이미다졸을 갖는 고분자의 촉매 반응의 Saturation Kinetics에 관한 연구

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Saturation Kinetics in Catalysis by Imidazole-Containing Polymers

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요 약 : 이미다졸(Im), poly[4(5)-vinylimidazole] (PVIm), 4(5)-vinylimidazole (95.5mole %)과 dodecyl methacrylate (4.5 mole %)의 공중합체 (CPVIm)를 촉매로 하여 활성화된 에스테르의 분해반응을 실시하였다. 기질로는 중성 그리고 음이온성이 선택되었으며 기질의 acyl 구룹의 길이도 변화시켜 보았다. 소수성 상호작용에 의해 CPVIm을 촉매로 하였을 때가 Im이나 PVIm을 촉매로 하였을 때 보다도 반응속도 상수값이 컸다. 중성기질을 사용하였을 때, PVIm을 촉매로 하였을 경우는 saturation kinetics가 나타나지 않았지만 보다 더 소수성인 CPVIm을 촉매로 하였을 경우는 saturation kinetics가 나타났다. 음이온성 기질을 사용하였을 때는 PVIm, CPVIm 모두 saturation kinetics를 나타냈다. 이 실험에서 Michaelis-Menten kinetics 의 관측은 효소작용에서와 같이 촉매—기질 복합체가 형성됐음을 나타낸다.

Abstract : The effects of the acyl chain length of activated substrates on the rate of hydrolysis of neutral substrates(Sn) and anionic substrates (Sn⁻) catalyzed by imidazole (Im), poly [4(5)-vinylimidazole] (PVIm), and the copolymer of 4(5)-vinylimidazole (95.5mole %) and dodecyl methacrylate (4.5mole %) (CPVIm) were investigated. They indicate the importance of hydrophobic interactions in the rate enhancements observed for CPVIm catalyzed reactions as compared to PVIm and Im catalyzed reactions. In case of neutral esters used as substrates, saturation kinetics were not observed with poly[4(5)-vinylimidazole] as a catalyst, but that was observed with the copolymer that has hydrophobic moiety. In case of anionic esters used as substrates, saturation kinetics were observed with both poly[4(5)-vinylimidazole] and CPVIm. The observation of Michaelis-Menten kinetics in these systems is indicative of the formation of a catalyst-substrate complex as in the case of enzyme systems.

INTRODUCTION

Looking into the labyrinth of enzymatic actions, a number of investigations have adopted model systems for the studies. In the mechanisms of reactions involving hydrolytic enzyme systems, the intramolecular multiple catalysis involving imidazole functions in hydrophobic environment has often enunciated. And this type of catalysis is considered to be closely related with the unique efficiency and specificity of the enzymes.[1] On this premises it seems justifiable to investigate the intramolecular cooperative catalysis of multifunctional catalysis systems involving imidazole in hydrophobic surroundings employing simplified model imidazole-containing polymers. In the past many investigations[2~4] had adopted various imidazole—derivatives and —polymers for model enzyme studies. Most of reported investigations have placed to emphasis on the enhanced activities of polymeric catalysts over their monomeric analogs. Recently there has been considerable interest in utilizing hydrophobic interactions to enhance the rate of ester hydrolysis by synthetic macromolecules.[5~9] In some cases it has been possible to observe saturation kinetics, formally characteristic of a catalyst-substrate complex, as is observed in enzyme-catalyzed reactions.[5,10~13]

In order to investigate further contribution of hydrophobic interactions for macromolecule-substrate complex formation and for catalytic activity, the polymeric catalysts, poly[4(5)-vinylimidazole] and the copolymer of 4(5)-vinylimidazole (95.5 mole%) and dodecyl methacrylate (4.5 mole%) were synthesized, and p-nitrophenyl acetate (PNPA), p-nitrophenyl hexanoate (PNPH), and p-nitrophenyl decanoate (PNPD) were prepared as neutral substrates, (Sn), and 3-nitro-4-acetoxybenzoic acid (NABA), 3-nitro-4-hexanoyloxybenzoic acid (NHBA), and 3-nitro-4-decanoyloxybenzoic acid (NDBA) were prepared as anionic substrates (Sn⁻). The hydrolysis reactions of S_n and S_n^- catalyzed by PVIm and CPVIm (relative to the catalytic activity of the monomeric analog, imidazole) were investigated.

