of substitution (DS) of alkyl and benzyl groups.



SCHEME 1. Poly(allylamine) derivatives and esters as substrates.

Substrates (IV–VI)

p-Nitrophenyl acetate (**IVa**) (Special Grade, Tokyo Kasei Co.) was recrystallized from ethanol. *p*-Nitrophenyl hexanoate (**IVb**) (Special Grade, Tokyo Kasei Co.) was used without further purification. *p*-Nitrophenyl benzoate (**IVc**) and *m*-nitrophenyl acetate (**V**) were synthesized by a method reported previously [34, 35]. 4-Acetoxy-3-nitrobenzoic acid (**VI**) was prepared according to the procedure of Overberger et al. [40]. The chemical formulas of the esters as substrates and their notations are also given in Scheme 1.

Spectrometry and Viscometry

UV/Vis spectra were determined with a double beam spectrophotometer (Hitachi 556). Stationary fluorometry was carried out by means of a Shimadzu RF-502 spectrofluorometer at 25°C [36]. Reduced viscosity was measured with an Ostwald viscometer at a concentration of 0.1 g/100 mL (25°C). Tris buffer (pH 8.74, μ = 0.05 mol·dm^{-3}) was used as solvent [36].

Kinetic Measurement and Kinetic Analysis

An acetonitrile solution (15 μL) of the substrates was mixed with 3 mL of Tris buffer (pH 8.74, μ = 0.05 mol·dm^{-3}) and used for kinetic hydrolysis measurement. The initial concentration of the substrate was 1×10^{-4} mol·dm^{-3}, and the temperature was kept constant at 25 ± 0.1°C. The reaction sequence was traced by

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determining the absorbance of *p*-nitrophenolate, *m*-nitrophenolate, and 4-carboxy-2-nitro-phenolate ions produced at 400 nm (ε = 18,500), 390 nm (ε = 3,300), and 408 nm (ε = 4,400), respectively. The values were monitored by a recorder (Model EPR100, Toa Electronics Co. Ltd) connected to the spectrophotometer.

The pseudo-first-order reaction constant, k_{obs}, was determined, and analysis was performed as described in our previous papers [34, 35].

RESULTS AND DISCUSSION

Hydrolysis of Carboxylic Acid Esters

In Fig. 1, k_{obs} versus polymer concentration is shown for **IVa**, **IVb**, and **IVc** as substrates/derivatives of **I** systems. All of the *N*-alkyl and *N*-benzyl derivatives accelerate the hydrolysis reaction to compare with unsubstituted **I**. In every case the hydrolysis of **IVa** apparently proceeds as a second-order reaction, and k_{obs} linearly increases with an increase in polymer concentration. For **IVb** and **IVc** the polymers containing hydrophobic groups give saturated type curves, suggesting Michaelis–Menten reactions. This is in contrast to polymer **I** in which hydrolysis proceeds by a second-order reaction. This tendency is clearer in cases of long alkyl chain groups and benzyl groups with a high degree of substitution (DS).

For polymers **I**, **IIc** (DS = 0.18), and **III** (DS = 0.75), hydrolyses of both the neutral substrates which have different steric structures and hydrophobicities (**IVa**, **IVb**, and **V**) and the ionic substrate (**VI**) were determined. The results are shown in Fig. 2. In the case of **I**, the ionic ester as **VI** easily hydrolyzes. On the other hand, in the cases of dodecylated and benzylated polymers the reaction of **IVc**, which has a larger acyl group than **IVa**, and especially the reaction of **IVb** are considerably accelerated.

From these results together with our previous data on the interaction between the hydrophobic poly(allyamine)s and small molecules [36–38], it is strongly suggested that the derivatives of **I** have a great ability to include hydrophobic substrates and that the resulting polymer/substrate complex enhances the reaction rates. To elucidate the mechanism of ester hydrolysis in these systems, a kinetic analysis of the rate curves of Figs. 1 and 2 was performed.

For **IVa**, **V**, and **VI**, both the second-order reaction rate constant k from the initial slope of the straight line and $\mathbf{k}'$, based on the residual amino group unit, were calculated. For **IVb** and **IVc**, which have enzyme-like saturation-type curves, the Lineweaver–Burk equation [41] was used to calculate the dissociation constant K_d and the reaction constant k_2. Table 1 gives the results for neutral substrates with different acyl groups, while Table 2 gives those of *m*,*p*-stereo isomeric and ionic substrates. k_2/K_d can be regarded as an apparent second-order reaction rate constant in an enzymatic reaction [42]. In this context, k_2/K_d, which is equivalent to k or k' in the **IVa** system, will hereafter be used as a measure of the total catalytic ability [34, 36].

Effects of Polymer Substituent and Its Degree of Substitution

In Fig. 3 the relationship between the chain length (DS is kept nearly constant as ca. 0.2) and k' (k_2/K_d) is given. As the carbon number of the side chain increases from 4 to 8 and then to 12, a drastic increase of k' is observed. The increase is

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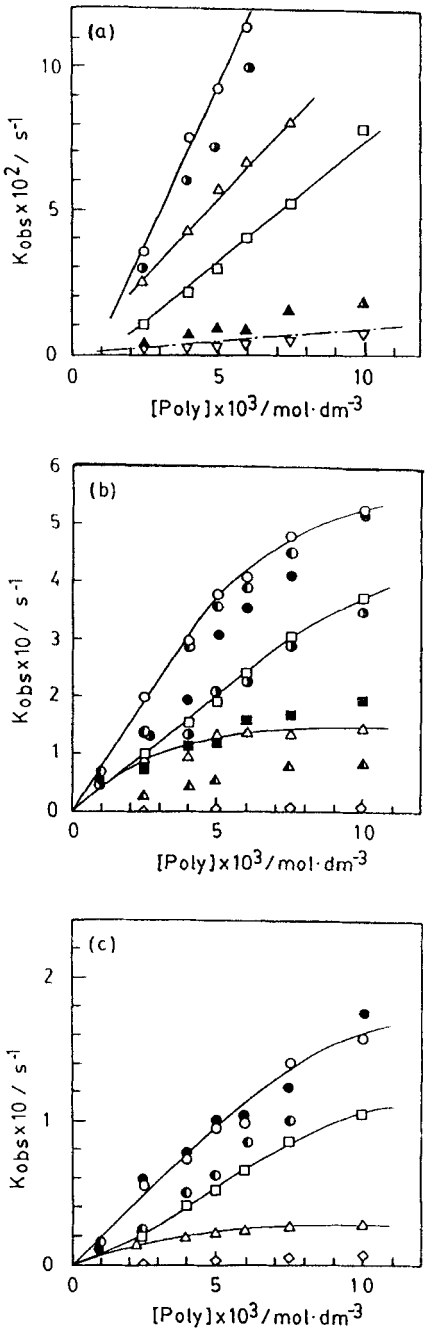

