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Stereoselective Dioxygenolysis of a Tryptophan Derivative Catalyzed by a Manganese Porphyrin Bound to Bovine Serum Albumin

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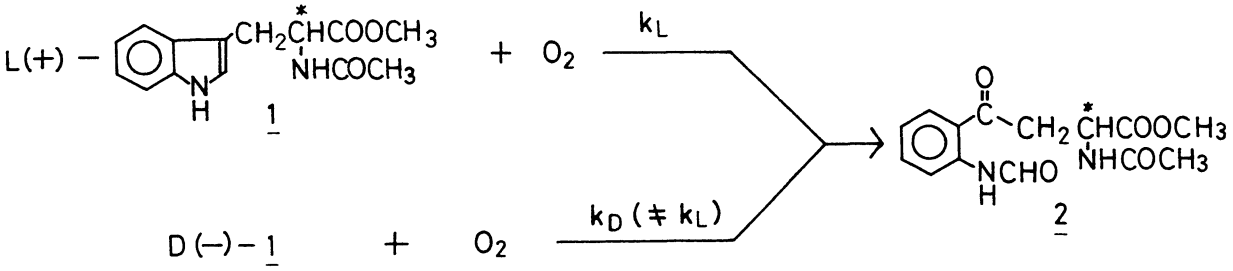
Stereoselective dioxygenolyses of the pyrrole ring in N-acetyl-L(+)- and D(-)-tryptophan methyl esters have been performed with a manganese porphyrin covalently bound to bovine serum albumin; the enantioselective ratio has achieved to 1.63 (k_L/k_D) in 18 vol% THF/H_2O (pH 9.3) under an O_2 atmosphere at 298 K.

Asymmetric mono-oxygenase model reactions have been investigated actively by using chiral transition metal complexes.[1] However, the stereoselective dioxygenase model reaction has only been reported on total dioxygen-inserted ring-opening of N-acetyl-L(+) and D(-)-tryptophan methyl esters (1) with manganese chiral porphyrins as a tryptophan-2,3-dioxygenase (TDO) model by us,[2] though many model studies have been made to understand the reaction process of TDO by using transition metal complexes.[3] On the other hand, metal complexes bound to proteins have been interested in connection with a metalloenzyme model, and enantioselective reactions by these metal complexes are expectable due to a chiral environment of the protein. However, there are only a few reports concerning enantioselective reactions catalyzed by metal complexes bound to proteins.[4] We report here that a newly prepared manganese porphyrin which is covalently bound to a carrier protein of bovine serum albumin (BSA) catalyzes the stereoselective dioxygenolyses of L(+)- and D(-)-tryptophan derivatives as a TDO model reaction.

The manganese porphyrin covalently bound to BSA were prepared in the following manner. Tetra(*p*-carboxyphenyl)porphyrin manganese(III) chloride (Mn^{III}ClTCPP, 2.7 x 10^{-4} mol) was dissolved in DMF (5 cm^3) at room temperature. N-Hydroxysuccinimide (5.4 x 10^{-4} mol) was added to the solution, followed by addition of dicyclohexylcarbodiimide (5.4 x 10^{-4} mol) at 273 K. After keeping the solution at the same temperature for 1 hour, the mixture was kept at room temperature for 1 day with stirring to give Mn^{III}ClTCPP-succinate and N,N'-dicyclohexylurea. Mn^{III}ClTCPP-



succinate was purified by washing with 1,4-dioxane to remove the unreacted N-hydroxysuccinimide. Mn^{III}ClTCPP-succinate (1.0×10^{-5} mol) and BSA (5.0×10^{-6} mol) were dissolved in aqueous $Na_2B_4O_7$ buffer (0.05 mol dm^{-3}, 20 cm^3) and stirred for 4 days at room temperature. The product, Mn^{III}ClTCPP-BSA, was purified by gel filtration with Sephadex G-50 and successive ultrafiltration with borax buffer solution (0.05 mol dm^{-3} $Na_2B_4O_7$ and 0.025 mol dm^{-3} NaCl) to remove the unbound Mn^{III}ClTCPP and the materials having molecular weights below 50 000, respectively. It was identified by electronic spectroscopy that equimolar Mn^{III}ClTCPP was bound to BSA and the aqueous solution of Mn^{III}ClTCPP showed spectral changes of the absorption decrease at 564 and 598 nm with newly appeared absorption bands at 572 and 608 nm (red-shift of Q maxima) by binding to BSA. The CD spectrum of BSA is known to appear at 208 and 220 nm as a maximal and shoulder peak, respectively. Conformational changes of BSA due to binding of the manganese complex to BSA may not occur, because changes of the CD spectra were not observed during the reaction.

The dioxygenolyses of L(+)- and D(-)-form of 1 (2.5×10^{-2} mol dm^{-3}) by Mn^{III}ClTCPP-BSA (2.5×10^{-4} mol dm^{-3}) under an O_2 atmosphere were carried out at room temperature in a mixture of aqueous $Na_2B_4O_7$ buffer (pH 9.3) and THF for 5 hours. The product 2 was isolated and identified by TLC.[2] The reaction was followed by monitoring the decrease of 1 with HPLC. The present reactions obeyed the pseudo-first-order kinetics as shown in Fig. 1, and the rate constants obtained were 1.37×10^{-5} s^{-1} and 8.35×10^{-6} s^{-1} for L(+)- and D(-)-form, respectively, in which the enantioselective rate ratio attained to 1.63. Turnover numbers also achieved to 25 and 14 for L(+)- and D(-)-form of 1, respectively.

