Enantioselectivity Enhancement of Ester Cleavage by a β-Sheet Polypeptide Containing Catalytic Triads in a Serine Protease

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Catalytic activity and enantioselectivity of Poly(Asp–Leu–His–Leu–Ser–Leu) with a β-sheet structure for the hydrolysis of chiral phenylalanine *p*-nitrophenyl esters were compared with those of the peptide hexamer, under various conditions. The relationship between the β-sheet structure of the polypeptide and the hydrolytic properties was also investigated. Both the activity and the enantioselectivity for the polypeptide were higher than those for the peptide hexamer. The activity increased with pH, but the enantioselectivity was independent of pH. An increase of ethanol content in reaction solution led to a relatively lower rate constant and a higher enantioselectivity. The enantioselectivity decreased with increasing temperature; the activity increased to 25 °C and it reduced above 35 °C. Because the nucleophilicity of the polypeptide and the concentration effect of the substrate reduced by a decrease of the β-sheet content with temperature, the regular secondary structure may play an important role in these hydrolytic reactions. In addition, the efficient hydrolytic activity and the enantioselectivity were derived from activation parameters. The polypeptide may provide a good model for studying the relationship between enzymatic activity and steric structural formation.

A natural enzyme is characterized by possessing both a high efficiency of reaction and a high degree of specificity. Therefore, the design and synthesis of artificial enzymes with enzyme-like catalytic activity and substrate specificity remains an elusive task of chemists. Artificial enzymes have attempted to mimic the biological functions of natural enzymes by using organic host molecules with functional groups,[1—3] molecular assembly such as micelle and vesicle containing oligopeptides,[4—6] or polymers with functional groups[7—10] in order to clarify the mechanism of enzyme reaction and develop artificial enzymes with more excellent functions.

In particular, because natural enzymes are copolypeptides composed of various amino acids, more recently, polypeptides with functional groups have been much studied as artificial enzymes.[11,12] Polypeptides are optically active polymers, so they are expected to exhibit enantioselective properties. Although most polymeric catalysts are considerably less efficient than natural enzymes, several analogies between natural and synthetic polymeric molecules have been revealed.[13—15] This high catalytic activity would be caused by intramolecular cooperative interactions of functional groups with the other groups in polymer chain. For example, it was reported that poly(vinylimidazole) has high efficiency of hydrolytic reactions due to the cooperativeness of two imidazole rings.[14,15]

On the other hand, the mammalian serine proteases have a catalytic triad of amino acid residues so positioned on the protein as to give hydrolase activity. X-Ray diffraction studies of α-chymotrypsin have indicated that aspartic acid residue (Asp^{102}) forms its β-carboxyl hydrogen bonding with the imidazole of the histidine residue (His^{57}). The imidazole ring possesses hydrogen bonding to the hydroxyl group of the serine residue (Ser^{195}); also, the oxygen of the hydroxyl group attacks the electropositive carbonyl carbon of the substrate. These three amino acid residues form catalytic triads, which play a key role in enzyme-catalyzed hydrolysis.[16—19]

Poly(Asp–Leu–His–Leu–Ser–Leu) was designed as a stable β-sheet alternating amphiphilic polypeptide and an artificial hydrolytic enzyme-model because it is expected that the stability of the β-sheet and the nucleophilicity of the histidine residue are increased by interactions among catalytic triads on one side of the β-sheet, as shown in Fig. 1.[20] It was proved that the copolypeptide could form a highly stable β-sheet conformation in aqueous solution as well as in aqueous/alcohol mixtures on account of alternating amphiphilicity. The β-sheet conformation may be stabilized by the side chain electrostatic interactions among hydrophilic residues, as well as by the hydrophobic interactions between leucine residues. In addition, previous investigation showed that the polypeptide had enhanced hydrolytic property of *p*-nitrophenyl derivatives more effectively than imidazole and Asp–Leu–His–Leu–Ser–Leu hexamer. This catalytic enhancement may not be caused by the formation of a charge relay system among three hydrophilic residues, which appear to be formed in catalytic triads in serine proteases, and it may be due to increasing the nucleophilicity of the histidine residue by the intramolecular cooperative interactions among the hydrophilic amino acid residues as well as condensation effect of substrates by the hydrophobic interactions, as shown in Fig. 1.