FIG. 1. Hydrolysis of phenyl esters catalyzed with derivatives of **I**: (a) **IVa**; (b) **IVb**; (c) **IVc**. (-·-,◇) **I**·HCl; (▽) **IIa** (DS = 0.23); (□) **IIb** (DS = 0.20); (■) **IIb** (DS = 0.38); (●) **IIc** (DS = 0.12); (○) **IIc** (DS = 0.18); (◐) **IIc** (DS = 0.30); (◑) **IIc**·HCl (DS = 0.45); (▲) **III** (DS = 0.23); (◭) **III** (DS = 0.48); (△) **III** (DS = 0.75).

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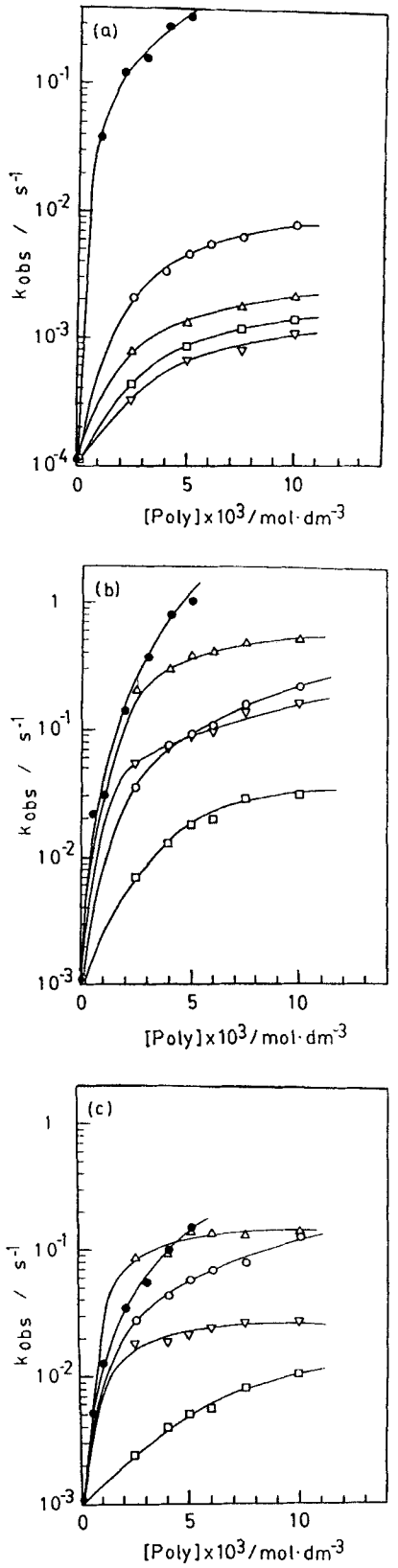

FIG. 2. Hydrolysis of phenyl esters catalyzed with derivatives of **I**: (a) **I**·HCl; (b) **IIc** (DS = 0.18); (c) **III** (DS = 0.75). (○) **IVa**; (△) **IVb**; (▽) **IVc**; (□) **V**; (●) **VI**.

TABLE 1. Parameters of the Hydrolysis of *p*-Nitorophenyl Esters[a]

Catalyst	Substrate	$k_2 \times 10$, s^{-1}	$K_d \times 10^2$, $mol \cdot dm^{-3}$	k_2/K_d, $mol^{-1} \cdot dm^3 \cdot s^{-1}$	k, $mol^{-1} \cdot dm^3 \cdot s^{-1}$
I·HCl	**IVa**				0.891
	IVb				0.219
	IVc				0.116
IIa	**IVa**				0.777
(DS = 0.23)	**IVb**				0.324
	IVc				0.218
IIb	**IVa**				5.85
(DS = 0.20)	**IVb**	25.4	5.66	44.9	
	IVc	7.89	6.42	12.3	
IIb	**IVa**				9.94
(DS = 0.38)	**IVb**	5.31	1.70	31.2	
	IVc	0.843	1.08	7.81	
IIc	**IVa**				16.5
(DS = 0.12)	**IVb**	14.4	1.82	79.1	
	IVc				18.7
IIc	**IVa**				22.9
(DS = 0.18)	**IVb**	15.3	1.65	92.7	
	IVc	3.74	1.49	25.1	
IIc	**IVa**				19.1
(DS = 0.30)	**IVb**	10.6	1.03	103	
	IVc				17.3
IIc·HCl	**IVa**				14.3
(DS = 0.45)	**IVb**	15.7	3.44	45.6	
	IVc				8.27
III	**IVa**				1.88
(DS = 0.23)	**IVb**				1.94
	IVc				0.815
III	**IVa**				6.05
(DS = 0.48)	**IVb**	2.73	2.22	12.3	
	IVc	0.475	1.35	2.81	
III	**IVa**				11.0
(DS = 0.75)	**IVb**	1.77	0.169	105	
	IVc	0.435	0.524	8.36	

[a][Substrate] = 1×10^{-4} $mol \cdot dm^{-3}$, pH 8.74, $\mu = 0.05$, Tris buffer in 0.5 vol% acetonitrile/water, 25°C.

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TABLE 2. Rate Constants of the Hydrolysis of Neutral and Anionic Esters[a]

Catalyst	k, $mol^{-1} \cdot dm^3 \cdot s^{-1}$ IVa	V	VI	$\frac{k(IVa)}{k(V)}$	$\frac{k(VI)}{k(IVa)}$
Propylamine	0.266	0.147		1.81	
I·HCl	0.891 (0.891)[b]	0.153 (0.153)	78.7 (78.7)	5.82	88.3
IIc (DS = 0.18)	22.9 (27.9)	3.07 (3.74)	314 (383)	7.46	13.7
III (DS = 0.75)	11.0 (44.5)	0.95 (1.16)	34.5 (140)	11.6	3.14

[a][Substrate] = 1×10^{-4} mol·dm^{-3}, pH 8.74, μ = 0.05, Tris buffer in 0.5 vol% acetonitrile/water, at 25°C.

