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Tryptophan 2,3-dioxygenase-like Activity of Monoclonal Antibody Anchored by a Manganese(III) Porphyrin Complex

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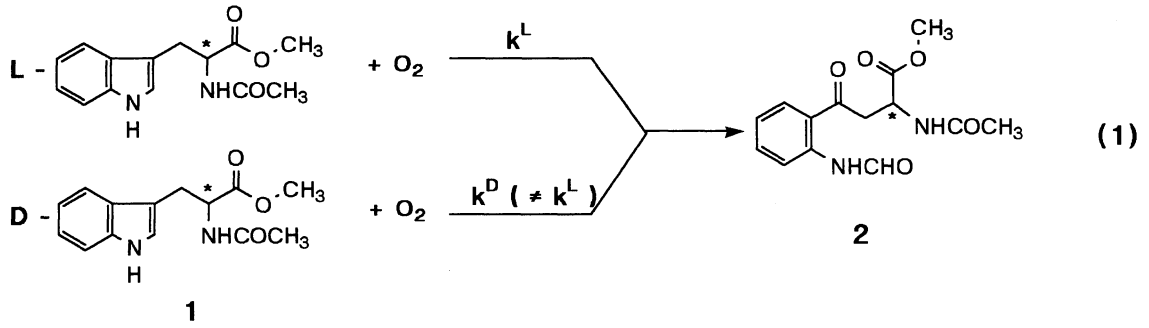
Anchoring of a manganese(III) porphyrin complex to monoclonal (or polyclonal) antibody performed with the *p*-nitrophenylamino moiety of the complex, and this antibody anchored by the metal complex showed efficient catalytic activity and stereoselective ability for tryptophan 2,3-dioxygenase like dioxygenolyses of N-acetyl- L(and/or D)-tryptophan methyl esters in THF/H_2O (pH 8.0) under atmospheric O_2 at 25 °C.

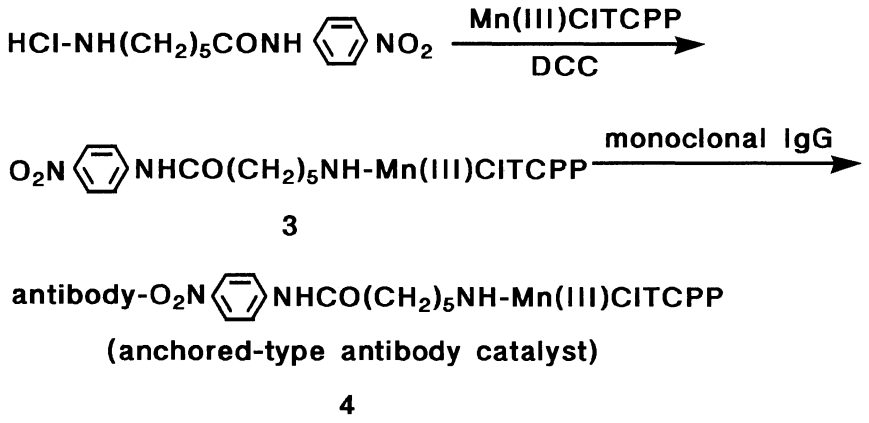
Catalytic nature of antibodies has recently received considerable attention as a semisynthetic enzyme, and their catalytic efficiencies are directly relied on the molecular structures of their haptens. In most cases, haptens have been designed as transition state analogs,[1] active site-substrate complex analogs,[2] or active site analogs.[3] This is the first report on the catalytic and stereoselective ability of monoclonal (or polyclonal) antibodies (IgG) anchored by manganese(III) porphyrin possessing a *p*-nitrophenylamino moiety in tryptophan 2,3-dioxygenase-like dioxygenolyses of N-acetyl- L(and/or D)-tryptophan methyl esters (Eq. 1).



(1)

In regard to the antibodies anchored by manganese(III) porphyrin including the *p*-nitrophenylamino moiety as a binding site toward

antibodies, they were produced by immunizing a mouse with a hapten of adipic acid *p*-nitroanilide which was attached to the carrier protein, bovine serum albumin (BSA). Polyclonal and monoclonal antibodies were obtained and purified in the usual way,[4] and the affinity of these antibodies for the BSA-*p*-nitroanilide conjugate were examined by an enzyme-linked immunosorbent assay (ELIZA). Manganese(III) porphyrin attached by the *p*-nitrophenylamino moiety (**3**) was synthesized as follows (Scheme 1): N,N'-dimethylformamide solution (4 cm^3) containing tetra(*p*-



Scheme 1.

carboxyphenyl)-porphyrin manganese(III) chloride (Mn(III)ClTCPP, 140 mg), 6-aminocaproic acid-*p*-nitroanilide·HCl (51.8 mg), triethylamine (0.042 cm^3), and dicyclohexylcarbodiimide (DCC, 33 mg) was stirred at 0 °C for 2 h and stirred again at 25 °C for 20 h, and the solution was stored in a refrigerater for 2 h. Precipitated dicyclohexylurea was removed by filtration, and the solvent was evaporated to yield crude crystals which were purified by washing several times with 0.6 mol dm^{-3} HCl and H_2O. Anal. Found: C, 60.57; H, 4.53; N, 7.79%. Calcd for $C_{60}H_{51}N_7O_{14}MnCl$; C, 60.84; H, 4.34; N, 8.28%.