In this paper, we describe the hydrolytic enantioselectivity of chiral substrates, *N*-protected *p*-nitrophenyl esters of the amino acid L- or D-phenylalanine, by poly(Asp–Leu–His–Leu–Ser–Leu) and

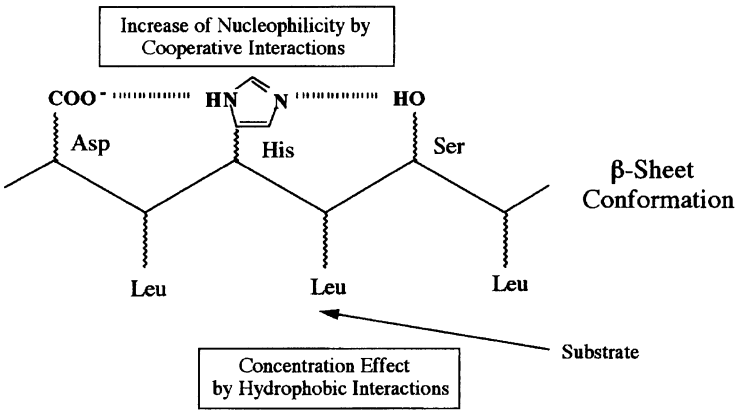

Fig. 1. Schematic model of catalytic triads formation of poly(Asp–Leu–His–Leu–Ser–Leu).

Asp–Leu–His–Leu–Ser–Leu hexamer as a function of pH, temperature or ethanol content. In addition, the relationship between the enantioselectivity and the secondary structure as well as the activation parameters is also discussed.

Experimental

Materials and Methods. The syntheses of the catalysts, poly(Asp–Leu–His–Leu–Ser–Leu) and Asp–Leu–His–Leu–Ser–Leu, were previously reported.[20] The intrinsic viscosity was 0.060 dL g^{-1} in dichloroacetic acid and the value corresponded to a molecular weight of 6800.[20] In addition, the size exclusion chromatogram showed that the molecular weight was approximately 6000 with 2.57 of the molecular weight distribution calculated on the basis of M_w/M_n.[20] The substrates: *p*-nitrophenyl acetate, *N*-benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (Z–L–Phe–ONp) and *N*-benzyloxycarbonyl-D-phenylalanine *p*-nitrophenyl ester (Z–D–Phe–ONp) were of high purity, commercially available, and were used without further purification.

Circular Dichroism (CD) Measurements. CD spectra were obtained under a constant flow of nitrogen on a Jovin Ivon CD6 spectropolarimeter equipped with an interface and a personal computer using a quartz cuvette of 1 mm pathlength. The instruments were calibrated with an aqueous solution of ammonium *d*-camphor-10-sulfonate.[21] Polypeptide stock solution was prepared at a concentration of approximately 3 mg mL^{-1} in distilled deionized water. The precise polypeptide concentration of the stock solutions was determined by ninhydrin analysis of a hydrolyzed polypeptide sample. CD samples were prepared by diluting the stock solutions either in water or ethanol. The observed ellipticity was expressed as mean residue ellipticity [θ], which was normalized to units of degrees centimeter squared per decimole. The content of a β-sheet conformation was calculated on the basis of the reported values of molar ellipticity of 100% β-sheet peptides: [θ] (217 nm) = −20000 deg cm^2 dmol^{-1}.[22]

Kinetic Measurements. Catalyst solutions of Asp–Leu–His–Leu–Ser–Leu hexamer and poly(Asp–Leu–His–Leu–Ser–Leu) were prepared at the residue molar concentration 5 mM, which is on the basis of total mole of histidine residue, by dissolving in water with 1 M tris(hydroxymethyl)aminomethane. The pH was adjusted with concentrated hydrochloric acid. The substrates: *p*-nitrophenyl acetate, Z–L–Phe–ONp and Z–D–Phe–ONp were dissolved in ethanol at a concentration of 10 mM. One hundred μL of catalyst solution (with or without catalyst) was mixed with 890 μL of solvent (water or water/ethanol mixture), and 10 μL of the substrate solution was added. Z–Phe–ONp precipitated at making the reaction solution, when the ethanol content of the reaction solution was less than 30 volume%, so the hydrolytic reactions of the chiral substrates were performed in solution containing 30 volume% ethanol or more.