[b]The k' value in parentheses was recalculated with respect to the content of amino groups in the polymer.

estimated as 6–28 times, 54–110 times, and 130–270 times for **IVa**, **IVc**, and **IVb**, respectively. These results indicate that the longer alkyl groups are favorable for formation in the local reaction medium, and consistent with that in the chemically modified PEI system [17, 29]. Polymer **III** (DS = 0.2) has a relatively low activity, and the contribution of the benzyl group corresponds to a five methylene chain length from graphical estimation.

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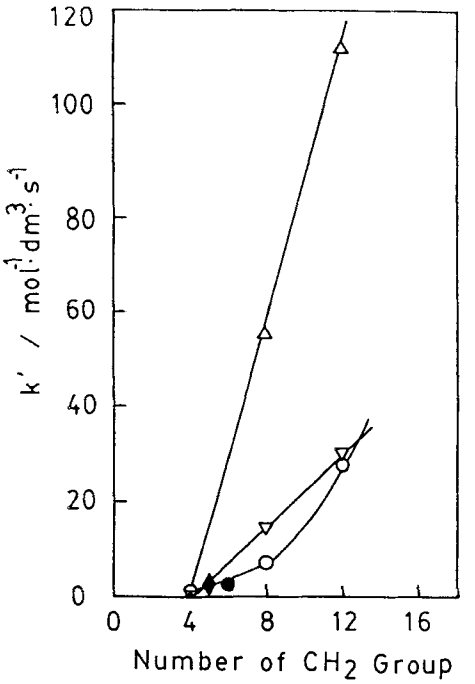

FIG. 3. Effect of side-chain length on the rate constant (k'). Open symbols; *N*-alkylated derivatives of **I** (**II**). Filled symbols: *N*-benzylated derivatives of **I** (**III**). (○) **IVa**; (△) **IVb**; (▽) **IVc**.

The relationship between DS and k' is shown in Fig. 4. The dodecylated polymer (**IIc**) with DS = 0.18 exhibits an enhancement of k' of 30 and 260 times for **IVa** and **IVc**, respectively. The same polymer with DS = 0.30 gives a maximum activity increase of 670 times for **IVb**. The benzylated polymers (**III**) above DS = 0.48 accelerate drastically for every substrate, especially **III** with DS = 0.75, which gives k' of 290 and 1900 times in comparison with that of **I** for **IVc** and **IVb**, respectively. The corresponding value of k' for **IVa** is approximately 49 times.

The sequences of the reaction rates of the neutral ester substrates with respect to the change of the acyl group are $C_5H_{11}CO > C_6H_5CO \geqq CH_3CO$ for the alkylated polymer (DS < 0.3) and $C_5H_{11}CO > CH_3CO \geqq C_6H_5CO$ for **IIb** and **IIc** of DS > 0.4 and the benzylated polymer. These sequences are $CH_3CO > C_5H_{11}CO > C_6H_5CO$ for poly(allylamine) (**I**).

From the above results it is suggested that hydrolysis action by chemically modified poly(allylamine) is strongly affected by 1) hydrophobic interaction between the local reaction fields formed near the amino groups and the substrates, and 2) the steric effect on interaction.

In the context of the above argument, our discussion is focused on the separation of substrate binding and reaction rate terms. Figure 5 is a plot of the dissociation constant of the complex between polymer and substrate, K_d against DS. The octylated and benzylated polymers give the enhanced binding constant ($1/K_d$) for

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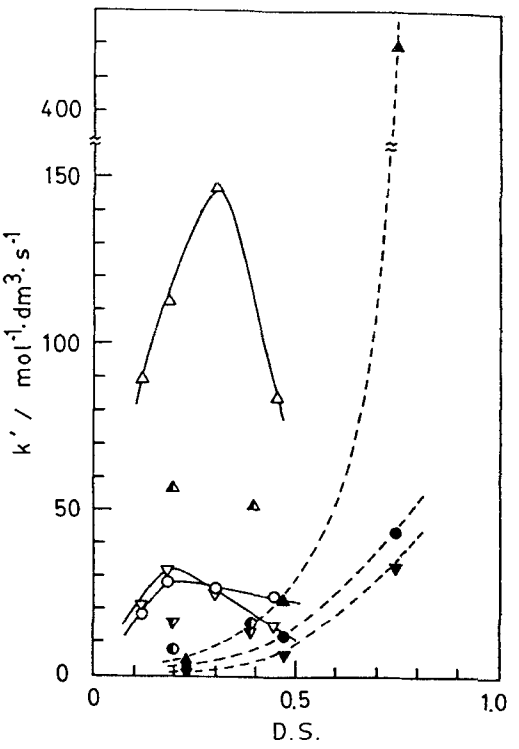

FIG. 4. Effect of DS of derivatives of **I** on the rate constant (k'). Open symbols: **IIc**. Half-filled symbols: **IIb**; Filled symbols: **III**. (○) **IVa**; (△) **IVb**; (▽) **IVc**.

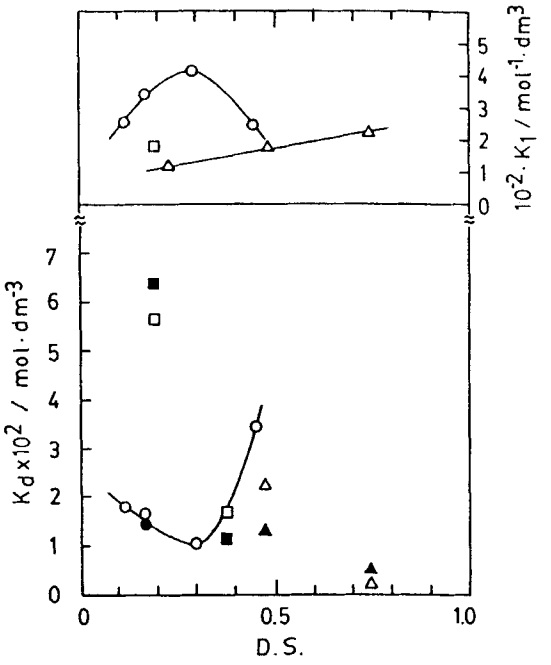

FIG. 5. Relationship between DS and the dissociation constant (K_d) of phenyl esters or the first binding constant (k_1) of dansyl acid. Open symbols: **IVb**. Filled symbols: **IVc**. (○) **IIc**; (□) **IIb**; (△) **III**.

