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A REMARKABLE STERIC CONTROL FOR THE ENANTIOSELECTIVE HYDROLYTIC-CLEAVAGE OF AMINO ACID ESTERS RESPONDING TO IONIC STRENGTH

Ryuichi Ueoka*, Masanori Cho, Yōko Matsumoto, Kōichi Goto,
Yasuo Kato, Kumiko Harada**, and Atsushi Sugii**

Department of Industrial Chemistry, Faculty of Engineering,
Kumamoto Institute of Technology, Ikeda, Kumamoto 860, Japan
**Faculty of Pharmaceutical Sciences, Kumamoto University,
Ohe-honmachi, Kumamoto 862, Japan

Summary: The dramatically beautiful effect of ionic strength on the enantioselective hydrolysis of amino acid esters by the active tripeptide was observed in the hybrid-assemblies composed of 59 mol% double-chain surfactant and 41 mol% single-chain one in mild conditions.

Stereoselective hydrolytic-cleavages of amino acid esters have attracted considerable attention in connection with understanding the origins of the stereoselectivity observed with proteolytic enzymes.[1-5]

In the course of our study on the enantioselective catalysis, we emphasized that the stereochemical control could be established by temperature regulation[6] and by changing the composition of the coaggregates (reaction field).[7] Particularly, the enantioselectivity for the hydrolytic cleavage of the enantiomeric substrates by the active tripeptide was well correlated with the apparent mean hydrodynamic diameter of coaggregates composed of synthesized double-chained vesicular surfactants and single-chained micellar ones.[8]

In this study we report on the dramatically beautiful effect of ionic strength (μ) on the enantioselective hydrolysis of p-nitrophenyl-D(L)-phenylalaninates (D(L)-S_{12}) by the tripeptide benzyloxycarbonyl-L-phenylalanyl-L-histidyl-L-leucine (Z-PheHisLeu) in the hybrid-assemblies composed of 59 mol% double-chain surfactant (ditetradecyldimethylammonium bromide : $2C_{14}Br$) and 41 mol% single-chain one (hexadecyltrimethylammonium bromide : CTABr) at pH 7.6 and 25 °C.

With respect to the morphology of coaggregates composed of 59 mol% $2C_{14}Br$ and 41 mol% CTABr, the presence of single- and double-walled vesicles, which reflect the morphology after being allowed to stand for 1 day at room temperature, was proved by electron microscopy as shown in Figure 1. We found that the size of the hybrid-assemblies after being

allowed to stand for 1 day in the condition of μ = 0.08 (R_h^{app} = 840 Å)[9] is about 2 times as large as that in the condition of μ = 0.02 (440 Å) on the basis of the dynamic light-scattering (dls) data and, interestingly, the relative size in the conditions of μ = 0.08 and 0.02 was in good agreement with the observed particle radii by electron microscopy (Figure 1).

The stability of coaggregates was examined by measuring the relationship between the time passed and size (R_h^{app}) change with dls at a fixed angle of 90°. The particle size gradually increased with time in the region of lower ionic strengths (μ = 0.01-0.08) and we obtained stable particles having a constant size after being allowed to stand for 8 days at room temperature. On the other hand, no-time dependent R_h^{app} changes were observed in the region of higher ionic strengths (μ = 0.1-0.2). This means that the large coaggregates remain very stable for at least 10 days. The radii (R_h^{app}) of the coaggregates after being allowed to stand for 1 day (usual preparation time for kinetics) and 4-8 days (equilibrium time) are shown in Figure 1.