The rates were studied in a Hitachi U-3210 spectrophotometer thermostated at various temperatures, by measuring the absorption (OD_t) of the 4-nitrophenolate ion at 400 nm as a function of time (t). At least 24 hours after starting the reaction, the absorption was measured for complete reaction (OD_∞).

The pseudo-first-order rate constants, k_1, were determined by the first order plot of the optical density at 400 nm according to Eq. 1.

The rate data obeyed a pseudo-first-order rate profile:

$$\log(OD_\infty - OD_t) = -k_1 t/2.303 + \log OD_\infty. \quad (1)$$

The second-order rate constants, k_{cat}, were obtained with the aid of Eq. 2 in which k_{measd} is the pseudo first-order rate constant measured in the presence of catalyst, k_{blank} is the pseudo first-order rate constant measured in its absence and $[Cat]_0$ is the initial concentration of the catalyst.

$$k_{cat} = (k_{measd} - k_{blank})/[Cat]_0. \quad (2)$$

The enantioselectivity is expressed as the ratio of the second-order rate constants obtained by employing Z–L–Phe–ONp and Z–D–Phe–ONp: k_L/k_D.

The hydrolysis of the substrates with the polypeptide catalyst was analyzed by the Michaelis-Menten kinetics which is described by Eq. 3:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P + E. \quad (3)$$

The change of initial rate V with changing catalyst concentration [E] was investigated, the substrate concentration $[S]_0$ being constant. Under the condition $[E] \gg [S]_0$, Eq. 3 is transformed into Eq. 4, where $K_m = (k_{-1}+k_2)/k_1$ (Michaelis constant). The reciprocal of V was plotted against the reciprocal of [E] to give a straight line. k_2 and K_m were determined from the intercept and the slope of the straight line. ($k_{measd} - k_{blank}$) was determined from the first-order plot of the optical density, and V was calculated from ($k_{measd} - k_{blank}$) by multiplying $[S]_0 = 1.0 \times 10^{-4}$ M.

$$1/V = 1/k_2[S]_0 + K_m/k_2[S]_0[E]. \quad (4)$$

The activation parameters for the hydrolytic reactions were calculated using the following equations: (1) the activation energy ($\Delta E^{\neq}$) from the slope of Arrhenius plot; (2) the activation enthalpy ($\Delta H^{\neq}$) from $\Delta H^{\neq} = \Delta E^{\neq} - RT$; (3) the activation free energy ($\Delta F^{\neq}$)

from $\Delta F^{\neq}=2.303RT\log(kT/hk_{cat})$; and (4) the activation entropy ($\Delta S^{\neq}$) from $\Delta S^{\neq}=(\Delta H^{\neq}-\Delta F^{\neq})/T$.

Results and Discussion

Effect of pH on the Hydrolytic Rates and the Enantioselectivity. The second-order rate constants and the enantioselectivity for the hydrolysis of the chiral substrates Z–Phe–ONp (L and D) with poly(Asp–Leu–His–Leu–Ser–Leu) and Asp–Leu–His–Leu–Ser–Leu hexamer at different pH values are summarized in Table 1. The rate constants increase with pH for both catalysts with two substrates because nucleophilicity of free base species of imidazolyl group is higher than that of its protonated species and the amount of its free base species increases with pH. Strong enhancements of the rate constants are observed at pH 8.0 compared with those form pH 6.5 to 7.5 for both substrate systems for the polypeptide catalyst. At pH 8.0, the rate constant, k_L, for the polypeptide is higher than that for the hexamer by a factor of approximately 4.0. It is likely that these results can be attributed to high nucleophilicity of histidine residues by electrostatic interactions as well as condensation effect of the substrates by hydrophobic interactions.[20]

The hydrolysis of Z–Phe–ONp with the polypeptide catalyst at pH 8.0 was analyzed by the Michaelis–Menten kinetics: A saturation of reaction rate with respect to the catalyst concentration was observed (data not shown). Figure 2 shows the Lineweaver–Burk plots, which have excellent linearity. For Z–L–Phe–ONp and Z–D–Phe–ONp as substrates, the intercepts give $k_2=2.08\times10^{-1}$ min^{-1} and 1.27×10^{-1} min^{-1}, respectively, and the slopes give $K_m=9.48\times10^{-4}$ and 7.85×10^{-4} M, respectively. These results indicate that the polypeptide catalyst behave as the Michaelis–Menten catalyst and the substrates might be strongly interacting with the polypeptide due to the hydrophobic interactions.