IVb and **IVc**, but not for **IVa**. The greater the degree of substitution of the polymer, the stronger the hydrophobic interaction. The measure of the hydrophobicity (π values by Hansch [43]) is twice as large for **IVc** (3.75) and **IVb** (3.80) as for **IVa** (1.64). On the other hand, dodecylated polymer with DS = 0.12 has a relatively small K_d for **IVb**, has a maximum binding ability at DS = 0.30, and increases at DS = 0.45. This suggests that for system **IIc** an assembled domain structure favorable for substrate binding is formed even at low DS values. For a higher degree of substitution, the long dodecyl groups are so crowded that the micelle-like compact structure is thought to be perturbed. In addition, steric hindrance by the closely aggregated chains may lower the inclusion of the substrate. This argument is supported by the fact that the relatively bulky substrate **IVc** does not show a clear saturated type of binding (Table 1).

It is worthwhile to point out that the benzylated polymer has a high ability for substrate binding at a higher DS ($\geqq$0.48). This is attributed to the formation of a hydrophobic void of the "face" conformation (as is known in paracyclophane [44, 45]); that is, pendant aromatic rings on the polymer chain are oriented parallel to each other [36–38]. The hydrophobic void thus formed may occur in a sterically stable inclusion with a substrate. In Fig. 5 the primary binding constant K_1 is plotted against DS for the interaction between derivatives of **I** and dansyl acid as a fluorescent probe [37]. Similar patterns of **IIc** (maximum type) and **III** (linear type) in the substrate binding behavior in esterolysis are observed. This fact supports the

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above argument of inclusion behavior in relation to the substituent on the polymer and its DS.

We now discuss the other important terms controlling the enzyme-like activity, i.e., the factors which influence the catalytic reaction itself. The intramolecular acyl group transformation reaction constant (k_2) of the polymer/substrate complex against DS is given in Fig. 6.

With **IVb** as substrate, **IIb** and **III** of higher DS give a smaller k_2, while **IIc** is approximately constant and has high values of k_2. This is because **IIb** and **III** are affected solely by the decrease of catalytic amino groups with increasing DS. On the other hand, dodecylated polymer forms a highly organized compact microdomain structure in which the amino groups are located near the included substrate molecules. The decrease of pK_a of the amino group in the hydrophobic atmosphere is a possible effect, giving rise of the free amino group concentration. However, this was not crucial in our previous work [36] where the effect of pH on activity did not clearly appear. The value of k_2 for the hydrolysis of the benzoate is one-quarter to one-sixth that of the hexanoate, especially for lower values for alkylated and benzylated polymers of high DS (Table 1). This suggests that substrate **IVc**, which has a highly restricted position in the microdomain structure due to the two aromatic rings, cannot find a sterically favorable position between the ester group of the substrate and the amino side group of the polymer. The fact that there is practically no difference of k_2 for **IVa** and **IVb** in system **III** at DS = 0.48 and 0.75 probably means that the mode of substrate inclusion favorable for reaction with the amino group compensates for the effect of a decrease of catalytic amino groups.

We conclude that the dodecyl derivatives (**IIc**), especially at DS = 0.18 and 0.30, have a superior substrate binding affinity and a compact hydrophobic domain

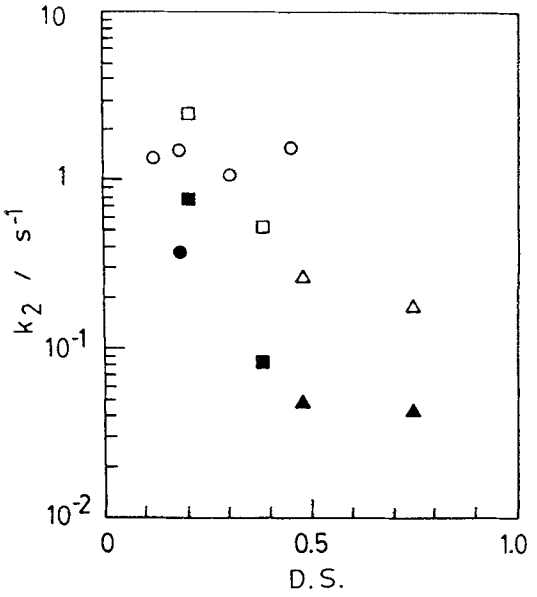

FIG. 6. Relationship between DS and the rate constant (k_2) of phenyl esters. Open symbols: **IVb**. Filled symbols: **IVc**. (○) **IIc**; (□) **IIb**; (△) **III**.

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which exhibits a high intramolecular reactivity. As a result, they have an extremely high hydrolysis reactivity: $k_2/K_d = 100\ \text{mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1}$.

The benzyl derivatives (**III**) maintain a high inclusion ability for hydrophobic substrates at higher DS values and retain a sterically favorable location for aromatic rings. However, as freedom in the transfer reaction of acyl group becomes lower, the pendant benzyl groups on the main chain molecules as a whole should be sterically more unfavorable compared with the alkyl groups. Thus, system **III** has a relatively lower catalytic ability in comparison to systems **IIb** and **IIc**, although system **IVb/III** (DS = 0.75) has a high binding constant and a remarkable high values of k_2/K_d ($=105\ \text{mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1}$).

The octyl derivatives (**IIb**) are moderate in both substrate binding ability and reactivity, which gives them total catalytic activities intermediate between systems **IIc** and **III**.

