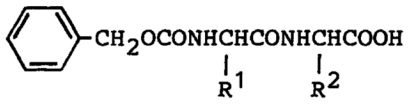
CHEMISTRY LETTERS, pp. 1227-1230, 1985.

ACCELERATED DEACYLATION OF ACYLATED INTERMEDIATE BY HYDROXYL FUNCTIONALIZED SURFACTANT MICELLES

Yasuji IHARA,* Mari OKAMOTO, Mamoru NANGO,† and Joichi KOGA†
Yamaguchi Women's University, 3-2-1 Sakurabatake, Yamaguchi 753
†Department of Applied Chemistry, University of Osaka Prefecture, Sakai, Osaka 591

The rate constants of both acylation and deacylation processes in the hydrolysis of p-nitrophenyl esters by dipeptide catalysts have been determined in the presence of hydroxyl functionalized surfactant micelles. The functional surfactants enhance the deacylation rate constants and the large rate differences are observed between the dipeptide catalysts which have the reverse sequence of amino acid residues.

The high catalytic action of α-chymotrypsin and related enzymes arises from the rapid formation and decay of the acyl enzyme intermediate.[1] The catalytic hydrolysis of p-nitrophenyl esters by micelles derived from functional surfactants normally occurs by a nucleophilic mechanism with subsequent decomposition of an acylated micellar intermediate.[2-4] We have previously[5] demonstrated the large rate enhancements in deacylation process during the hydrolysis of p-nitrophenyl esters by N-acylhistidine in the presence of functional surfactant micelles. This communication describes a large deacylation effect in the hydrolysis of p-nitrophenyl acetate (PNPA), hexanoate (PNPH), and dodecanoate (PNPL) by dipeptide catalysts (I) in the presence of hydroxyl functionalized surfactant (II) micelles at pH 7.30 (0.02 M phosphate buffer) and 25 °C. Both the histidyl and leucyl groups are involved in the catalysts and it should be interesting to compare the catalytic differences between the dipeptides which have the reverse sequence of amino acid residues since the specific catalysis of the enzymes has been shown to depend on the intramolecular interaction of side chain functional groups.

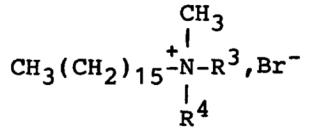


(I)

Z-Leu-His(Ia), $R^1=CH_2CH(CH_3)_2$; $R^2=CH_2$-Im

Z-His-Leu(Ib), $R^1=CH_2$-Im; $R^2=CH_2CH(CH_3)_2$

Im=4-imidazolyl



(II)

CTAB(IIa), $R^3=R^4=CH_3$

CTAOH(IIb), $R^3=CH_3$; $R^4=(CH_2)_2OH$

$CTAOH_2$(IIc), $R^3=R^4=(CH_2)_2OH$

The catalytic process for hydrolysis of p-nitrophenyl esters by II can be described by Eq. 1, where C_{Im} designates the dipeptide catalyst, AcONp is the ester, Ac-C_{Im} is the acylated intermediate, and k_a and k_d represent the rate constants for acylation and deacylation process, respectively.

$$C_{Im} + AcONp \xrightarrow{k_a} \underset{+}{\text{Ac-}C_{Im}} \text{ (p-nitrophenol)} \xrightarrow{k_d} C_{Im} + AcOH \qquad (1)$$

The kinetics were studied under single turnover conditions, [surfactant] > [catalyst] > [ester], at pH 7.30 (0.02 M phosphate buffer) and 25 °C. The acylation rate constant (k_a) was determined by monitoring the release of p-nitrophenol at 410 nm. The deacylation rate constants (k_d) were directly measured spectrophotometrically by following slow decrease in absorption at 245 nm in the case of $k_a >> k_d$. In the case of $k_a < k_d$, we independently prepared an acyl intermediate in the presence of CTAB[6] and this intermediate was then quickly injected into a buffered micellar solution to measure the deacylation rate constants (at 245 nm). In both cases, the kinetics were first order and good least-squares rate constants were obtained ($r > 0.999$). We also carried out some experiments under burst conditions, [surfactant] > [ester] > [catalyst]. In the presence of CTAOH or $CTAOH_2$, however, the burst kinetics could not be observed, probably due to faster deacylation.