EXPERIMENTAL

Synthesis of Substrates

p-Nitrophenylacetate (PNPA) (S_2) : PNPA obtained from Sigma Co., was used without purification.

p-Nitrophenylhexanoate (PNPH) (S_6) : Hexanoyl chloride (2.02 g, 0.015 mole in 15 ml of dried ethyl ether) which was obtained by chlorination[4] of hexanoic acid was added slowly to a stirred solution of p-nitrophenol (2.09g, 0.015mole) and triethylamine (1.52g, 0.015mole) in 75ml of dried ethyl ether. After the addition, the milky solution was stirred at room temperature for 5hr. The precipitated triethylamine hydrochloride was filtered off and the filtrate evaporated by means of a rotary evaporator under reduced pressure. The crude product could be purified by vacuum distillation, bp145℃.

p-Nitrophenyldecanoate (PNPD) (S_{10}) : Decanoyl chloride (0.015 mole) which was obtained by chlorination of decanoic acid, p-nitrophenol (0. 015 mole) and triethylamine (0.015 mole) were used. The synthetic procedure was same as above procedure. The crude product was recrystallized from ethanol, mp 35~36℃.

3-Nitro-4-Acetoxybenzoic Acid (NABA) (S_2^-) : NABA was prepared by the procedure of Overberger at al[2], mp 152~153℃.

3-Nitro-4-Hexanoyloxybenzoic Acid(NHBA) (S_6^-): 3-Nitro-4-hydroxybenzoic acid (0.015mole), mp 183~184℃, which was obtained by nitration of p-hydroxybenzoic acid, and hexanoyl chloride (0.015mole) and triethylamine (0.015mole) were used. The synthetic procedure was same as that described above. The crude product was recrystallized from cyclohexane-aquous ethanol (1 : 1 v/v), then cyclohexane again, mp 89~90℃.

3-Nitro-4-Decanoyloxybenzoic Acid(NDBA) (S_{10}^-): 3-Nitro-4-hydroxybenzoic acid, decanoylchloride and triethylamine were used. The synthetic procedure was same as above procedure. The crude product could be purified by two recrystallizations from cyclohexane and then finally from methanol, mp. 71~72℃.

Synthesis of Catalysts

Poly[4(5)-vinylimidazole] (PVIm) : A solution of

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4(5)-vinylimidazole[14](0.5g, 5.3mmoles) and azobis-isobutyronitrile (0.9mg) in 10ml of methanol or benzene was heated at 65℃ for 24hr. in a polymerization tube. The polymer prepared in methanol remained in solution while that prepared in benzene precipitated during the polymerization. This solution was added to a large excess of benzene, and the precipitated polymer was collected by filtration. This polymer was dissolved in methanol and reprecipitated into benzene. And then the polymer was dried in vacuum oven. The ultraviolet spectrum of poly[4(5)-vinylimidazole] in 28.5vol % 1-propanol-water at pH 1 showed absorption maximum at 214nm ($\varepsilon=3300$ liters mole^{-1}cm^{-1}).

The Copolymer of 4(5)-vinylimidazole and dodecyl methacrylate (CPVIm) : The polymerization procedure was same as above procedure. 4(5)-vinylimidazole (0.5 g, 5.3 mmole) and dodecyl methacrylate (0.05 g, 0.2 mmole) and AIBN (0.9 mg) were used.