On the other hand, the enantioselectivities are independent of pH for both catalysts, and k_L/k_D values for the polypeptide and the peptide hexamer are approximately 1.5 and 1.2, respectively. These results would indicate that both catalysts formed more stable transition states with L-phenylalanine moiety than with D-phenylalanine moiety, because the catalysts were comprised of L-amino acids, and the β-sheet structural formation of poly(Asp–Leu–His–Leu–Ser–Leu) may be significant for higher enantioselectivity.

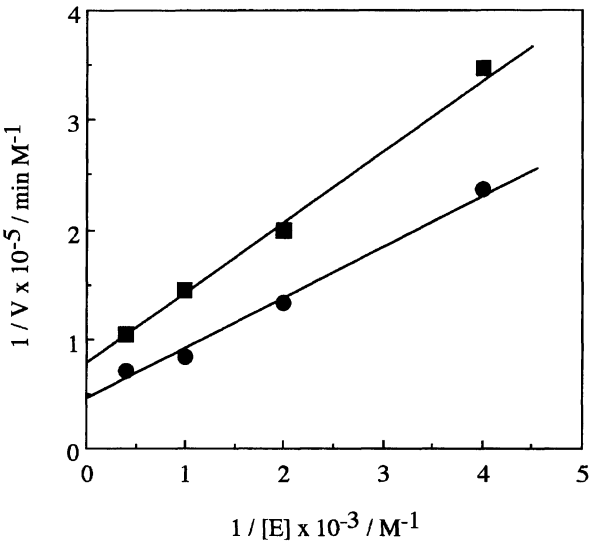

Fig. 2. The Lineweaver-Burk Plots of hydrolysis of Z–L-Phe–ONp (●) and Z–D-Phe–ONp (■) by poly(Asp–Leu–His–Leu–Ser–Leu) in water containing 30 vol% ethanol at 25 °C. [E]$=1.0\times10^{-3}-2.5\times10^{-4}$; [S]$=1.0\times10^{-4}$.

Effect of Ethanol Content on the Hydrolytic Rates and the Enantioselectivity. Table 2 summarizes the second-order rate constants and the enantioselectivity for the hydrolysis of the chiral substrates Z–Phe–ONp (L and D) with poly(Asp–Leu–His–Leu–Ser–Leu) and Asp–Leu–His–Leu–Ser–Leu hexamer at different ethanol contents of the reaction solution at 25 °C. The rate constants and the enantioselectivity (k_L/k_D) for the polymeric catalyst are superior to those for the other catalysts in all cases. The enhanced hydrolysis of the polypeptide may be caused by increasing nucleophilicity and condensation effect. And it is likely that high enantioselectivity of the polypeptide is caused by hydrophobic interactions with the β-sheet structure.

Table 1. Dependence of Rate Constants (k_{cat} (M^{-1} min^{-1})) and Enantioselectivities (k_L/k_D) for Hydrolysis of Z–Phe–ONp by Poly(Asp–Leu–His–Leu–Ser–Leu) or Asp–Leu–His–Leu–Ser–Leu Hexamer in Water Containing 30 Volume% Ethanol at 25 °C on pH[a)]

Catalysts	pH	k_L	k_D	k_L/k_D
Poly (DLHLSL)	6.5	2.89	1.92	1.51
	7.0	15.96	11.01	1.45
	7.5	40.88	27.44	1.49
	8.0	151.53	102.94	1.47
DLHLSL Hexamer	6.5	2.20	1.83	1.20
	7.0	13.92	11.32	1.23
	7.5	17.71	14.64	1.21
	8.0	38.54	31.12	1.24

a) Catalysts concentrations are 5.0×10^{-4} M and substrates concentration are 1.0×10^{-4} M.