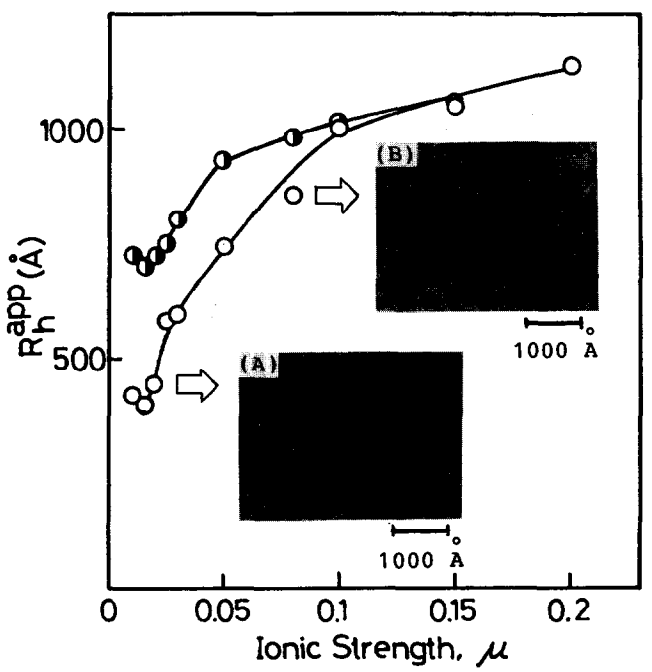

Figure 1. Apparent hydrodynamic radii (R_h^{app}) of the coaggregates after being allowed to stand for 1 day (○) and 4-8 days (◑) versus ionic strength. The coaggregates contain 59 mol% $2C_{14}Br$ and 41 mol% CTABr. Electron micrographs, negatively stained with uranyl acetate, for μ = 0.02 (A) and μ = 0.08 (B).

Table I. Rate Constants (k_2, $M^{-1}s^{-1}$) and Enantioselectivity (k_2^L/k_2^D)[a]

Ionic Strength	1 day[b]			4-8 days[b]		
(μ)	L-S_{12}	D-S_{12}	L/D	L-S_{12}	D-S_{12}	L/D
0.01	1100	30	37	1800	31	58
0.015	1100	1.4	790	1600	28	57
0.02	1000	1.0	1000	1400	0	∞
0.025	1200	11	110	1800	36	50
0.05	1200	15	80	1500	73	21
0.08	1100	21	52	1400	71	20
0.10	1200	43	28	1300	81	16
0.15	1200	68	18	1200	63	19
0.20	1100	52	20	1300	72	18

(a) The second-order rate constant k_2 was evaluated by eq.1 : $k_2 = (k_t - k_s)/[Cat]_0$ (1), where k_t and k_s denote the first-order rate constants with and without catalyst, respectively, and $[Cat]_0$ indicates the initial catalyst concentration. The rate constants have maximum errors of ±4%. Conditions: 25 °C, pH 7.6, 0.01-0.20 Tris-KCl buffer (μ = 0.01-0.20), 3% (v/v)CH_3CN-H_2O, [Z-PheHisLeu]=5×10^{-5} M, [D(L)-S_{12}] =1×10^{-5} M, [$2C_{14}$Br]=1×10^{-3} M, [CTABr]=7×10^{-4} M.

(b) The days correspond to those as shown in Figure 1.

L- or D-S_{12} was cleavaged by the active tripeptide (Z-PheHisLeu) in pure micelles, pure vesicles, or coaggregates composed of micellar and vesicular surfactants.[7,8] Second-order cleavage rate constants (k_2, $M^{-1}s^{-1}$) for the hydrolysis of D(L)-S_{12} by Z-PheHisLeu in the $2C_{14}$Br (59 mol%) / CTABr (41 mol%) coaggregates are summarized in Table 1. The control of stereoselectivity responding to ionic strength is a most attractive feature indeed! In particular, a remarkably high enantioselectivity (k_2^L/k_2^D = 1000) and the exclusively preferential cleavage of the L-isomer (k_2 = 1400 $M^{-1}s^{-1}$) but without the detectable cleavage of the D-isomer (k_2 = 0) was attained in the ionic strength (μ) of 0.02 for the catalytic solution after being allowed to stand for 1 day and for that after 8 days, respectively. It is also worthy to note that the enantioselectivity was gradually enhanced as the ionic strength decreased in the range of μ = 0.20-0.02 and was finely inverse to the radii of coaggregates.

Significantly, the effect of ionic strength in this study would lead to a hypothesis that the stereoselectivity might be inverse to the size of

coaggregates in opposition to the case of the composition effect of coaggregates in 0.08 M Tris-KCl buffer.[8] Here, we should pay close attention to the fact that the stereoselectivity was optimized around a kind of fluctuation of membranes or morphology-change which should occur in a specific condition (μ = 0.02)[10] as well as at temperatures and compositions close to phase boundaries and that this might be related to the activation of native enzymes at the boundary between stable and unstable regions.

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(9) The dynamic light-scattering measurements were peformed as described in R. A. Moss, Y. -C. Chang, Y. Hui, J. Am. Chem. Soc., 106, 7506 (1984). The R_h^{app} values have maximum errors of ±6%.
(10) The CD spectra of Z-PheHisLeu between 200 and 240 nm closely resembled those of α-helical peptides as described in ref. 8 and were sharply changed in the region of μ = 0.015-0.020. This result suggests that the optimum conformational change of Z-PheHisLeu for enhancing the stereoselectivity might occur around μ = 0.02 and this should be induced by the change in some physical properties of the coaggregates.

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