Determination of Copolymer (CPVIm) Composition

From the ultraviolet spectrum of the homopolymer solution and the copolymer solution at 214nm, stoichiometric composition of the copolymer was determined. The molar extinction coefficient of protonated imidazole was first obtained from the optical density of homopolymer at its λ_{max} at pH 1. Stoichiometric composition of imidazole-containing copolymer was then determined from its ultraviolet absorption at the corresponding wavelength. The composition of copolymer thus obtained is given in Table 1.

Kinetic Measurements of Hydrolysis Reactions
A) Catalyst solutions were prepared in 33% 1-propanol-water buffered with 0.02 M tris (hy-droxymethyl) aminomethane and hydrochloric acid, sufficient potassium chloride was added to adjust the ionic strength to 0.02. The substrates were dissolved in 1-propanol. In all cases, a 10-fold molar excess of catalyst over substrate was used. The catalyst solution (3.0ml) and the substrate solution (200μl) were mixed in a quartz cell and the hydrolytic rates were studied on Simadzu UV-240 spectrophotometer at 23℃ by measuring the absorption(A_t) of corresponding phenolate ion as a function of time(t) at 400nm for Sn : at 410nm for Sn$^-$. The alcohol composition and concentrations of catalyst and substrate reported are those of the final 3.2ml solutions. After at least 10 half-lives the absorption was measured for complete reaction(A_∞). Catalysis by buffer only(blank) was measured in the same manner. The measured data were treated as pseudo first-order kinetics by plotting $A_\infty-A_t$ on a logarithm scale vs. t. The slope after plotting was taken as k_{measd}. The slope(k_{measd}) was corrected by subtracting the blank rate to obtain the obsered rate constant k_{obs}. The second-order rate constant k_{cat} was then calculated by dividing k_{obs} by catalyst concentration.

$$k_{cat} = k_{obs} / [catalyst]$$

B) Initial rates were determined from plots of optical density vs. time on Shimadzu UV-240 spectrophotometer. Calculations of initial rate values, V_{measd}, were made from

$$V_{measd} = V_{measd} - V_{blank} \ [A_t - A_o]/t \cdot \varepsilon$$

where ε is the molar extinction coefficient of the substrates being used.

$$V_{obsd} = V_{measd} - V_{blank}$$

RESULTS AND DISCUSSION

Hydrolysis of Sn and Sn$^-$ Catalyzed by Imidazole (Im)

Table 2 shows the results of the Im catalyzed

Table 1. Composition of CPVIm.

| Polymer	Concentration[a]	A(λ_{max}, nm)[b]	ε	Mole % of vinylimidazole
PVIm	1×10^{-4}mole / L (in imidazole)	0.330(214)	3300	100%
CPVIm	0.0088g / L	0.274(214)		95.5 %

[a] 28.5% 1-Propanol-water as a solvent.
[b] At pH 1.

Table 2. Influence of Chain Length of Activated Substrates on the Effective Second-Order Rate Constants(k_{cat}) for the Im-Catalyzed Hydrolysis of S_n and S_m^-.

Neutral substrates	k_{cat} $M^{-1}min^{-1}$	Negative substrates	k_{cat} $M^{-1}min^{-1}$
S_2	7.79	S_2^-	15.71
S_6	3.33	S_6^-	4.04
S_{10}	2.77	S_{10}^-	3.55

$[Im]=2.7\times10^{-4}M$, $[S_n]=[S_n^-]=2.7\times10^{-5}M$, $\mu=0.02$, $[Tris]=0.02M$, pH$=8.0$, $23°C$.
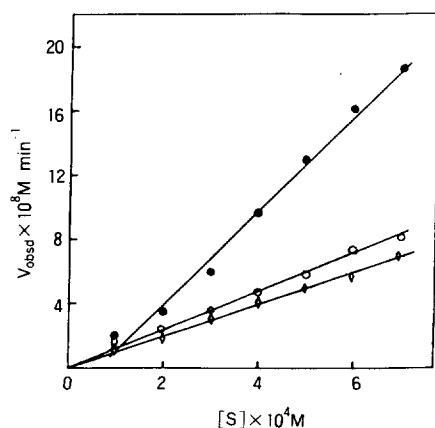
Fig. 1. PVIm-catalyzed initial hydrolysis rates of $S_2(\bullet)$, $S_6(\bigcirc)$, $S_{10}(\diamondsuit)$ in 33% 1-propanol-water : $[PVIm]=2.7\times10^{-5}M$, $\mu=0.02$, $[Tris]=0.02$ M, pH$=8.0$, $23°C$.