Table 2. Rate Constants (k_{cat} (M^{-1} min^{-1})) and Enantioselectivities (k_L/k_D) for Hydrolysis of Z–Phe–ONp by Poly(Asp–Leu–His–Leu–Ser–Leu) or Asp–Leu–His–Leu–Ser–Leu Hexamer in water (pH 8.0)/Ethanol Mixtures at 25 °C[a)]

Catalysts	Ethanol content (Volume%)	k_L	k_D	k_L/k_D
Poly(DLHLSL)	30%	151.53	102.94	1.47
	40%	93.67	55.10	1.70
	50%	41.07	23.88	1.72
DLHLSL Hexamer	30%	38.54	31.12	1.24
	40%	18.23	14.83	1.23
	50%	7.74	6.43	1.20

a) Catalysts concentrations are 5.0×10^{-4} M and substrates concentration are 1.0×10^{-4} M.

Table 3. Rate Constants (k_{cat} (M^{-1} min^{-1})) and Enantioselectivities (k_L/k_D) for Hydrolysis of Z–Phe–ONp by Poly(Asp–Leu–His–Leu–Ser–Leu) or Asp–Leu–His–Leu–Ser–Leu Hexamer in Water (pH 8.0) Containing 30 Volume% Ethanol at Various Temperatures[a)]

Temp	Poly(DLHLSL)			DLHLSL Hexamer		
°C	k_L	k_D	k_L/k_D	k_L	k_D	k_L/k_D
5	62.66	24.66	2.54	15.76	12.11	1.30
10	86.92	36.87	2.36	19.64	14.99	1.31
15	108.54	51.74	2.10	25.53	20.43	1.25
20	128.91	73.96	1.74	30.42	24.68	1.23
25	151.53	102.94	1.47	38.54	31.12	1.24
35	96.12	69.21	1.39	44.61	37.23	1.20
45	75.95	58.75	1.29	46.10	38.16	1.21
55	66.53	55.72	1.19	47.08	39.01	1.21

a) Catalysts concentrations are 5.0×10^{-4} M and substrates concentration are 1.0×10^{-4} M.

The rate constants of both catalysts with both chiral substrates decrease with increasing ethanol content in the reaction solution. Three factors might be responsible for the decrease in the rate constants. (1) Free movements of catalysts and substrates may decrease because addition of ethanol to water lowers dielectric constant and the state of orderliness of water molecules increases. (2) The amount of free base species of a imidazolyl group may lower, because its pK_a may increase by addition of ethanol. (3) The hydrophobic interactions between the catalysts and the substrates may be depressed by the increase in the ethanol content; consequently, the substrates find it difficult to approach to the active site of the catalysis.

The enantioselectivity increases with increasing the ethanol content for the polypeptide, while that for the other catalyst is not affected by the ethanol content. Because hydrophobic interactions between the catalyst and the substrates decrease with increasing ethanol content, an increase of enantioselectivity for the polypeptide with ethanol content may be due to the lower magnitude of the decrease for L-substrate than for D-substrate. Although hydrophobic interactions would also play an important role in the hydrolytic reaction for the peptide hexamer in analogy with the polypeptide, it dose not form a regular secondary structure; therefore, enantioselectivity would be affected by the steric structure.

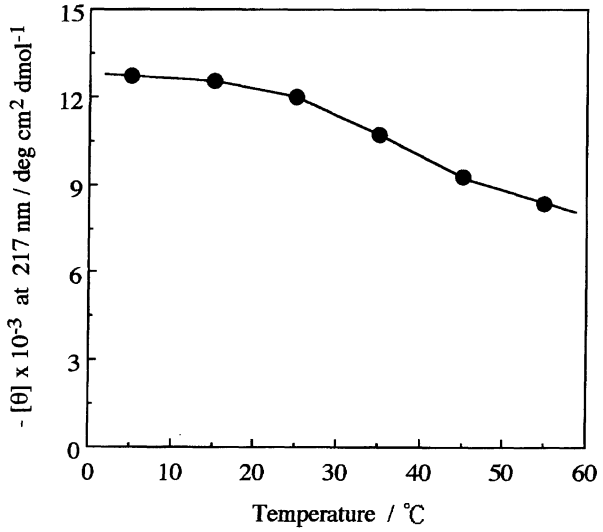

Fig. 3. Dependence of β-sheet contents for poly(Asp–Leu–His–Leu–Ser–Leu) in water (pH 8.0) containing 30 vol% ethanol on temperature.

