Amphiphiles with Polypeptide-Head Groups III. Regulation of Enantioselectivity in Micellar Hydrolysis

Hiroataka IHARA, Toshio IDE, Osamu HOTTA,
Hakaru UCHIO,[†] Chuichi HIRAYAMA,
and Kimiho YAMADA*

Department of Synthetic Chemistry, Faculty of Engineering, and

[†]*Education, Kumamoto University, Kumamoto 860, Japan*

(Received October 7, 1985)

ABSTRACT: Chiral ester cleavage by achiral hydroxamic acids was investigated in the presence of chiral matrix micelles formed from amphiphiles with poly-L-lysine-head groups. The results are summarized as follows. (1) The enantioselectivity was not observed in the alkaline hydrolysis in chiral micelles only. This matrix possesses no selectivity. When an achiral hydroxamic acid was added to the system, selectivity was induced. (2) The direction of selectivity is reversed by the conformational change of the secondary structure formed by the peptide moiety of matrix micelles. L-Selectivity in the random coil and D-selectivity in the α -helix condition were observed. (3) The degree of selectivity is dependent on the chemical structure of hydroxamic acid; for example, an N-H type hydroxamic acid gives higher selectivity than an N-methyl type one. Thus, it is supposed that enantioselectivity is introduced by the chiral interaction between chiral micelles and achiral nucleophiles, in which the conformation of polypeptide moiety plays an important role.

KEY WORDS Amphiphile / Surfactant / Micelle / Enzyme Model /
Hydroxamate / Enantioselectivity / Polypeptide / Poly-L-lysine / α -Helix /
Random Coil /

Many hydrolysis reactions of chiral esters catalyzed by amino acid-derivatives have been studied in micellar systems in order to mimic the enantioselectivity of enzyme reactions.^{1–3} These studies have proposed or proved that the structural fitting between a catalyst and a substrate is a significant factor to give high enantioselectivity. However, in spite of the recognition that the secondary structure of an enzyme plays a part in the specificity, the relation between the secondary structures and enantioselectivity is not yet clear. On the other hand, we investigated the property of chiral assemblies formed from amphiphiles with polypeptide-head groups. It was previously

reported that compounds **1a–1d** can form micelles with structural asymmetries such as α -helical⁴ and β -sheet peptide-head groups,⁵ and **2** can form helical superstructures composed of single-walled bilayer membranes.⁶

1a: R = (CH₂)₄NH₂ ($m+n=3,8,13$ and 18)

1b: = CH₃ ($m=1, n=10$)

1c: = CH₂C₆H₅ ($m=1, n=12$)

1d: = CH₂CH(CH₃)₂ ($m=9, n=9$)

* To whom correspondence should be addressed.

2

In this study, the effects of chiral matrix micelles on cleavage reactions of chiral esters were investigated, using these polypeptide amphiphiles. As nucleophiles, hydroxamic acids **3a**–**3c** were used. The use of these achiral nucleophiles made the chiral effects from matrix micelles clear.

EXPERIMENTAL

All amphiphiles were synthesized by the polymerization of *N*-carboxyanhydride of *N*-carbobenzoxy-L-lysine with corresponding single-chain alkyl amines as initiators.^{4,6} Hydroxamic acids¹ and substrates² used were prepared by the previous procedures. The structures were confirmed by NMR and IR spectra and elemental analysis.

CD spectra of amphiphiles **1a**–**1d** in aqueous solutions were measured at $25.0 \pm 0.1^\circ\text{C}$ and pH 7.2 ± 0.1 , using a JASCO J-500C spectrophotometer. The concentration of amphiphiles was $4.0 \times 10^{-3} \text{ mol dm}^{-3}$, and quartz cells having path length from 1 mm to 0.1 mm were used.