The dioxygenolyses of 1 catalyzed by manganese (II or III) porphyrins in the presence of BSA (*i.e.* Mn^{III}ClTCPP/BSA, Mn^{II}TPP/BSA and Mn^{III}ClTPP/BSA; TPP = tetraphenylporphyrin) were performed for comparison, and the results are listed in Table 1 with the result for Mn^{III}ClTCPP-BSA. We have already reported that the manganese complexes in these systems might be included in a hydrophobic region of BSA due to the hydrophobicity of the complexes.[2b] Hydrophobic Mn(II and III)TPP complexes may be included in BSA rapidly compared with the hydrophilic TCPP complex. In

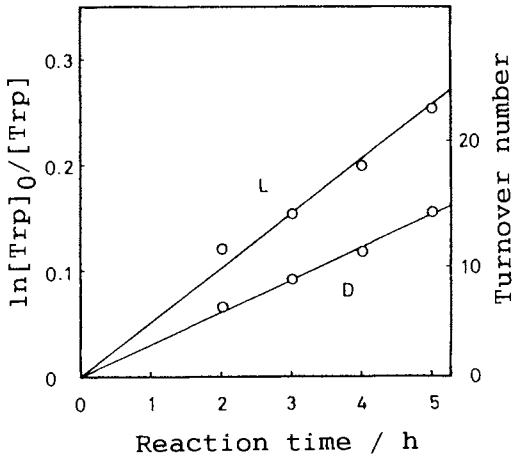


Fig. 1. The pseudo-first-order plots in the dioxygenolyses of L(+)- and D(-)-1 by Mn^{III}ClTCPP bound to BSA.

Table 1. Rate constants (k) and the enantioselective rate ratio (k_L/k_D) for the stereoselective dioxygenolysis of 1[a)]

Catalyst	10^5k / s^{-1}		k_L/k_D
	L	D	
Mn^{III}ClTCPP-BSA	1.37	0.835	1.63
Mn^{III}ClTCPP/BSA[b)]	2.44	1.93	1.13
Mn^{II}TPP/BSA[b)]	1.68	1.44	1.17
Mn^{III}ClTPP/BSA[b)]	1.89	1.64	1.15

a) In 18 vol.% THF of 0.05 mol dm^{-3} $Na_2B_4O_7$ buffer (pH 9.3) and under an O_2 atmosphere at 298 K. b) *In situ* prepared from the Mn complex (2.5×10^{-4} mol dm^{-3}) and BSA (2.5×10^{-4} mol dm^{-3}).

fact, inclusion of Mn^{III}ClTCPP was confirmed to occur very slowly by monitoring time dependent electronic spectra changes and it takes 4 days until completion due to the hydrophilic ligand, though the Mn^{II}TPP and Mn^{III}ClTPP only took about several minutes for inclusion. It was found that the reaction rates and the enantioselectivities of the hydrophobic TPP complexes were slightly slower and higher than those of hydrophilic TCPP complex, respectively, in the *in situ* prepared manganese porphyrin/BSA complexes. This suggests that the enantioselective dioxygenolysis proceeds with the manganese porphyrin included in the hydrophobic region in BSA. It is also noteworthy that the manganese porphyrin bound to BSA, Mn^{III}ClTCPP-BSA, shows larger enantiomer rate ratio than those of the *in situ* prepared manganese porphyrin/BSA complexes, though the reaction rate was slow. The fact suggests that fixation of a metal complex to protein is effective for molecular recognition in a catalytic reaction.

In order to obtain information for the binding position of the manganese porphyrin in BSA, the fluorescence spectra of an aqueous Mn^{III}ClTCPP-BSA solution (pH 9.3) was measured at 298 K. Excitation and emission wavelengths were 295 and 340 nm, respectively. Mn^{III}ClTCPP bound to BSA remarkably quenched the tryptophan (Trp-134 and 212) fluorescence, suggesting that an efficient energy transfer from the excited tryptophan to manganese porphyrin took place. The distance between Trp-134 (or Trp-212) and bound Mn^{III}ClTCPP was estimated to be 2.48 nm from the Förster equation which exhibited the relation between distance and quenching

efficiency.[5] Although the crystal structure of BSA has not been known yet, the position of the manganese porphyrin can be estimated from that of human serum albumin (HSA)[6] which has a very similar structure. Consequently, the obtained distance is consistent with the results that the manganese porphyrin complex is located in the hydrophobic region.

It has been concluded that the BSA hybrid manganese porphyrin, Mn^{III}ClTCPP-BSA, resulted in high enantioselectivity compared with the *in situ* prepared manganese porphyrin/BSA complexes. Michaelis-Menten analyses to investigate the origin of enantioselectivity in the systems are now in progress.

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