Substrate Specificity

We investigated the *m*- and *p*-substituent effects of nitrophenyl acetates on hydrolysis. The hydrophobicities (π-value [43]) of the phenyl esters used are practically equivalent. As shown in Table 2, **IIc** (DS = 0.18) and **III** (DS = 0.75) have excellent accelerating effects compared with **I** for both of **IVa** and **V**. In both systems the values of k and k' are **IVa** > **V** despite the binding affinity of the substrate with polymer being of almost the same order. The ratios of the rate constants [k(**IVa**)/k(**V**)] are **I** (5.8) < **IIc** of DS = 0.18 (7.5) < **III** of DS = 0.75 (11.6). This is attributed to the high selectivity of **IVa** as well as the low nucleophilic reactivity of **V**, as reported in our previous paper [34]. An additional reason for this tendency is the steric regulation of the reaction of **V** due to its strong inclusion in the hydrophobic domain. This is clearly observed in benzyl derivatives. The amino groups suffer from screening or steric hindrance effects by the aromatic side groups.

When the polymer has charged groups, the electrostatic effect on the inclusion mechanism also plays a role [31, 37]. In Table 2 the rate constants k and k' of **VI** with an anion constituent are shown as 88 times as large for **IVa** as for **I**, 14 times for **IIc** (DS = 0.18), and more than 3 times for **III** (DS = 0.75). In contrast to the cases of **IVb**, **IVc**, and **V**, the activity of **III** of DS = 0.75, which has a smaller residual amino group content, is lower than that of **I**. From these observations we estimate that derivatives of **I** with a low DS will have a strong ability to concentrate the anionic substrate by electrostatic interaction, leading to high catalytic hydrolysis. **IIc** with DS = 0.18 is a special case, and it gives a very high value of the initial rate constant, k ($=314\ \text{mol}^{-1}\cdot\text{dm}^3\cdot\text{s}^{-1}$). The reason for this is the synergistic effect of its high inclusion ability and electrostatic substrate binding ability. From the above results, the selective reactivities of five substrates in systems **I**, **IIc** (DS = 0.18), and **III** (DS = 0.75) are summarized as follows. The overall hydrolysis catalytic ability, k or k_2/K_d, is in the sequences

I:	**IVc** ≈ **V** < **IVb** < **IVa** ≪ **VI**
IIc (DS = 0.18):	**V** < **IVa** ≦ **IVc** < **IVb** < **VI**
III (DS = 0.75):	**V** < **IVc** ≦ **VIa** < **VI** < **IVb**

In system **I** the hydrolysis rate of **VI** is extremely high compared with any other neutral substrate. **IVb** and **IVc** are slower than **IVa**, which indicates that

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polymer **I** has no effective reaction field for hydrophobic inclusion near the main chains, and that substrate binding is mainly due to electrostatic interaction.

In system **IIc** with DS = 0.18, the activity for every substrate is higher than in system **I**, especially for hydrophobic **IVb** and **IVc** which bind strongly. The large contribution of the hydrophobic interaction to hydrolysis is clear. The formation of the hydrophobic domain with a compact and ordered structure is suggested. In addition, electrostatic binding due to the charged groups densely distributed in the reaction fields also plays a role.

IVb is more easily hydrolyzed than **IVa** and **VI** when **III** has DS = 0.75. This explains why the inclusion of the anionic substrate by the small amount of charged amino groups is not as effective as systems **I** and **IIc** (DS = 0.18). In this system the domain formed by benzyl side groups makes a large contribution to the hydrophobic interaction and includes the substrates. Furthermore, the steric hindrance of the 3-NO_2 group in **VI** to the interaction and intramolecular reaction reduces the reactivity of **VI**. The hydrolysis rate constants, k_{un}, of **IVc** ($2.9 \times 10^{-6}\ s^{-1}$) and **V** ($8.7 \times 10^{-5}\ s^{-1}$) are smaller than that of **IVa** ($1.15 \times 10^{-4}\ s^{-1}$) in alkaline conditions, and the order is the same as in the activity (k value) in system **I**. In addition, we have already observed that the bulky **IVc** shows a steric hindrance effect on the binding ability ($1/K_d$) and the reactivity of acyl transformation (k_2) in the hydrophobic derivatives of **I**. In spite of these observations the selectivity of **IVc** in **IIc** (DS = 0.18) and **III** (DS = 0.75) is higher than that of **V**. This result reflects the important role of inclusion by the hydrophobic interaction.

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Substrate Binding in Relation to Polymer Structure

In the above section we discussed the effects of a hydrophobic organized structure on the inclusion and hydrolysis reaction of a substrate. In this section the discussion focuses on the relationship between the catalytic action and the micropolarity of the reaction milieu [16, 46], as well as the conformation of the polymer chain [30].

Figure 7 shows the micropolarity parameter (E_T) [36] estimated by use of Methyl Orange as a probe and the emission maximum (λ_{max}) [37] as a measure of the hydrophobicity of the environment as determined by a fluorescent probe of dansyl acid for derivatives of **I**. In all cases of **IIb**, **IIc**, and **III**, an increase of DS leads to a decrease in E_T and a blue shift of λ_{max}, which indicates the organized structure of the polymer becomes more hydrophobic with an increase of DS. **IIc** (DS = 0.12), **IIb** (DS = 0.2), and **III** (DS = 0.5) are estimated to have a hydrophobic milieu at a methanol level of $E_T \approx 56$.

Figure 5 is replotted as K_d vs E_T in Fig. 8 and indicates a decrease in E_T causes a decrease in K_d for **IIb** and **III**. This means that a decrease in the polarity of the microdomain strengthens hydrophobic interaction with the substrate. An interesting pattern is observed in the **IIc** system. Up to DS = 0.3, a decrease in hydrophobicity leads to a low K_d; however, at DS = 0.45, the inclusion ability decreases abruptly. To explain this behavior it is necessary to consider the conformational factor in relation to substrate binding. The reduced viscosity of the polymers, η_{sp}/C, against K_d is also given in Fig. 8(b). From the figure it is seen that system **IIb** has a low viscosity for all of the degrees of substitution examined. The improvement in substrate inclusion ability can be attributed to the decrease of the polarity of the