Ester cleavage was carried out at 25.0

$\pm 0.1^\circ\text{C}$ and a range of pH 7.0 to 8.5 in the presence of NaClO_4 ($0\text{--}1.0 \text{ mol dm}^{-3}$) or NaCl ($0\text{--}0.2 \text{ mol dm}^{-3}$), and was spectrophotometrically followed using the absorption of the product (*p*-nitrophenolate anion, λ_{max} 400 nm). The pseudo-first-order rate constant (k_L and k_D , where L or D stands for the chirality of the substrate used) attributable to hydroxamic acids was evaluated from k_{total} in the presence of hydroxamic acids and k_{spont} in the absence of hydroxamic acids in the usual way.

$$k_L \text{ or } k_D = k_{\text{total}} - k_{\text{spont}}$$

Enantioselectivity was expressed by the ratio of k_L to k_D in this study.

RESULTS AND DISCUSSION

Conformation of Peptide-Head Groups in Micelles

Amphiphiles with poly-L-lysine-head groups (**1a**–**1d**) have the cmc of typical surfactants. The micelles are cationic at pH 7.0–8.5, because pK_a of the lysine residue is 9.7 to 10.0.⁴ The cmc values are in the range of 2.0 to $0.5 \times 10^{-3} \text{ mol dm}^{-3}$ in aqueous solution at pH 7.2. It was confirmed that the cmc was lowered by more than a factor of 10 in the presence of more than 0.2 mol dm^{-3} of NaCl or NaClO_4 . The hydrolysis reaction of chiral esters is carried out in the presence of these additional ions which are used to regulate the conformation of polypeptide-head groups and suppress the contribution of spontaneous hydrolysis by adjusting ionic strength.

Conformation of the poly-L-lysine moiety in these micelles was investigated by the CD method. The spectrum shown by the dotted

Figure 1. CD spectra of amphiphile **1a** ($n=13$) in the presence of NaClO_4 . **1a**: 4.0 mol dm^{-3} , 25.0°C , pH 7.2.

line in Figure 1 indicates that the conformation of amphiphile **1a** is a random coil at pH 7.2 and $4.0 \times 10^{-3} \text{ mol dm}^{-3}$ (above cmc). The CD pattern changed from that of the random coil to α -helix by the addition of NaClO_4 (solid lines in Figure 1). The induction of α -helix is attributed to lowering of the electrostatic repulsion among the lysine residues ($-\text{NH}_3^+$) by salt formation with ClO_4^- .⁷ On the contrary, strong electrolytes such as NaCl and KCl do not induce the conformational change. The relationship between the helix-formation and concentration of NaClO_4 is shown in Figure 3b. The apparent helix content (x^H %) was evaluated from the value of $[\theta]_{222}$. The value of x^H reaches saturation by the addition of $0.8\text{--}1.0 \text{ mol dm}^{-3}$ of NaClO_4 . The saturation in the helical content (x^H_{max}) is related to the degree of polymerization (n) in peptide amphiphiles. Figure 2 shows the relation-

Figure 2. Relationship between helicity (x^H) and the degree of polymerization (n) of amphiphile **1a**. **1a**: 4.0 mmol dm^{-3} , 25.0°C , pH 7.2 —○—, 1.0 M-NaClO_4 ; —●—, 0.2 M-NaCl .

Table I. Conformation of polypeptide-head groups in micelles^a

Amphiphile	Conformation of head-groups	
	0.2 M-NaCl	1.0 M-NaClO ₄
1a	Random	Helical
1b	Random	Helical
1c	Random	Helical
1d	Helical	Insoluble

^a 25.0°C, pH 7.2. Amphiphile, above cmc.

ship between the helix content ($x_{\max}^H$) and degree of polymerization (n) of amphiphile **1a**. It is known that the degree of helix-formation in oligopeptides is dependent on that of polymerization (n).^{4,8} The transition from a random coil to α -helix by the addition of NaClO₄ was similarly induced in the case of amphiphiles **1b** and **1c**. On the other hand, in amphiphile **1d**, the α -helix form ($[\theta]_{200}$, -18000; $[\theta]_{218}$, -10000 at pH 7.2) is partially present even in the absence of NaClO₄. It is reported that poly-L-leucine ($R = CH_2CH(CH_3)_2$ in **1d**) can maintain the α -helix form because of its hydrophobicity.⁹

It is very significant that the conformational change similar to usual poly-L-lysine is observed even in the micelle formation. On the basis of CD measurements, the conformation of peptide-head groups in micelles can be summarized approximately as in Table I.