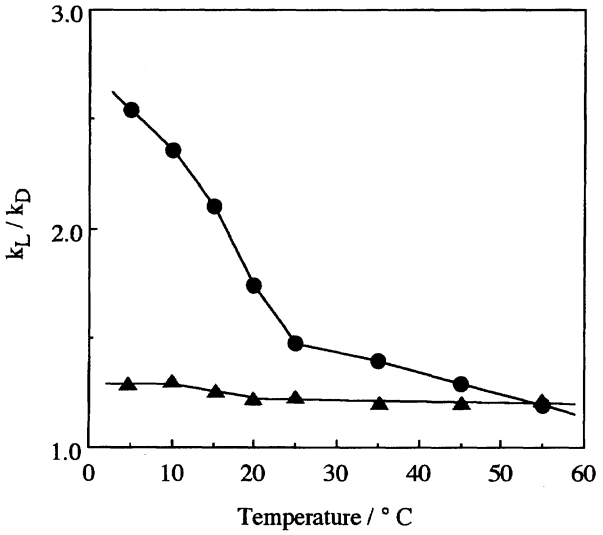

Fig. 4. Dependence of enantioselectivity for hydrolysis of Z–Phe–ONp with poly(Asp–Leu–His–Leu–Ser–Leu) (●) and Asp–Leu–His–Leu–Ser–Leu hexamer (■) in water (pH 8.0) containing 30 vol% ethanol on temperature. [E]= 5.0×10^{-4}; [S]=1.0×10^{-4}.

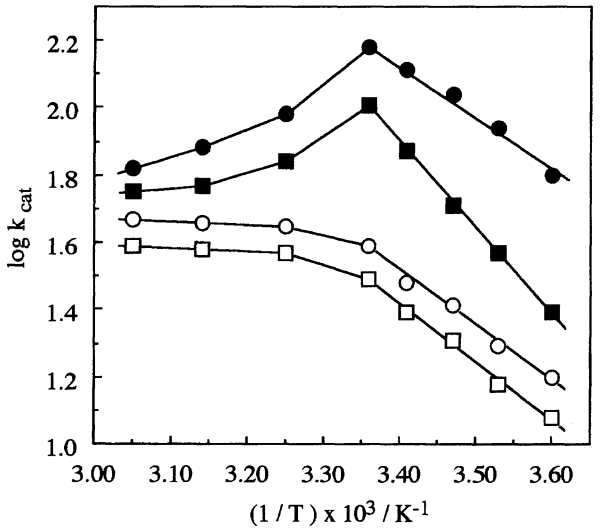

Fig. 5. Arrhenius plots for hydrolysis of Z–L-Phe–ONp (●) and Z–D-Phe–ONp (■) with poly(Asp–Leu–His–Leu–Ser–Leu), and Z–L-Phe–ONp (○) and Z–D-Phe–ONp (□) with Asp–Leu–His–Leu–Ser–Leu hexamer in water (pH 8.0) containing 30 vol% ethanol. [E]=5.0×10^{-4}; [S]=1.0×10^{-4}.

Effect of Temperature on the Hydrolytic Rates and the Enantioselectivity. The effect of the temperature on the hydrolytic activity of poly(Asp–Leu–His–Leu–Ser–Leu) or Asp–Leu–His–Leu–Ser–Leu hexamer toward the chiral

Table 4. Activation Parameters for Hydrolysis of Z–Phe–ONp Catalyzed by Poly(Asp–Leu–His–Leu–Ser–Leu) or Asp–Leu–His–Leu–Ser–Leu Hexamer in Water Containing 30 Volume% Ethanol[a)]

