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STEREOSELECTIVE HYDROLYSIS OF ENANTIOMERIC AMINO ACID ESTERS CATALYZED BY PENTAAMMINE-L-HISTIDINERUTHENIUM(III) AND ANIONIC SURFACTANTS

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Stereoselective hydrolysis of enantiomeric N-acyl-L(or D)-phenylalanine p-nitrophenyl ester, $H-(CH_2)_{n-1}CONHCH(CH_2Ph)CO_2C_6H_4-NO_2-p$ (n=2, 10, or 16), was carried out with pentaammine-L-histidineruthenium(III) and anionic surfactants. The highest selectivity of 4.2 was obtained for n=10, by using bilayer membranes, di-2-ethylhexyl sodium sulfosuccinate.

Though a few transition-metal histidine complexes have been applied to stereoselective hydrolysis of enantiomeric esters to mimic esterase activity,[1)] histidine complexes prepared in situ were used in such works and the reported selectivities were rather low; the highest value was 3.49.[1)] In this study, the isolable pentaammine-L-histidineruthenium(III), $[Ru(NH_3)_5(L\text{-histidine})]^{3+}Cl_3$ (Ru-his), was used as a catalyst in hydrolysis of enantiomeric N-acyl-L(or D)-phenylalanine p-nitrophenyl ester, $H-(CH_2)_{n-1}CONHCH(CH_2Ph)CO_2C_6H_4-NO_2-p$ (Phe-S_n; n=2, 10, or 16), because the histidine ligand of Ru-his becomes a deprotonated active form at lower pH than the other metal complexes.[3)] The emphasis, and point of departure from previous works, of this work lies in the use of anionic surfactants to mimic the reaction atmosphere of an enzyme and to improve the catalytic activity of Ru-his.

Ru-his and Phe-S_n were prepared according to previous reports.[2,3)] Commercially available sodium dodecyl sulfate (SDS) and di-2-ethylhexyl sodium sulfosuccinate (Aerosol OT) of extra pure grade were used without further purification. The hydrolysis of L- or D-Phe-S_n (5×10^{-5} mol/dm^3) was carried out by Ru-his (1×10^{-3} mol/dm^3) with or without surfactant (SDS=$2\sim8\times10^{-2}$ mol/dm^3; Aerosol OT=$1\sim4\times10^{-3}$ mol/dm^3) in 3 vol% CH_3CN-H_2O solution at 25°C and pH=9.0 (Borax buffer; μ=0.02). The deacylation rate was followed by spectroscopic determination of p-nitrophenolate concentration (400nm). The rate constant per active species of catalyst was obtained from the observed pseudo-first order rate constant after abstracting the contribution of the hydrolysis by OH^-.

In the absence of surfactant, little stereoselectivity, defined by enantiomer rate ratio (k^L_{cat}/k^D_{cat}), was found in both hydrolysis catalyzed by Ru-his and L-histidine (L-His) (see Table). However, the presence of either SDS or Aerosol OT increases the rate constant and stereoselectivity in the Phe-S_{10}/Ru-his reaction system. SDS and Aerosol OT may concentrate the hydrophobic Phe-S_{10} and cationic Ru-his in the Stern layer at the micelle-water interface. This concentration effect should accelerate hydrolysis. The simultaneous improvements of the reaction rate and stereoselectivity suggest that improvement of the selectivity may originate from the incorporation of Phe-S_{10} and the binding of Ru-his to micelles. Two possible factors are considered;

Table Rate constant, k_{cat} ($dm^3/mol \cdot s$), and enantiomer rate ratio, k^L_{cat}/k^D_{cat}, in hydrolysis of Phe-S_n

Sub-strate	Sur-factant	L-His			Ru-his		
		k^L_{cat}	k^D_{cat}	k^L_{cat}/k^D_{cat}	k^L_{cat}	k^D_{cat}	k^L_{cat}/k^D_{cat}
Phe-S_2	none	7.00	6.95	1.0	16.2	14.8	1.1
	SDS a)	0.70	0.81	0.9	14.0	11.9	1.2
Phe-S_{10}	none	0.23	0.22	1.0	2.56	1.76	1.5
	SDS a)	0.06	0.06	1.0	13.2	6.3	2.1
	AOT b)	—	—	—	1.88	0.57	3.3
Phe-S_{16}	SDS a)	—	—	—	1.00	0.72	1.4

a) $[SDS]=2.0\times10^{-2}$ mol/dm^3. b) AOT=Aerosol OT, $[AOT]=2.0\times10^{-3}$ mol/dm^3. $k_{cat}=k_{obsd}/[Ru\text{-}his]$.

the concentration of L-Phe-S_{10} in the Stern layer is larger than that of D-Phe-S_{10} due to Ru-L-his present on the micelle surface. Alternatively, the above-mentioned incorporation and binding results in less flexibility of Phe-S_{10} and Ru-his, leading to a better stereoselective orientation between L-Phe-S_{10} and Ru-L-his. Details are still obscure and further study is necessary. On the other hand, SDS little improved the catalytic activity of Ru-his in the Phe-S_2 hydrolysis(Table). Since Phe-S_2 has too short N-acyl chain(CH_3CO-), the benzyl group of the alanine part is the most hydrophobic in Phe-S_2 and might be incorporated into micelle. The incorporation of the benzyl group would make the ester part too closer to the micelle surface, for the ester part is adjacent to the benzyl group. This situation for Phe-S_2 seems unfavorable for the nucleophilic attack of Ru-his, because the active site of Ru-his, i.e., the negatively charged imidazolyl group, is pushed away from the negatively charged micelle surface. Though Phe-S_{16} has a long N-acyl chain, both rate constant and selectivity are rather low. The longer N-acyl chain than the alkyl chain of SDS may put the ester part of Phe-S_{16} far away from the micelle surface, which decreases the approximating effect, leading to the decreased reaction rate and the decreased stereospecific situation. Thus, Phe-S_{10}, possessing not too short and not too long chain length, is the most appropriate in the examined substrates. In Phe-S_2/L-His and Phe-S_{10}/L-His systems, SDS remarkably retarded the hydrolysis without improvement of selectivity. In these cases, Phe-S_2 and Phe-S_{10} are concentrated into the micelle, but L-His is considered to be in the bulk water phase because of its hydrophilicity. Thus, SDS suppresses the hydrolysis. Finally, the influence of the type and concentration of surfactant was investigated. As shown in Figure, the highest stereoselectivity, 4.2 in Aerosol OT and 2.9 in SDS, was obtained at moderately higher concentration of surfactants than cmc. The selectivity of 4.2 is the highest value reported in the ester hydrolysis catalyzed by transition-metal histidine complexes.$^{1)}$ From these results, the high selectivity is expected to be obtained by using the moderately high concentration of tight membranes, such as Aerosol OT.

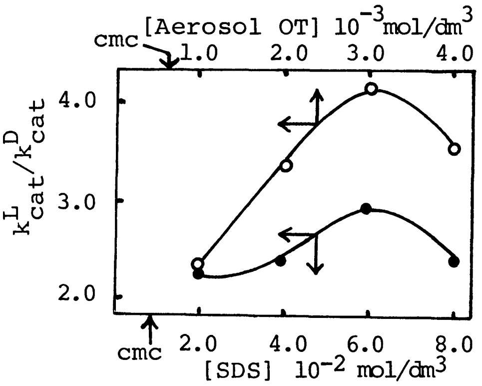

Figure Enantiomer rate ratio vs. surfactant concentration in the Phe-S_{10}/Ru-his reaction system

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