Ester Cleavage

It is already confirmed that the micelles of amphiphile **1a** show a typical saturated kinetic pattern similar to an enzyme reaction toward the hydrolysis of esters **4** and **5** in the range of pH 9–12.⁴

In this case, esters **4** and **5** are bound to cationic peptide micelles (ionized partially) by hydrophobic and electrostatic interactions, respectively, and are hydrolyzed by concentrated OH⁻ and neutral amino groups of lysine residues.⁴

In this study, the cleavage of chiral esters (L-S and D-S) was examined in mixed micellar systems with achiral hydrophobic hydroxamic acids, **3a–3c**, and the matrix micelles formed from poly-L-lysine amphiphiles, **1a–1d**. The pH of the solution was adjusted to 7.0–8.5 in order to lessen the contribution of OH⁻ and neutral amino groups of lysine residues as much as possible. The hydroxamate anion, a well known reagent for enzyme model reaction, is highly nucleophilic in the presence of cationic micelles¹⁰ and has no selectivity for cleavage of chiral esters in non-micellar solutions.¹ By the use of these achiral nucleophiles, the effects of matrix asymmetries due to α -helix and a random coil on enantioselectivity were investigated.

Hydrolysis reaction was carried out in the presence of NaCl when NaClO₄ was not added to the system. Additional NaCl was used to regulate the hydrolysis rate without conformational change of the polypeptide-head groups. The concentration of NaCl in the system was adjusted to 0.2 mol dm⁻³ because of precipitation at more than 0.3 mol dm⁻³. First order rate constants observed in the

Table II. First-order rate constant (k_{spont}) and enantioselectivity (k_L/k_D) in alkaline hydrolysis^a

Matrix	$k_{\text{spont}}/\times 10^2 \text{ s}^{-1}$						
	0.2 M-NaCl			1.0 M-NaClO ₄			
	L*	D*	L/D*	L*	D*	L/D*	$x^H/\%$
1a ($n=3$)	5.10	5.21	1.0	0.83	0.86	1.0	15
1a ($n=8$)	4.11	3.84	1.1	0.73	0.74	1.0	43
1a ($n=13$)	3.83	3.80	1.0	0.48	0.49	1.0	57
1a ($n=18$)	3.51	3.69	1.0	0.94	0.97	1.0	63
1b	2.44	2.48	1.0	0.51	0.62	0.8	81
1c	3.16	2.32	1.4	0.52	0.65	0.8	60
1d	2.99	2.84	1.1	Insoluble			

^a x^H , the content of helix (%), 25.0°C, pH 7.2; amphiphile, 4.0 mmol dm⁻³; substrate, 0.04 mmol dm⁻³.

* L and D stand for the chirality of the substrate used.

Table III. First-order rate constant (k_L or k_D) and enantioselectivity (k_L/k_D) in the presence of **3a**^a

Matrix	First-order rate constant/ $\times 10^2 \text{ s}^{-1}$						
	0.2 M-NaCl			1.0 M-NaClO ₄			
	L	D	L/D	L	D	L/D	$x^H/\%$
1a ($n=3$)	3.04	2.21	1.4	2.17	2.33	0.9	15
1a ($n=8$)	3.12	1.49	2.1	1.17	1.65	0.7	43
1a ($n=13$)	7.83	3.40	2.3	1.15	1.57	0.7	57
1a ($n=18$)	5.38	2.43	2.4	1.37	1.92	0.7	63
1b	5.38	1.69	3.2	1.98	1.63	1.2	81
1c	6.64	2.52	3.6	1.21	0.60	2.0	60
1d	7.26	7.20	1.0	Insoluble			

^a 25.0°C, pH 7.2. Amphiphile, 4.0 mmol dm⁻³; **3a**, 0.4 mmol dm⁻³; substrate, 0.04 mmol dm⁻³.

chiral ester cleavage are summarized in Tables II and III.