Table 1 summarizes the rate constants for acylation and deacylation of p-nitrophenyl esters by two dipeptide catalysts in the presence of surfactant micelles. For the acylation process, the catalysis by Ia was slightly stronger than that by Ib in all esters used, although both catalysts tend to decrease the rate with increasing the hydrophobicity of the side chains of the esters. These differences in reactivity must be due to the basicities and the steric hindrance in the reaction of imidazolyl group in the catalysts. In the presence of CTAB, Ib is

Table 1. Rate Constants for the Hydrolysis of p-Nitrophenyl Esters in the Presence of Surfactant Micelles[a)]

Catalyst	Ester	$10^3 k_a/s^{-1}$			$10^3 k_d/s^{-1}$			Rel. k_d	
		CTAB	CTAOH	$CTAOH_2$	CTAB	CTAOH	$CTAOH_2$	CTAOH/CTAB	$CTAOH_2$/CTAB
Z-Leu-His (Ia)	PNPA	3.59	3.10	2.54	0.743	16.9	21.5	22.7	28.9
	PNPH	3.20	3.11	2.65	0.231	4.23	10.4	18.3	45.0
	PNPL	2.88	2.69	2.02	0.193	4.48	11.7	23.2	60.6
Z-His-Leu (Ib)	PNPA	3.12	2.95	1.89	0.960	22.6	62.6	23.5	65.2
	PNPH	2.18	2.22	1.68	0.519	7.09	16.8	13.7	32.4
	PNPL	1.82	1.70	1.31	0.490	7.70	16.7	15.7	34.1

a) At pH 7.30, 0.02 M phosphate buffer, and 25 °C; [catalyst] = 1.00×10^{-3} M; [surfactant] = 1.00×10^{-2} M; [ester] = 1.0×10^{-4} M. All of the amino acids in the catalyst are of the L form. From three or more independent experiments, we estimate that rate constants are reproducible within ±4%.

uniformly more reactive than Ia in deacylation process, in contrast with their reactivities in the acylation process. The deacylation rate constants of Ia and Ib are about 4.8 and 3.3 (PNPA), 13.9 and 4.2 (PNPH), and 14.9 and 3.7 (PNPL) times smaller than the corresponding acylation rate constants. Thus, the k_a/k_d values of Ia increase with increasing alkyl side chains of the esters, while those of Ib are relatively small and are almost independent of the esters used. These results indicate that the deacylation process is sensitive to the structures of the catalysts and esters. This probably reflects the steric environment of the acylated intermediate in micelles.

The hydroxyl functionalized surfactants (CTAOH, $CTAOH_2$) enhance the deacylation rates constants although the acylation rate constants are almost independent of the surfactants used. These large deacylation enhancements can be ascribed to intermolecular acyl transfer from the catalyst imidazole to the surfactant hydroxyl groups. This observation has been made in several laboratories.[2-4)] The most interesting result in this experiment is the large differences in deacylation rate between the catalysts Ia and Ib in the presence of $CTAOH_2$. In the case of catalyst Ia, the largest deacylation rate acceleration , based on CTAB, was observed from the acyl intermediate of PNPL which has the longest acyl chain among the esters

used (The deacylation is 60.6 times faster than that in CTAB). On the other hand, in the case of Ib, the largest deacylation rate acceleration was observed from the intermediate of PNPA which has the shortest acyl chain among them. These results thus suggest 1) that the deacylation involves geometrical interactions between $CTAOH_2$ and the acyl intermediates from the dipeptide catalysts which have reverse sequence of the amino acid residue, and 2) that the acyl intermediates of N-dodecanoyl-Ia and N-acetyl-Ib are then optimally positioned for attack by the hydroxyl function of $CTAOH_2$ in micellar phase, resulting in the largest acceleration of deacylation rate (Table 1).

In conclusion, this study demonstrates the large rate differences in deacylation process between the catalysis by Ia and Ib in the presence of functional surfactants. This catalytic action should be of considerable interest in connection with studies on enzyme reaction since the combination of hydrophobic side chains and histidyl group plays an important role in the catalysis.

References

1) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Benjamin, New York (1966), Vol. 1, and references cited therein.

2) C. Gitler and A. Ochoa-Salano, J. Am. Chem. Soc., 90, 5004 (1968); R. A. Moss, R. C. Nahas, and S. Ramaswami, ibid., 99, 627 (1977); "Micellization, Solubilization, and Microemulsions," ed by K. L. Mittal, Plenum Press, New York (1977), p. 603; U. Tonellato, J. Chem. Soc., Perkin Trans. 2, 1977, 821.

3) J. Sunamoto, H. Okamoto, H. Kondo, and Y. Murakami, Tetrahedron Lett., 1975, 2761; T. Kunitake, Y. Okahata, and T. Sakamoto, Chem. Lett., 1975, 459 ; J. Am. Chem. Soc., 98, 7799 (1976).

4) W. Tagaki, S. Kobayashi, and D. Fukushima, J. Chem. Soc., Chem. Commun., 1977, 29; W. Tagaki, D. Fukushima, T. Eiki, and Y. Yano, J. Org. Chem., 44, 555 (1979).

5) Y. Ihara, M. Nango, Y. Kimura, and N. Kuroki, J. Am. Chem. Soc., 105, 1252 (1983).

6) Conditions: pH 7.3 (0.02 M phosphate buffer), 25 °C, 17% v/v CH_3CN, [catalyst] = 5.0×10^{-3} M, [CTAB] = 1.0×10^{-2} M, [ester] = 2.0×10^{-3} M.

(Received May 29, 1985)