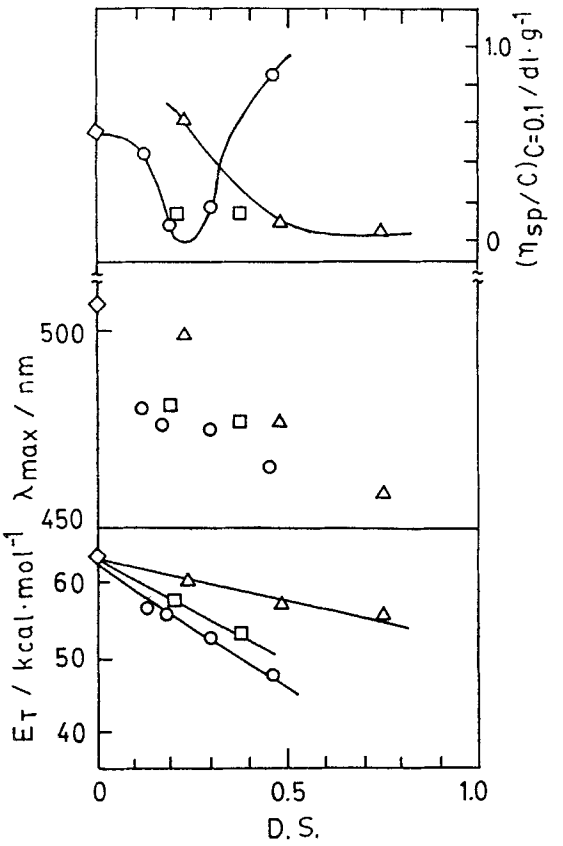

FIG. 7. Relationship between DS and E_T value, λ_{max} of dansyl acid, and reduced viscosity. (○) **IIc**; (□) **IIb**; (△) **III**.

microenvironment. In the case of **III**, a considerable increase of the binding constant corresponding to the decrease of the reduced viscosity is observed at DS > 0.48. The viscosity value is smaller than that of the alkylated polymer and approximately corresponds to the globular protein, 0.04–0.07 [30]. It is suggested that a highly rigid and less polar microdomain is formed by hydrophobic interaction between aromatic side chains. Furthermore, steric adaptability of the hydrophobic void thus formed contributes to the effective inclusion and fixation of the substrate.

In the case of **IIc**, an organized structure with high hydrophobicity and low viscosity is formed up to DS ≈ 0.3. A further increase of DS to 0.45 leads to an increase of the viscosity because of the increase of K_d. This is probably because of perturbation of the domain structure caused by crowded dodecyl chains as described above [36, 37].

We now turn to a discussion on substrate selectivity. In a low DS of the octyl derivative, there is practically no difference in K_d between **IVb** and **IVc**. However, at DS = 0.38 the polymer forms a compact microstructure with low polarity (E_T ≈ 53), which has a good adaptability to **IVc** and gives a high affinity. For the benzylated polymer of DS = 0.48, the binding availability to **IVc**, which has two aro-

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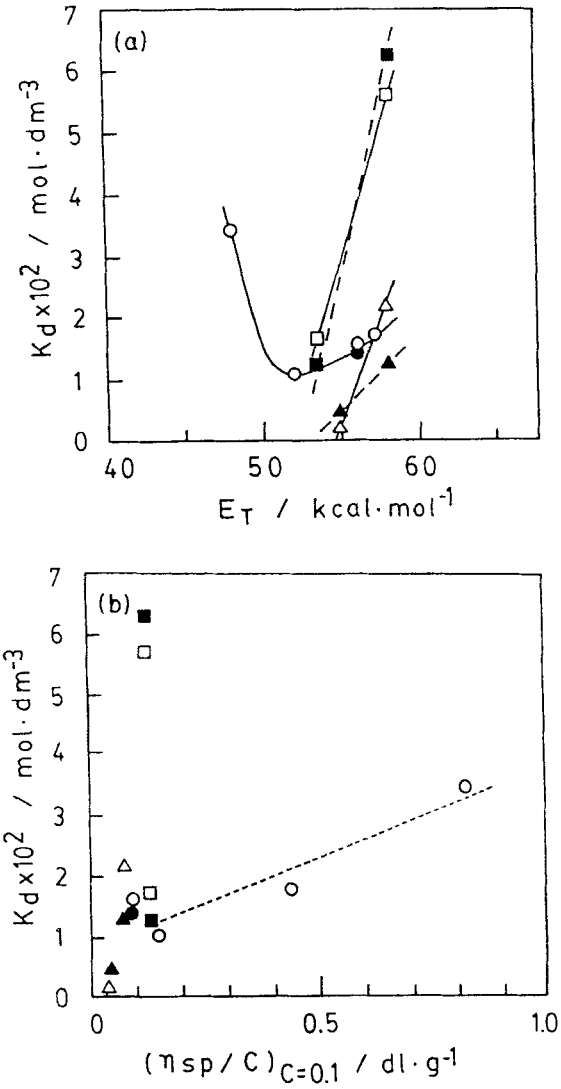

FIG. 8. The dissociation constant (K_d) of phenyl esters against (a) E_T value and (b) reduced viscosity. Open symbols: **IVb**. Filled symbols: **IVc**. (○) **IIc**; (□) **IIb**; (△) **III**.

matic rings, is higher than that of **IVb**. This is again attributable to the face-to-face type of effective inclusion of the substrate. In the case of DS = 0.75, the increase in the binding constant of **IVc** is 2.6 times whereas **IVb** is about 13 times. This large difference in the selective inclusion of **IVb** compared with that of **IVc** may be caused by a steric recognition of the more bulky **IVc** molecule.

The dodecylated polymer with a viscosity value of higher than 0.15 has no clear K_d for **IVc** even at high hydrophobicity. This fact may also be ascribed to the steric adaptability in the microdomain. It is worthwhile to point out that this long-chain alkylated polymer has high affinity and recognition of the hydrophobic sub-

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strate because **IVb** has an aliphatic acyl group in contrast to the aromatic rings of the polymer. In this context it can be concluded that poly(allylamine)s with hydrophobic groups can, to some extent, molecularly recognize the substituent's structure as well as the polarity of the substrate.

Catalytic Reactivity in Relation to Polymer Structure

Figure 9(a) and 9(b) show the relationship among total catalytic activity (k') for three neutral substrates and micropolarity and viscosity, respectively. As the hydrophobicity becomes higher in the **III** system, yielding a more rigid polymer structure, the hydrolysis activity is considerably enhanced. This result corresponds to the change of K_d values of Fig. 8, and indicates that the increase of the inclusion ability overcomes the decrease of the intramolecular reactivity.