In the absence of **3a**, no enantioselectivity was observed regardless of conformational change of matrix micelles ($L/D=1$ in k_{spont} as shown in Table II). The chirality of matrix seems to play no role. It is presumed that the ester is hydrolyzed by OH⁻ concentrated onto the micelles, since amino group-residues of amphiphiles are almost completely protonized at pH 7.2.

On the contrary, the selectivity was induced by the addition of hydroxamic acid **3a** with increase of k_{total} . The values of k_L and k_D were

obtained by subtraction of k_{spont} from k_{total} and are shown in Table III. Since **3a** is achiral and has no enantioselectivity, it may be considered that the selectivity is introduced by co-operation between the chiral micelles and achiral nucleophiles. We cannot explain this mechanism, although many speculations for the mechanism of enantioselectivity have been suggested in various micellar systems.³ However, it could be supposed from the following results that the secondary structural asymmetry introduced from the poly-L-lysine moiety is important for enantioselectivity. First, the selectivity for L-S and D-S was changed

Figure 3. Relationship between the concentration of NaClO_4 and selectivity (a) or helicity (b) at 25.0°C , pH 7.2 (a) **1a**: 4.0 mmol dm^{-3} with **3a**: 0.4 mmol dm^{-3} (b) **1a**: 4.0 mmol dm^{-3} .

by the addition of NaClO_4 . For example, the value of k_L/k_D changed from 2.4 to 0.7 in the case of amphiphile **1a** ($n=18$). As shown in Figure 3, the selectivity change is related to the conformational change from a random coil to α -helix upon the addition of NaClO_4 . A similar result is obtained in the addition of poly(acrylic acid). The presence of poly(acrylic acid) promotes the helix-formation of amphiphile **1a** and causes the selectivity change. The content of helix increased from 0 to 80% and k_L/k_D changed from 2.4 to 0.6 by the addition of 0.25 wt% of poly(acrylic acid) at pH 7.2. Poly(acrylic acid) is also one of the additive ions which causes induction of the

Table IV. Enantioselectivity of some nucleophiles in the presence of **1a** ($n=8$)^a

Nucleophile	First-order rate constant/ $\times 10^2\text{ s}^{-1}$					
	0.2 M- NaCl			1.0 M- NaClO_4		
	L	D	L/D	L	D	L/D
None	4.11	3.84	1.1	0.73	0.74	1.0
3a	3.12	1.49	2.1	1.17	1.65	0.7
3b	1.49	1.04	1.4	0.91	1.08	0.8
3c	2.65	0.88	3.0	1.05	1.62	0.6

^a 25.0°C , pH 7.2; substrate, 0.04 mmol dm^{-3} . **1a**, 4.0 mmol dm^{-3} ; **3a** and **3b**, 0.4 mmol dm^{-3} ; **3c**, 1.0 mmol dm^{-3} .

helix formation of poly-L-lysine.¹¹ Secondly, the selectivity increases with the degree of polymerization (n) of head-groups of the matrix amphiphiles. It is known that the conformation of a low-molecular-weight polypeptide is dependent on n .^{4,8} Thirdly, the enantioselectivity and activity changed by the structural differences of hydroxamates added. This result indicates that the achiral hydroxamate possesses chiral selectivity due to interaction with the chiral matrix. Hydrogen bonding between the hydroxamate and micelles is considered to induce chirality, because the degree of the selectivity is higher in the hydroxamate of N-H type than those of N-methyl type, as shown in Table IV. This supposition is not yet proved, since we could not detect the ICD of hydroxamate **3c** by the chiral matrix.