Catalyst	Substrate	$\Delta E^{\neq}$	$\Delta H^{\neq}$	$\Delta F^{\neq}$	$T\Delta S^{\neq}$	$\Delta S^{\neq}$
Poly(DLHLSL)	Z–L–Phe–ONp	6.8	6.2	16.7	−10.5	−35.8
	Z–D–Phe–ONp	11.7	11.1	17.0	−5.9	−20.0
DLHLSL	Z–L–Phe–ONp	7.4	6.8	17.7	−10.9	−37.2
Hexamer	Z–D–Phe–ONp	7.8	7.2	17.8	−10.6	−36.1

a) The units are in kcal per mole for all values except $\Delta S^{\neq}$ and in e.u. for $\Delta S^{\neq}$ (1 cal=4.184 J). Catalysts concentrations are 5.0×10^{-4} M and substrates concentration are 1.0×10^{-4} M.

Table 5. Rate Constants for Hydrolysis of *p*-Nitrophenyl Acetate by Poly(Asp–Leu–His–Leu–Ser–Leu) or Asp–Leu–His–Leu–Ser–Leu Hexamer in Aqueous Solution (pH 8.0) at Various Temperatures[a)]

Temp	k_{cat} / M^{-1} min^{-1}	
°C	Poly(DLHLSL)	DLHLSL Hexamer
5	29.51	9.66
10	37.58	12.83
15	47.82	17.27
20	59.57	22.74
25	69.50	25.65
35	70.79	45.73
45	66.07	44.56
55	61.44	43.91

a) Catalysts concentrations are 5.0×10^{-4} M and substrates concentration are 1.0×10^{-4} M.

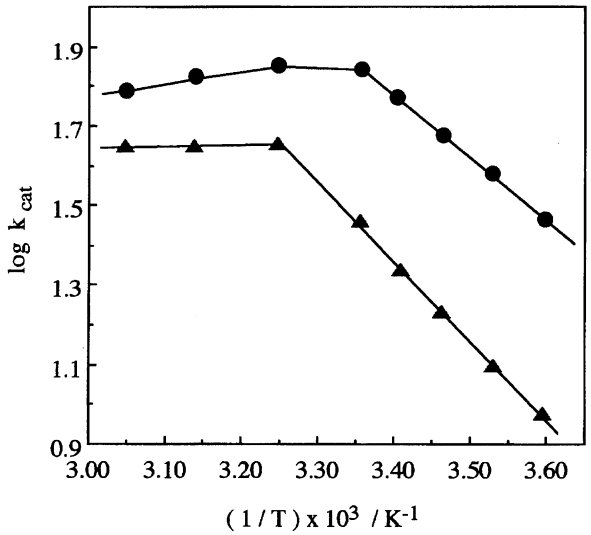

Fig. 6. Arrhenius plots for hydrolysis of *p*-nitrophenyl acetate in water (pH 8.0) by poly(Asp–Leu–His–Leu–Ser–Leu) (●) and Asp–Leu–His–Leu–Ser–Leu hexamer (▲). [E]=5.0×10^{-4}; [S]=1.0×10^{-4}.

Table 6. Activation Parameters for Hydrolysis of *p*-Nitrophenyl Acetate Catalyzed by Poly(Asp–Leu–His–Leu–Ser–Leu) or Asp–Leu–His–Leu–Ser–Leu Hexamer[a)]

Catalysts	$\Delta E^{\neq}$	$\Delta H^{\neq}$	$\Delta F^{\neq}$	$T\Delta S^{\neq}$	$\Delta S^{\neq}$
Poly(DLHLSL)	7.0	6.4	17.2	−10.8	−37.0
DLHLSL Hexamer	7.8	7.2	17.8	−10.6	−36.3

a) The units are in kcal per mole for all values except $\Delta S^{\neq}$ and in e.u. for $\Delta S^{\neq}$ (1 cal=4.184 J). Catalysts concentrations are 5.0×10^{-4} M and substrates concentration are 1.0×10^{-4} M.

substrates Z–Phe–ONp (L and D) in 30% ethanol solution is shown in Table 3. For the peptide hexamer, the rate constants increase with temperature from 5 to 35 °C, while above 35 °C, they remain approximately constant. Similar behavior is observed for enzymes that frequently display a maximum activity at a certain temperature. The enantioselectivity is kept almost constant throughout the experimental temperature. It is likely that the enantioselectivity is not affected by temperature, since the hexamer does not exist in regular secondary structure.