IIc has a maximum activity at $E_T \approx 55$, which corresponds to the maximum substrate binding ability. When the reduced viscosity becomes lower, K_d decreases and the reactivity k' increases for all of the substrates investigated. This suggests that the more compact the hydrophobic microstructure, the stronger the substrate binding. The reason why the k' of DS = 0.18 is smaller than when DS = 0.3 is due to the lower hydrophobicity. These results imply that polymer conformation has important roles in the selective inclusion of the substrate and the subsequent intramolecular catalytic reactivity, just like the micropolarity of the domain.

System **IIb** has an increased substrate binding ability, but improvement of the hydrolytic activity is not observed except for **IVa**. The less polar substrate which is included and firmly fixed in the hydrophobic domain is difficult for the amino group to attack because of its decreased local concentration. This argument is supported by Table 1 in which the k_2 values for DS = 0.38 are one-fifth (**IVb**) and one-ninth (**IVc**) for DS = 0.20.

It is worthwhile to compare the selective catalytic reactivities of various substrates in relation to the polymer structures. In the case of **IIc** up to $E_T \approx 53$, the sequence of k' is **IVb** $\gg$ **IVc** $\geqq$ **IVa**. The effect of the selective binding of hydrophobic substrates, especially for aliphatic acyl derivatives, is clearly reflected by the selectivity of the catalytic reaction. The reason for the change of the sequence to **IVb** $\gg$ **IVa** $>$ **IVc** at $E_T \approx 48$ is ascribed to the steric effect of the more compact aggregated formation on both inclusion and intramolecular reactions of **IVc** with the bulky benzoyl group.

The sequence of the catalytic reactivity of **III** is **IVb** $\gg$ **IVa** $>$ **IVc** for all E_T values and viscosities. Again, **IVb**, which has a large hydrophobic binding ability in the inclusion process and a high reactivity in acyl transformation, is preferentially selected. **IVa**, which has a small acyl substituent group, has a relatively high activity. This may be due to the steric adaptability of the domain for this substrate. **IVc** has a higher binding ability compared with **IVa** and is subject to a large restriction due to guest fixation, which results in lower intramolecular reactivity. The steric hindrance also affects the reactivity. Because of these reasons, the selectivity ratio [k'(**IVb**)/k'(**IVc**)] of **III** of DS = 0.75 has a large value (12.6) compared with those of **IIb** of DS = 0.38 (ca. 4) or **IIc** of DS = 0.30 (ca. 6), even though the hydrophobic nature (π value [43]) of both substrates is at the same level. The values of k'(**IVb**)/k'(**IVa**), where both ester substrates have a different hydrophobicity, are 7.7, 5.4, and 9.5 for **IIb** (DS = 0.20), **IIc** (DS = 0.30), and **III** (DS = 0.75),

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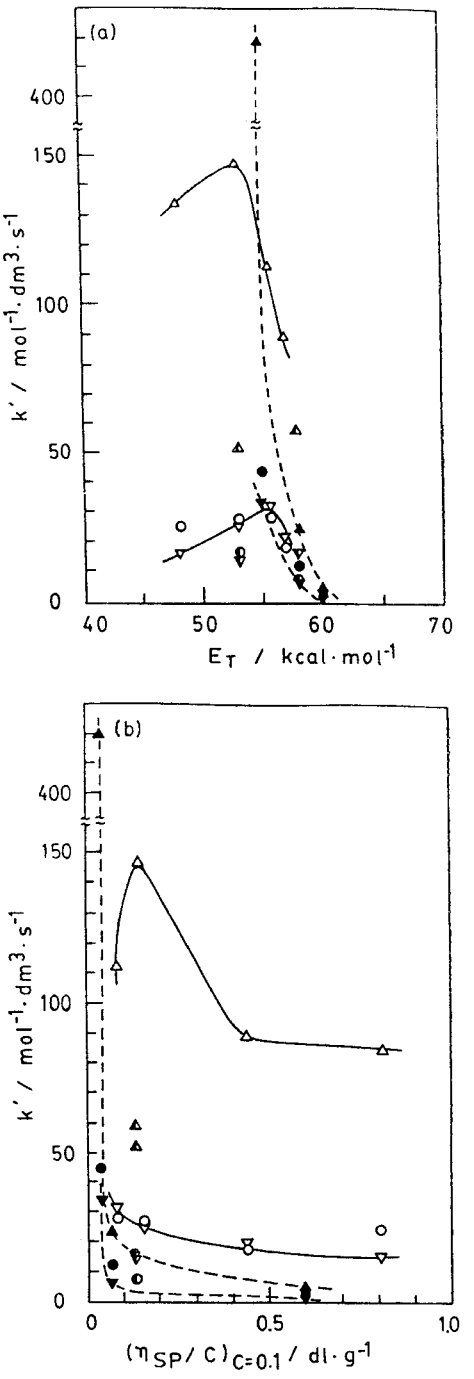

FIG. 9. The rate constant (k') of phenyl esters against (a) E_T value and (b) reduced viscosity. Open symbols: **IIc**. Half-filled symbols: **IIb**. Filled symbols: **III**. (○) **IVa**; (△) **IVb**; (▽) **IVc**.

respectively. This suggests that control of the substrate selective catalytic reaction takes place at the molecular level.

The order of catalytic activity of polymers with the same microenvironment is **IIc** > **IIb** > **III** at $E_T > 55$, and **III** > **IIc** > **IIb** at $E_T < 55$. The activity of polymers which have the same reduced viscosity are in the sequence **IIc** > **IIb** > **III** at $\eta_{sp}/C > 0.1$ and **III** > **IIc** > **IIb** at $\eta_{sp}/C < 0.1$. We conclude that the necessary conditions for polymers which have a high inclusion ability for substrates and a high catalytic reactivity are 1) the formation of a hydrophobic microdomain ($E_T \approx 55$) and 2) an organized compact conformation through which the reactive site approaches the catalytic site.