The enantioselectivity (k_L/k_D) is also sensitive to structural differences of the peptide moiety in matrix amphiphiles. When a spacer such as L-phenylalanine or L-alanine was introduced between the long-chain alkyl group and poly-L-lysine-head group, the ratio of k_L/k_D increased from 2.1 to 3.2 under the random coil condition and changed from D-selectivity ($k_L/k_D=0.7$) to L-selectivity ($k_L/k_D=2.0$) in the α -helix condition. The CD spectra of amphiphiles **1b** and **1c** at pH 7.2 are similar to that of amphiphile **1a**. These results indicate that the interaction be-

tween the matrix and hydroxamate produces at the spacer site varying chiral environments by the introduction of a different amino acid. On the other hand, in the case of **1d** by which the block of poly-L-leucine (which forms α -helix) was introduced as a spacer, the selectivity disappeared from 2.1 to 1.0 under the random coil condition. Therefore, it is clear that the local chirality based on the structure of the peptide moiety as well as matrix asymmetry plays a part in the enantioselective reaction.

In conclusion, this study provides a typical example of the relation between the secondary structure of polypeptide(s) and enantioselectivity in micellar catalysis. Selectivity is induced by an achiral hydroxamate in the chiral environment and is regulated by conformational change of matrix micelles.

The mode of chiral interaction is still obscure, but we suppose that the reaction site in micelles is near the root of polypeptide-head groups, and the peptide conformation plays an important role in enantioselective reactions.

Acknowledgements. We thank Mr. Fukumoto for his capable experimental assistance. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. K. Yamada, H. Shosenji, and H. Ihara, *Chem. Lett.*, 491 (1979); K. Yamada, H. Shosenji, H. Ihara, and S. Ono, *Tetrahedron Lett.*, **20**, 2592 (1979); H. Ihara, S. Ono, H. Shosenji, and K. Yamada, *J. Org. Chem.*, **45**, 1623 (1980).
2. S. Ono, H. Shosenji, and K. Yamada, *Tetrahedron Lett.*, **22**, 2371 (1981).
3. K. Ohkubo, K. Sugahara, K. Yoshinaga, and R. Ueoka, *J. Chem. Soc., Chem. Commun.*, 637 (1980); K. Ohkubo, H. Ogata, K. Yamaki, and K. Yamashita, *Makromol. Chem.*, **185**, 891 (1984); Y. Ihara, *J. Chem. Soc., Chem. Commun.*, 984 (1978); Y. Ihara, N. Kunikiyo, T. Yunimasa, N. Nango, and Y. Kuroki, *Chem. Lett.*, 667 (1981); R. A. Moss and G. O. Bizzigotto, *J. Am. Chem. Soc.*, **103**, 6512 (1981).
4. H. Uchio, H. Ihara, T. Izuno, T. Ide, K. Taniguchi, and K. Yamada, *Yukagaku*, **33**, 38 (1984).
5. K. Yamada, H. Ihara, T. Ide, T. Fukumoto, and C. Hirayama, *Chem. Lett.*, 1731 (1984).
6. H. Ihara, T. Ide, K. Taniguchi, K. Yamada, and H. Uchio, *Polym. Prepr. Jpn.*, **33**, 361 (1984).
7. E. Peggion, A. Cossani, M. Tervojecvichi, and G. Borin, *Biopolymers.*, **11**, 633 (1972).
8. A. Yaron, E. Katchalski, and A. Berger, *Biopolymers.*, **10**, 1107 (1971).
9. E. R. Blout, "Polyamino Acids, Polypeptide, and Proteins," The University of Wisconsin Press, Madison, 1962.
10. T. Kunitake, Y. Okahata, and T. Sakamoto, *Chem. Lett.*, 459 (1975); I. Tabushi, Y. Kuroda, and S. Kita, *Tetrahedron Lett.*, 643 (1974); I. Tabushi and Y. Kuroda, *ibid.*, 3613 (1974).
11. A. B. Zevin, V. V. Lutsenko, V. B. Rogacheva, O. A. Aleksina, R. I. Kalyuzhnaya, V. A. Kavanov, and V. A. Kiargin, *Vyskomol. Soedin. Ser., A*, **14**, 772 (1972).