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STEREOSELECTIVE PREFERENCE OF BOVINE SERUM ALBUMIN IN THE BURST PHASE OF REACTION WITH AMINO ACID p-NITROPHENYL ESTERS

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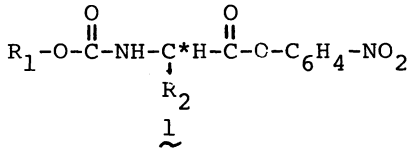
Bovine serum albumin exhibits remarkable acylation efficiency and stereoselectivity in the hydrolysis of optically active amino acid p-nitrophenyl esters. A stereoselective ratio, k_D/k_L = 66 has been observed with added Triton-x. The hydrolysis rate and stereoselectivity depend on the structure of the substrate and of added surfactants.

In the cleavage of the appropriate substrates, proteolytic enzymes exhibit high catalytic efficiency and stereoselectivity. Bovine serum albumin(BSA) or human serum albumin(HSA), normally not classified as a proteolytic enzyme, also has hydrolytic[1)] and stereospecific activity.[1c,1d)] However, those activities of BSA or HSA are poor when compared with those of a proteolytic enzyme,[1)] where the acylation step is faster than the spontaneous hydrolysis of the substrate but the deacylation step of the acylated albumin is slower than the hydrolysis of the substrate.[1e)]

This article describes the results of the stereoselective hydrolyses of optically active amino acid p-nitrophenyl esters by BSA with and without added surfactants and reveals that BSA exhibits remarkable acylation activity and stereoselectivity, comparable with those of proteolytic enzyme, where the acylation rate and stereoselectivity are affected by added surfactants. This study provides some insights into characteristic enzyme-like behavior of BSA.

The hydrolysis of amino acid p-nitrophenyl esters(1) was followed spectrophotometrically by measuring the increasing absorbance of p-nitrophenolate ion at 400 nm.[2)] For fast hydrolyses, the stopped-flow technique was used.[3)] In all experiments the conditions were [protein] > [substrate], 0.01 M Bis-tris buffer, pH 7.3, 25 °C.



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1a: CBZ-Ala(R_1 = $PhCH_2$, R_2 = CH_3), 1b: CBZ-Phe(R_1 = $PhCH_2$,$PhCH_2$),
1c: BOC-Ala(R_1 = $(CH_3)_3C$, R_2 = CH_3), 1d: BOC-Leu(R_1 = $(CH_3)_3C$, R_2 = $(CH_3)_2CHCH_2$),
1e: BOC-Phe(R_1 = $(CH_3)_3$, R_2 = $PhCH_2$), 1f: MOC-Phe(R_1 = CH_3, R_2 = $PhCH_2$)

Pesudo-first-order rate constants(k_{obsd}) for the hydrolysis of D- and L-amino acid p-nitrophenyl esters by BSA and α-chymotrypsin are listed in Table 1. As is apparent in Table 1, a pronounced stereoselectivity in the hydrolysis for D- and L-esters(1) in the presence of BSA and α-chymotripsin was observed. The hydrolysis rate and stereoselectivity of CBZ-Ala(1a) by BSA correspond approximately to those of CBZ-Ala(1a) by α-chymotrypsin, although BSA hydrolyzes the D-esters preferencially and α-chymotrypsin hydrolyzes the L-esters preferencially. Interestingly, the highest rate for the hydrolysis of the esters by BSA is observed for D-CBZ-Ala where the rate is enhanced by about 6×10^4 fold when compared with the observed hydrolysis rate in the presence of BSA. Furthermore, the highest stereoselective ratio(D/L = 34) is observed for the hydrolysis of BOC-Ala(1c). The variation in the hydrolysis rate and stereoselective ratio among the esters is fairly large, indicating that the hydrolysis rate and stereoselectivity are affected by the specific interaction of BSA and the amino acid side chains of the substrate. Nevertheless, it is apparent that BSA prefers the D-enantiomer of the substrates in all cases.

More extensive kinetic measurements were also made with D- and L-CBZ-Ala (1a). Pseudo-first-order rate constants for the hydrolysis of the esters, as a function of concentration of BSA under condition of excess of protein were analyzed by an equation similar to that used in enzymatic catalyses as described in Eqs. 1 and 2:[3,4]

$$S + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S\,C \xrightarrow{k_2} \text{p-nitrophenol} + \text{Acyl-albumin} \qquad (1)$$

$$K_M = (k_2 + k_{-1})/k_1 \qquad (2)$$

where C represents one catalytic site on the protein, S is the substrate, and k_1, k_{-1}, and k_2 are the respective rate constants. For certain sets of the experiments, the initial concentration of catalytic sites, C_0 was kept in great excess over that of substrate, S_0. Under these conditions, $C_0 \gg S_0$, the steady-state rate expression for the mechanism of Eq. 1 becomes

$$k_{obsd} = k_2 C_0/(K_M + C_0) \qquad (3)$$

Values of these constants are assembled in Table 2. As is apparent in Table 2, BSA distingushes between D- and L-structures for the CBZ-Ala(1a) both with respect to k_2 and K_M, and the stereoselective ratio is significantly different for k_2 compared to that for K_M. It is likely, therefore, that the kinetic activation step, represented by k_2, is crucial for the effect on the hydrolysis rate and stereoselective ratio for the hydrolysis of the ester by BSA, indicating that hydrolysis rate and stereoselective control are mainly determined by acyl transfer to the catalytic functional groups at the active site of BSA.[1d]