Fig. 2. PVIm-catalyzed initial hydrolysis rates of S_2^- ($\bullet$) S_6^- ($\bigcirc$), S_{10}^-($\diamondsuit$) in 33% 1-propanol-water; $[PVIm]=2.7\times10^{-5}M$, $\mu=0.02$, $[Tris]=0.02M$, pH$=8.0$, $23°C$.

hydrolysis of Sn and Sn$^-$ in 33 vol% 1-propanol-water. In this solvent composition, the second-order rate constants (k_{cat}) decreased as the chain lenth of the substrates increased. This indicates steric hindrance to Im-attack at the carbonyl carbon by the hydrophobic alkyl chain of the acyl portion of the substrates. This type of steric effect has been demonstrated by Fife[15] for the Im catalysed hydrolysis of a series of alkyl esters of *p*-nitrophenol.

Hydrolysis of Sn and Sn$^-$Catalyzed by Poly[4(5)-vinylimidazole](PVIm) and the Copolymer of 4(5)-Vinylimidazole with Dodecyl Methacrylate (CPVIm)

The hydrolysis reactions of Sn by PVIm and S_2 by CPVIm were a strict second-order process
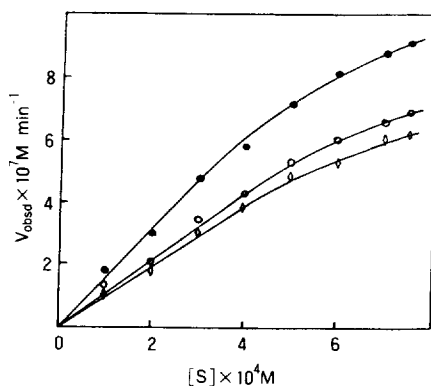
in 33 vol % 1-propanol-water, but had a different kinetic pattern on the PVIm catalysis of Sn$^-$ in 33 vol % 1-propanol-water where electrostatic forces between the partially protonated imidazole groups in PVIm and anionic esters become significant, and on the CPVIm catalysis of $S_6 \cdot S_{10}$ and Sn$^-$ 33 vol % 1-propanol-water where electrostatic and hydrophobic forces between CPVIm and activated esters become significant. The results indicate saturation and thus can be treated according to the Michaelis-Menten[16] mechanism. Fig.1 and Fig.3 for PNPA (S_2) show that the lack of limiting initial rates with increased substrate concentrations could perhaps be accounted for by the fact that the substrate concentrations were not sufficiently high and / or that hydrophobic forces are not sufficient to accumulate a high local concentration of the neutral esters in the vicinity of the polymers.

Fig.2 shows that the protonated sites on the polymer chain, which do not contribute to the hydrolysis of substrates, serve as binding sites for the anionic esters, thereby accumulating them in a high local concentration of catalytically active imidazole functions. This electrostatic effect would be expected to facilitate the saturation of the partially protonated polymer (PVIm) when high concentrations of substrates are employed.

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Fig.3 shows that the evidence for the involvement of hydrophobic interations for complex formation in the hydrolysis of PNPH (S_6) and PNPD (S_{10}) for CPVIm was obtained by the saturation phenomenon. When we compare PVIm catalyzed reactions with CPVIm catalyzed reactions for Sn, the PVIm catalyzed reactions for Sn did not show saturation, but the CPVIm catalyzed reactions for S_6 and S_{10} showed saturation. This means that hydrophobic interactions between the hydrophobic dodecyl methacrylate backbone and the hydrophobic alkyl chain of the acyl group of the substrates are responsible for saturation kinetics.