In regard to the anchored-type antibody catalyst (**4**) which is conveniently *in situ* prepared by mixing **3** and the monoclonal antibody, the complete inclusion of **3** by the antibody was confirmed from the decrease in the emission (340 nm) intensity of the tryptophan (Trp) residues in the antibody (Trp-35(V_L), 35(V_H), 148(C_L), and 148(C_H1) in one of the F_{ab} region[5]) by the anchoring of the complex **3**; from the change in the fluorescence intensity of the Trp residues in the antibody by the formation of **4** through the energy transfer from the photo-excited Trp residues to **3**, the average distance between the Trp residues and **3** was estimated to be 4.81 nm by the Förster equation.[6] Thus, the evaluated

distance supports that the position of the catalytically active Mn(III)ClTCPP portion in **4** is located inside the F_{ab} resion of the antibody.

The rate of stereoselective dioxygenolyses of L- and D-**1** (1.0×10^{-4} mol dm^{-3}) in the presence of **3** (1.0×10^{-6} mol dm^{-3}) and antibody (1.0×10^{-5} mol dm^{-3} for monoclonal and polyclonal IgG)[7] in 10 vol% THF/H_2O (pH 8.0) under atmospheric O_2 at 25 °C were determined as pseudo-first-order rate constants; the reaction was monitored by the decrease of **1** with HPLC (JASCO Finepack SIL C_{18}, UV 280 nm, and eluent 40 vol% $MeOH/H_2O$ with retention times of 15.9 min for **1** and 10.7 min for **2**). The evaluated rate constants are listed in Table 1. The monoclonal IgG antibody exhibited

Table 1. Stereoselective dioxygenolysis of **1** by the anchored-type antibody (**4**)[a]

Antibody	10^6k / s^{-1}		k^L/k^D
	L	D	
monoclonal IgG	8.88	4.44	1.98
polyclonal IgG	29.2	26.5	1.10
none (Mn(III)ClTCPP)[b]	62.5	62.5	1.00

a) L (or D)-**1** (1.0×10^{-4} mol dm^{-3}), **3** (1.0×10^{-6} mol dm^{-3}), and antibody (1.0×10^{-5} mol dm^{-3}) in 10 vol% THF/H_2O (pH 8.0) under atmospheric O_2 at 25°C. b) Mn(III)ClTCPP (1.0×10^{-6} mol dm^{-3}) in 30 vol% THF/H_2O.

substantial dioxygenolysis activity (turnover number of 14.6 for 5 h in L-**1**), but its catalytic activity was found lower as compared with that of the polyclonal IgG antibody (turnover number 40.9 for 5 h in L-**1**) or Mn(III)ClTCPP *per se* (turnover number 67.5 for 5 h in L-**1**). Since the present monoclonal antibody catalyst was not designed as a receptor of the enantiomeric **1** substrates, the antibody framework did not directly contribute the substrate incorporation so as to prevent the approach of **1** toward the active Mn(III)ClTCPP site from the bulk solution. However, stereoselective ability (defined by enantiomer rate ratio, k^L/k^D) of the monoclonal antibody was higher than that of the polyclonal one which involves the present monoclonal antibody in the extent of *ca.* 10%. In this sense, the polyclonal antibody catalyst is, as a matter of fact,

ineffective for the present stereoselective dioxygenolysis of **1** because it hardly takes the structure of the present anchored-type antibody.

Anyway, the catalytic and stereoselective ability of the anchored-type monoclonal antibodies might be improved by the use of monoclonal antibodies supplied from such a hapten as L-tryptophan-coordinated manganese porphyrin complex, which is now under progress.

References

1) A. Tramontano, K. D. Janda, and R. A. Lerner, *Science,* **234**, 1566 (1986); S. J. Pollack, J. W. Jacobs, and P. G. Schultz, *ibid.,* **234**, 1570 (1986); T. Kitazume, J. T. Lin, T. Yamamoto, and T. Yamazaki, *J. Am. Chem. Soc.,* **113**, 8573 (1991).
2) B. L. Iverson and R. A. Lerner, *Science,* **243**, 1184 (1989).
3) A. G. Cochran and P. G. Schultz, *J. Am. Chem. Soc.,* **112**, 9414 (1990); E. Keinan, S. C. Sinha, A. Sinha-Bagchi, E. Benory, M. C. Ghozi, Z. Eshhar, and B. S. Green, *Pure Appl. Chem.,* **62**, 2013 (1990); A. Harada, K. Okamoto, and M. Kamachi, *Chem. Lett.,* **1991**, 953.
4) G. Kohler and C. Milstein, *Nature,* **256**, 495 (1975); E. Engvall, *Methods Enzymol.,* **70**, 419 (1980).
5) G. M. Edelman, B. A. Cunningham, W. Einar Gall, P. D. Gottlieb, U. Rutishauser, and M. J. Waxdal, *Proc. Natl. Acad. Sci. U. S. A.,* **63**, 78 (1969); R. J. Poljak, L. M. Amzel, H. P. Avey, B. L. Chen, R. P. Phizackerley, and F. Saul, *ibid.,* **70**, 3305 (1973); E. A. Padlan, *Quarterly Reviews of Biophysics,* **10**, 35 (1977).
6) T. Förster, *Ann. Phys. (Leipzig),* **2**, 55 (1948).
7) Protein molarity was determined by absorbance at 280 nm by using $E_{1\ cm}^{0.1\%} = 1.40$ and a molecular weight of 150000 for IgG.

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