Pseudo-first-order rate constants for the hydrolysis of the esters of CBZ-Ala(1a) and BOC-Ala(1c) by BSA in the presence of various surfactants are listed in Table 3. The corrected rate constant, k_{corr} may be extracted from the observed ones(k_{obsd}) after removal of the contribution of the surfactant, $k_{obsd\ su}$, obtained from a separate measurement with surfactant alone:

$$k_{corr} = k_{obsd} - k_{obsd\ su} \qquad (4)$$

As is apparent in Table 3, the variation in the hydrolysis rate and stereoselect-

Table 1. Pseudo-First-Order Rate Constant(k_{obsd}) of Hydrolysis of D- and L-Amino Acid p-Nitrophenyl Esters(1) by Bovine Serum Albumin(BSA) and α-Chymotrypsin(Chy)[a]

Protein	Substrate	D-Ester k_{obsd}/s^{-1}	(k_{rel})[b]	L-Ester k_{obsd}/s^{-1}	(k_{rel})	$k_{obsd}(D)/k_{obsd}(L)$
BSA	CBZ-Ala(1a)	2.11	(703)	0.795	(398)	2.7
	CBZ-Phe(1b)	0.12	(40)	0.039	(19.5)	3.1
	BOC-Ala(1c)	1.91	(663)	0.056	(28)	34
	BOC-Leu(1d)	0.032	(10)	0.014	(7)	2.3
	BOC-Phe(1e)	0.010	(3.3)	7.00×10^{-3}	(3.5)	1.4
	MOC-Phe(1f)	3.00×10^{-3}	(1)	2.00×10^{-3}	(1)	1.5
Chy	CBZ-Ala(1a)	0.482		2.57		0.19[c]
None	CBZ-Ala(1a)	3.30×10^{-5}				

a) Reaction condition: concentration of BSA = 4.5×10^{-5} M, concentration of Chy = 5×10^{-5} M, concentration of substrate = 2×10^{-5} M, pH 7.3, 0.01 M Bis-tris buffer, 25 °C.

b) $k_{rel} = k_{obsd}(1a\text{-}1e)/k_{obsd}(1f)$ for BSA.

c) $k_{obsd}(L)/k_{obsd}(D) = 5.3$.

Table 2. Kinetic Parameters for Hydrolysis of D- and L-CBZ-Ala p-Nitrophenyl Esters(1a) by Bovine Serum Albumin(BSA)[a]

	D-Ester	L-Ester	D/L
k_2/s^{-1}	3.48	0.476	7.3
$K_M/10^{-5}$ M	1.91	1.16	1.6
$k_2/K_M/10^{-5}\ s^{-1}M^{-1}$	1.82	0.406	4.6

a) Reaction condition: pH 7.3, 0.01 M Bis-tris buffer, 25 °C, concentration of substrate = 5×10^{-6} M, concentration of BSA = $0.1\text{-}1\times10^{-4}$ M.

ivity among the surfactants is fairly large, indicating that the hydrolysis rate and stereoselective ratio are affected by specific interactions of BSA-surfactants. The binding behavior of cationic surfactants by BSA corresponds with the effect of these surfactants on the hydrolysis rate(data not shown).[5] Thus, interactions between polymer and surfactants may be crucial for an effect on the hydrolysis rate in polymer-surfactant solutions.[2,5] Interestingly, the highest stereoselective ratio(D/L = 66) is observed for hydrolysis of BOC-Ala(1c) by BSA in the presence of Triton-x. Furthermore, the effect of surfactant for the hydrolysis of the esters by BSA also depends upon the structure of substrate.

It will be of interest to see if enzyme-like activity of BSA is manifested with other substrates and whether it can be affected by alternative local micro-environments.

Table 3. Pseudo-First-Order Rate Constant(k_{corr}) of Hydrolysis of D- and L-Amino Acid p-Nitrophenyl Esters(1) by Bivine Serum Albumin(BSA) in the Presence of Various Surfactants[a)]

Surfactants	k_{corr}/ s^{-1}					
	CBZ-Ala(1a)			BOC-Ala(1c)		
	D-ester	L-ester	D/L	D-ester	L-ester	D/L
None	2.11	0.795	2.7	1.91	0.056	34
SDS[b)]				0.043	6.00×10^{-3}	7.2
CTAB[c)]	4.43	0.840	5.3	1.78	6.00×10^{-2}	28
Triton-x	3.49	0.710	4.9	1.78	0.027	66

a) Reaction condition: pH 7.3, 0.01 M Bis-tris, 25 °C, concentration of BSA = 4.5×10^{-5} M, concentration of substrate = 2×10^{-5} M, concentration of surfactant = 1×10^{-4} M. k_{corr}: see Eq. 4 in text.
b) Sodium dodecylsulfate. c) Cetyltrimethylammonium bromide.

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