Temperature Effect on Catalytic Action

To obtain further insight into the catalytic action discussed above, we investigated the temperature dependence of the reaction of **IVb**/polymer systems. The polymers used were **IIc** (DS = 0.30) as the alkyl derivative and **III** (DS = 0.75) as the benzyl derivative. Because system **III** with its rigid domain structure gives a straight line for the van't Hoff plot of K_d, the thermodynamic parameters in the binding process were calculated by use of the following equations.

$$\Delta G = -2.303RT \log(1/K_d) \quad (1)$$

$$\Delta H = -2.303R[d \log(1/K_d)/d(1/T)] \quad (2)$$

$$\Delta S = (\Delta H - \Delta G)/T \quad (3)$$

The binding free energy, ΔG, is a large negative value, -16 kJ·mol^{-1} (Table 3), which is about 80% of the free energy of transfer from water to *n*-octanol (~ -21 kJ·mol^{-1}) [43]. This value suggests that the microdomain of **III** with DS = 0.75 is a milieu having a hydrophobic nature a little bit less than the hydrophobicity of an *n*-octanol. Thus, in the catalytic action of poly(allylamine) carrying a hydrophobic group, the binding mechanism of the substrate is supposed to be a mode similar to the case of α-chymotrypsin [47].

The heat of binding, ΔH, and the entropy of binding, ΔS, are negative values, which indicates that an additional mechanism of binding, π–π interaction, and/or hydrogen bonding interaction mechanism should be considered. In system **IIc** (DS = 0.30), no definite parameters could be obtained because $\Delta H > 0$ at $T < 30$°C and $\Delta H < 0$ at $T > 30$°C. This complicated behavior is attributed to a decrease of hydrophobic interaction with a rise in temperature [48].

TABLE 3. Thermodynamic Parameters of the Hydrolysis of **IVb** Catalyzed with **III** (DS = 0.75)

T, K	$1/K_d$, mol^{-1}·dm^3	ΔG, kJ·mol^{-1}	ΔH, kJ·mol^{-1}	ΔS, J·K^{-1}·mol^{-1}
288	800	−16.0		
298	625	−15.9	−18.8	−10.4
303	446	−15.3		
308	424	−15.5		

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In the case of Arrhenius plots of the polymer/substrate complex reaction, both **IIc** (DS = 0.30) and **III** (DS = 0.75) give a straight line. The thermodynamic parameters in the transition state were calculated by Eqs. (4)–(7), and the results are given in Table 4.

$$E_a = -2.303R[d \log k_2/d(1/T)] \quad (4)$$

$$\Delta G‡ = 2.303RT \log(RT/Nh) - 2.303RT \log k_2 \quad (5)$$

$$\Delta H‡ = E_a - RT \quad (6)$$

$$\Delta S‡ = (\Delta H‡ - \Delta G‡)/T \quad (7)$$

The activation energies (E_a) are 53.9 and 80.8 kJ·mol^{-1} for **III** of DS = 0.75 and **IIb** of DS = 0.30, respectively, indicating more stabilization in the former case.

The entropy change, $\Delta S‡$, is more negative in **III** (DS = 0.75) than in **IIc** (DS = 0.30). The benzylated polymer has a less charged group and a high substrate binding ability. Because the nucleophilic reaction of the catalytic group to the phenyl ester accompanys a charge separation in the transition state, the hydrophobic catalytic site is not favorable for the reaction. As a result, the hydrophobic interaction is considerably perturbed, giving a negative entropy change [49, 50]. On the other hand, as the domain of **IIc** with a high amino group content forms a sufficient ionic atmosphere, the entropy contribution in the intramolecular reaction is maintained, giving a positive value to $\Delta S‡$.

Here we compare these thermodynamic parameters with the values of the **IVb**/dodecylated *l*-PEI system. The free energy change, $\Delta G‡$, in the substrate binding process, −28.6 kJ·mol^{-1}, is larger than in polymer **III** of DS = 0.75. The activation energy of the complex formation, E_a, 67.2 kJ·mol^{-1}, lies between **III** (DS = 0.75) and **IIc** (DS = 0.30). It is interesting to compare this value with that in the acyl group transformation reaction of *p*-nitrophenyl laurate by paracyclophane having an oxime group [50]. This compound is known as an enzyme analog. Its

TABLE 4. Thermodynamic Parameters of the Hydrolysis of **IVb**

T, K	k_2, s^{-1}	E_a, kJ·mol^{-1}	$\Delta G‡$, kJ·mol^{-1}	$\Delta H‡$, kJ·mol^{-1}	$\Delta S‡$, J·K^{-1}·mol^{-1}
			III (DS = 0.75)		
288	0.0807	53.9	76.4	51.4	−87.0
298	0.177		77.2		
303	0.233		77.9		
308	0.350		78.2		
			IIc (DS = 0.30)		
283	0.286	80.8	72.1	78.3	22.9
288	1.01		70.4		
293	0.736		72.4		
298	1.06		72.8		
303	2.96		71.5		
308	8.57		70.0		

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values are E_a = 62.3 kJ·mol^{-1}, $\Delta G\ddagger$ = 89.0 kJ·mol^{-1}, $\Delta H\ddagger$ = 59.8 kJ·mol^{-1}, and $\Delta S\ddagger$ = −94 J·K·mol^{-1}, which are similar to those of **III** having DS = 0.75. This is supporting evidence for the argument that the benzylated system of a high DS has a hydrophobic substrate binding space similar to the hydrophobic void of paracyclophane and shows a steric adaptability to the inclusion of the substrate and a catalytic action similar to the inclusion complex-type catalyst.

From the above thermodynamic discussion, it is concluded that poly(allyamine) having hydrophobic groups shows an enzyme-like catalytic reaction mechanism. The contribution of the hydrophobic interaction is manifest in the substrate binding and in the transition state of the intramolecular reaction process. The polarity and steric structure of the microenvironment also have an important role.

CONCLUSION

To elucidate the structure–reactivity relationship for poly(allylamine) derivatives, the hydrolysis of charged, hydrophobic, and stereoisomeric esters was examined. It was found that the unmodified polymer accelerates the hydrolysis of the anionic ester due to an electrostatic effect. On the other hand, the catalytic activity of hydrophobic esters was considerably enhanced by an amphiphilic polymer which has lower polarity and a compact structure capable of including substrate molecules. Kinetic and thermodynamic analyses indicated that alkylated and benzylated polymers have different modes of contributing to the catalytic activity and the selective recognition of the substrates.

To shed more light on the molecular design of synthetic enzymes, we are investigating the hydrolysis reaction of optical isomers of esters in polymer systems with optically active sites.

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