The involvement of electrostatic and hydrophobic interations for complex formation in the hydrolysis of Sn^- catalyzed by CPVIm was observed by the saturation phenomenon shown in Fig.4.

Although the PVIm catalyzed reactions for Sn^- and the CPVIm catalyzed reactions for S_6, S_{10} and Sn^- in this study showed a deviation from strict second-order kinetics, a k_{cat} value was calculated and is reported as an "apparent" second-order rate constant (see Table 3). This was done as a means of quantifying the data in order to probe the nature of the forces operating in these systems. When the acyl group of substrates become larger, the steric hindrance in the nucleophilic reaction at the carbonyl group will increase. At the same time hydrophobicity of the substrates will also increase. In the hydrolysis of Sn and Sn^- by PVIm, the second-order rate constants decreased as the chain length of the substrates increased, indicating increase in steric hindrance.

In order to investigate the extent of hydrophobic interations operative in the presently investigated systems, the hydrolysis of Sn and Sn^- catalyzed by CPVIm was performed at pH 8.00. And the results are summarized in Table 3. The order of reactivity based on steric factors which was observed in the Im and PVIm catalysis of Sn and Sn^- is no longer present with the copolymer of 4(5)-vinylimidazole and dodecyl methacrylate. As the chain length of substrates increased, the second-order rate constants increased, in spite of the increase in steric hindrance of substrates. It indicates that the dodecyl methacrylate backbone possesses significant hydrophobic character.

The r value (ie, rate enhancement) is defined as k_{cat} for PVIm or CPVIm / k_{cat} for Im, for the particular substrate. In all cases investigated the r values were greater than one. This is caused
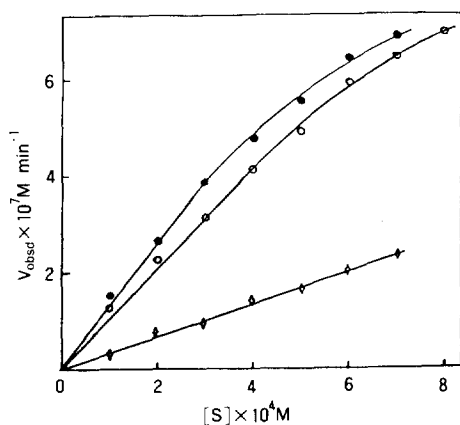

Fig. 3. CPVIm-catalyzed initial hydrolysis rates of $S_2(\diamond)$, $S_6^-(\bigcirc)$, $S_{10}^-(\bullet)$ in 33% 1-propanol-water ; [CPVIm]$=2.7\times10^{-5}$M (in imidazole), $\mu=0.02$, [Tris]$=0.02$, pH$=8.0$, 23℃.
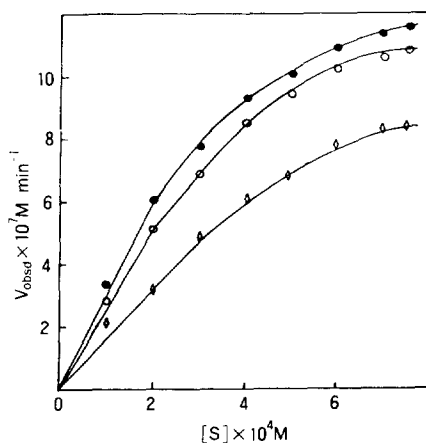
Fig. 4. CPVIm-catalyzed initial hydrolysis rates of $S_2^-(\diamond)$, $S_6^-(\bigcirc)$, $S_{10}^-(\bullet)$ in 33% 1-propanol-water ; [CPVIm]$=2.7\times10^{-5}$M (in imidazole), $\mu=0.02$, [Tris]$=0.02$, pH$=8.0$, 23℃.

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Table 3. Influence of Chain Length of S_n and S_n^- on the Apparent Second-Order Rate Constants (k_{cat}) for the CPVIm, PVIm and Im-Catalyzed Hydrolysis of S_n and S_n^-.