In contrast, the rate constants and enantioselectivity are influenced by the reaction temperature for the polypeptide, which affects the mobility of the substrates and catalysts as well as its β-sheet content. Both rate constants increase with temperature from 5 to 25 °C, whereas these decrease with temperature above 35 °C. The enantioselectivity values decrease with increasing temperature.

Figure 3 shows dependence of ellipticity at 217 nm for the polypeptide on temperature. The β-sheet content decreases slowly with temperature to 25 °C, and above 35 °C the content diminishes rapidly. A decrease of hydrolytic activity above 35 °C, as shown in Table 3, may be correlated with the drastic decrease of β-sheet content above 35 °C.

Figure 4 shows dependence of the enantioselectivity on temperature for both catalysts (see Table 3). The enantioselectivity reduces with temperature; in particular, above 35 °C the strong reduction is observed. The high enantioselectivity may require high β-sheet content of the polypeptide, because temperature of the drastic decrease of enantioselectivity is in accord with that of reduction of β-sheet content of the polypeptide.

Figure 5 shows Arrhenius plots for the hydrolytic reactions of Z–Phe–ONp (L and D) with poly(Asp–Leu–His–Leu–Ser–Leu) or Asp–Leu–His–Leu–Ser–Leu hexamer in 30% ethanol solution. Close linear Arrhenius plots are obtained from 5 to 25 °C for these four plots, while a strong deviation of all plots from linearity above 35 °C is observed. Activation parameters for the hydrolytic reactions from 5 to 25 °C calculated from Figure 5 are summarized

in Table 4. The characteristic features of the reaction will be reflected in the activation parameters. The relative low rate constant of D-substrate, would be reflected in the relative larger activation energy ($\Delta E^{\neq}$). The smaller (or negatively larger) activation enthalpy ($\Delta H^{\neq}$) and activation entropy ($\Delta S^{\neq}$) values of L-substrate, as compared with those of D-substrate, indicate the preferential attack of the catalyst on the L-substrate and more effective interaction between the catalyst and the L-substrate.

Table 5 shows dependence of hydrolytic rate constants of *p*-nitrophenyl acetate by both catalysts on temperature. The polypeptide has higher hydrolytic rate constants than the other catalyst under all experimental temperatures. For the polypeptide catalyst, the rate constant increases with temperature to about 35 °C, and it decreases slightly with temperature above 35 °C. This behavior is similar to the temperature dependence of the rate constants of Z–Phe–ONp, so β-sheet content of the polypeptide may be a dominant factor to determine the rate constants. In contrast, for the hexamer, the rate constants rises with temperature to 35 °C in analogy with the polypeptide catalyst, but above 35 °C, it stays almost constant.

Figure 6 shows Arrhenius plots for hydrolysis of *p*-nitrophenyl acetate by both catalysts; almost linear plots are observed from 5 to 25 °C for both catalysts. Activation parameters for the hydrolysis from their linear plots are summarized in Table 6. The relative high rate constant for the polypeptide catalyst compared with the other catalysts would be reflected in the relative smaller activation energy ($\Delta E^{\neq}$). It is likely that the smaller activation enthalpy ($\Delta H^{\neq}$) and activation entropy ($\Delta S^{\neq}$) values for the polypeptide exhibit relatively high rate constants through its more efficient attack on the substrate by hydrophobic interaction between leucine residues domain of the polypeptide and *p*-nitrophenyl group of the substrate.

The amphiphilic polypeptide poly(Asp–Leu–His–Leu–Ser–Leu) forms β-sheet structure under the hydrolytic reactions, and the β-sheet content affects the second-order rate constant and enantioselectivity because β-sheet formation induces efficient nucleophilicity and condensation effect of substrates. These results may indicate that formation of regular steric conformation for the polypeptide play an important role in these hydrolytic reactions in analogy with natural enzymes. The polypeptide may provide a good model for investigating relationships between enzymatic activity and steric structural formation.

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