Neutral[b] substrates	Catalysts[a]	k_{cat} $M^{-1}min^{-1}$	r[c]	Negative[b] substrates	Catalysts,	k_{cat} $M^{-1}min^{-1}$	r
S_2	CPVIm	11.88	$r_1=1.52$	S_2^-	CPVIm	63.98	$r_1=4.07$
	PVIm	7.88	$r_2=1.01$		PVIm	51.50	$r_2=3.29$
	Im	7.79			Im	15.71	
S_6	CPVIm	13.91	$r_1=4.17$	S_6^-	CPVIm	73.56	$r_1=18.19$
	PVIm	4.12	$r_2=1.24$		PVIm	18.36	$r_2=4.54$
	Im	3.33			Im	4.04	
S_{10}	CPVIm	14.80	$r_1=5.34$	S_{10}^-	CPVIm	77.85	$r_1=21.92$
	PVIm	3.83	$r_2=1.38$		PVIm	16.25	$r_2=4.58$
	Im	2.77			Im	3.55	

[a]. $[CPVIm]=[PVIm]=[Im]=2.7\times10^{-4}$.
[b]. $[S_n]=[s_n^-]=2.7\times10^{-5}$, $u=0.02$, $[Tris]=0.02M$, pH=8.0, 23℃.
[c]. $r_1=k_{cat}CPVIm/k_{cat}Im$, $r_2=k_{cat}PVIm/k_{cat}Im$.

by two types of effects, viz., the increased concentration of the substrate in the polymer domain by electrostatic and / or hydrophobic interactions and the bifunctional interactions between the catalytically active pendent groups and the substrate.

Deviations from Pseudo-First-Order Kinetics

There is convincing evidence for the formation of a catalyst-substrate complex in enzyme systems[1] and some synthetic catalysts appear to operate by a similar mechanism.[4,6] This complexation is reflected by the observation of "saturation" kinetics. In its simplest form the kinetic expression for an enzymatic reaction can be represented by the Michaelis-Menten mechanism, which can be written in the form of eq 1.

$$E+S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\longrightarrow} E+P \qquad (1)$$
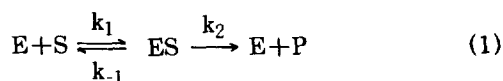
The mechanism is based on the assumption that the enzyme, E, reacts reversibly with the substrate, S. to form an enzyme-substrate complex, ES, which is then decomposed, giving back the free enzyme and a product or products, P. The steady-state equation for initial rate is

$$V = \frac{k_2[E][S]}{K_m+[S]} = \frac{V_{max}[S]}{K_m+[S]} \qquad (2)$$

where K_m is the Michaelis constant ($K_m=(k_{-1}+k_2)/k_1$) and V_{max} is the maximum rate ($V_{max}=k_2[E]$) Equation 2 can be rearranged to give eq 3.

$$\frac{1}{V} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}} \qquad (3)$$

By utilizing the Lineweaver-Burk plot. $1/V$ can be plotted against $1/[S]$ (Fig. 5, 6, 7). A straight line is obtained with a slope K_m/V_{max} and an intercept of the vertical axis of $1/V_{max}$. The value of K_m can also be obtained by extending the graph to the S axis. The values of V_{max} determined in this study can be used to calculate the constant k_2, sometimes called the "turnover number", since V_{max} is defined by $k_2[E]$. From a least-square treatment of these data, all of the kinetic parameters obtained and summarized in Table 4. Table 4 shows that the Michaelis constants increased and the turnover unmber decreased with a increase of chain length of substrates (Sn) catalyzed by PVIm, indicating increase in steric hindrance. But the Michaelis constants decreased and the turnover number increased with a increase of chain length of substrates (Sn, Sn⁻) catalyzed by CPVIm. It indicates that the interactions
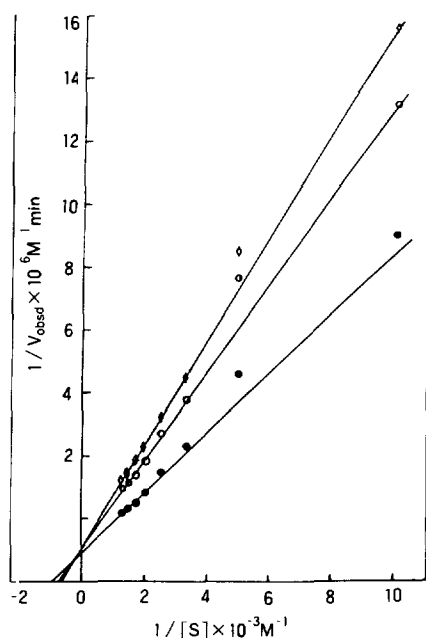
Fig. 5. The Lineweaver-Burk plots for the PVIm-catalyzed hydrolysis of S_2^- (●), S_6^- (○), S_{10}^- (◇).
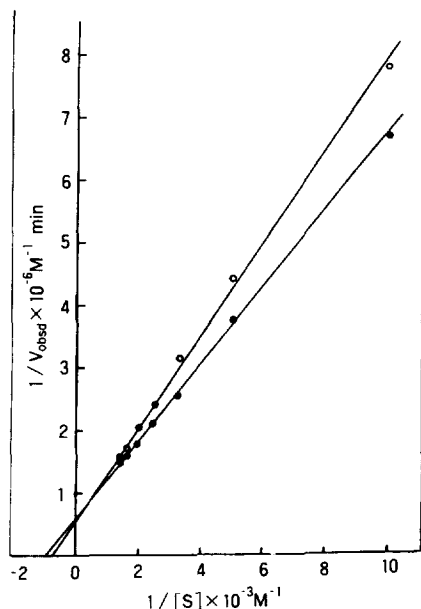

Fig. 6. The Lineweaver-Burk plots for the CPVIm-catalyzed hydrolysis of S_6^- (○) and S_{10} (●).
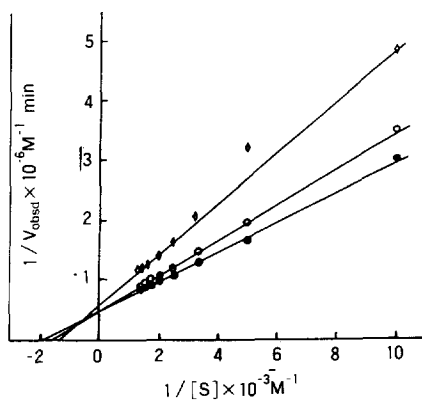

Fig. 7. The Lineweaver-Burk plots for the CPVIm-catalyzed hydrolysis of S_2^- (◇), S_6^- (○) and S_{10}^- (●).

Table 4. Kinetic Parameters for the PVIm and CPVIm-Catalyzed Hydrolysis of S_n and S_n^-.

Catalysts	Substrates	$K_m \times 10^4 M$	$k_2 \times 10^2 min^{-1}$
PVIm	S_2^-	12.94	4.55
	S_6^-	13.60	3.46
	S_{10}	16.38	3.67
CPVIm	S_6	12.63	6.42
	S_{10}	10.95	6.59
	S_2^-	6.90	5.86
	S_6^-	6.32	7.80
	S_{10}^-	5.18	7.66

pH=8.0, μ=0.02, 33% 1-propanol-water, 23℃.

between the dodecyl methacrylate backbone and the hydrophobic alkyl chain of the acyl group of the substrates in the CPVIm-substrate comples increased as the chain lenth of the substrates increased. These results suggest that the K_m value is associated with the magnitude of the hydrophobic and electrostatic interactions as a function of substrate chain length and that k_2 value is associated with not only the bifunctional catalytic interactions, but also with the magnitude of the hydrophobic and electrostatic interactions in the polymer-substrate complex.

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