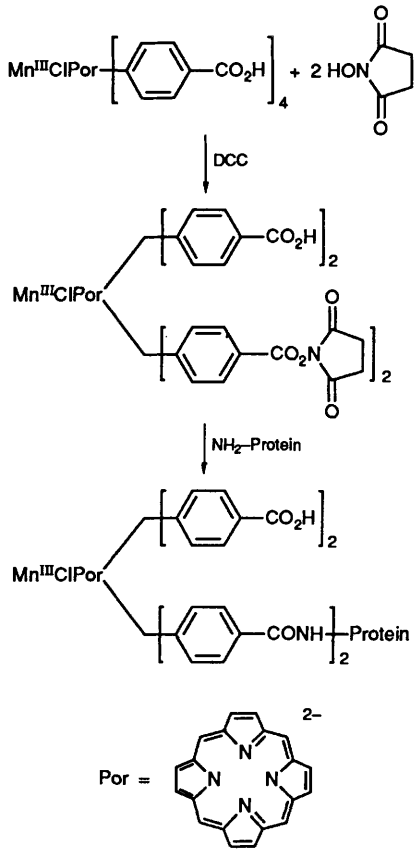
Enzymatic Tryptophan 2,3-Dioxygenase-like Activity of a Manganese Porphyrin Bound to Bovine Serum Albumin Modified with Poly(ethylene glycol)

Takashi Sagawa,[a] Hitoshi Ishida,[a] Kenji Urabe,[a] Kohji Yoshinaga[b] and Katsutoshi Ohkubo*,[a]
[a] *Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kumamoto 860, Japan*
[b] *Department of Applied Chemistry, Faculty of Engineering, Kyushu Institute of Technology, Kitakyushu 804, Japan*

Stereoselective dioxygenolyses of *N*-acetyl-L(+)- and D(−)-tryptophan methyl esters (**1**) have been performed with manganese porphyrins [*meso*-tetrakis(*p*-carboxyphenyl)porphyrinato manganese(III) chloride (Mn^{III}ClTCPP), *meso*-tetrakis(phenyl)porphyrinato manganese(II) (Mn^{II}TPP), and *meso*-tetrakis(phenyl)porphyrinato manganese(III) chloride (Mn^{III}ClTPP)] bound and/or included to a carrier protein, bovine serum albumin (BSA) in THF–H_2O (pH 9.3; $Na_2B_4O_7$ buffer) under an O_2 atmosphere at 25 °C. The maximum stereoselectivity, which is evaluated to be an enantiomer rate ratio 1.63 (k_L/k_D), is observed in the covalently bound system, and the fixation of a manganese porphyrin to BSA *via* a covalent bond is found to be effective for enantiomeric molecular recognition in the stereoselective dioxygenolyses of tryptophan derivatives. The modification of the BSA hybrid catalyst by poly(ethylene glycol) (PEG) has also been performed, and this PEG modification enhances the solubility and stability of the BSA hybrid catalyst in organic–aqueous solvents. The PEG-modified BSA hybrid Mn^{III}ClTCPP retains catalytic activity and stereoselectivity for the stereoselective dioxygenolyses of L(+)- and D(−)-**1** with a higher content of organic solvent in aqueous solution.

Various asymmetric oxygenations of alkenes using chiral metalloporphyrins[1] or optically active Co (or Mn) Schiff base complexes[2] have been well investigated as mono-oxygenase models. However, the stereoselective dioxygenase model reaction has only been reported on total dioxygen-inserted ring-opening of the pyrrole ring in *N*-acetyl-L(+) and D(−)-tryptophan methyl esters with chiral manganese porphyrins as a tryptophan 2,3-dioxygenase (TDO) model by us,[3,4] though many studies have been made to understand the reaction process of TDO by using transition-metal complexes such as Co^{II} Schiff base,[5] Mn^{II} phthalocyanine,[6] Cu^{I}Cl(pyridine),[7] Co^{II}TPP,[8] Fe^{II}TPP(pyridine)$_2$,[9] and Fe^{III}ClTPP[10] complexes. On the other hand, catalytically active metal complexes bound to proteins which have hydrophobic domains capable of entrapping the organic compound have hitherto received considerable attention in connection with metalloenzyme models, and the protein hybrid metal complexes would be expected to catalyse the desired specific reactions which involve substrate recognition by a chiral environment of the protein. There have appeared a few reports concerning enantioselective reactions catalysed by metal complexes binding to proteins; such a synthetic metalloenzyme-like catalyst is extremely limited to the *in situ*-prepared catalytic system of Rh^{I} phosphine complex and avidin (by use of strong coordination affinity between biotin and avidin),[11] Co^{III} pentaammine, Mn^{III} (or Fe^{III}) porphyrin complex and antibody,[12] and of OsO_4 and BSA.[13] We report here tryptophan 2,3-dioxygenase (TDO)-like activity of a BSA-attached manganese porphyrin, which is covalently bound to BSA, in the stereoselective dioxygenolyses of L(+)- and D(−)-**1** and the efficiency of amphiphilic poly(ethylene glycol) (PEG)-modified BSA for catalysing the TDO-like reaction with a high content of organic solvent in an aqueous solution.



Scheme 1

Results and Discussion

Synthesis of Manganese Porphyrin bound to Bovine Serum Albumin (Mn^{III}ClTCPP–BSA).—Introduction of the manganese porphyrin complex to BSA was carried out by the synthetic route in Scheme 1. Coupling of Mn^{III}ClTCPP-succinate and BSA was conducted using dicyclohexylcarbodiimide (DCC) in pH 9.3 borax buffer. After removing the unbound Mn^{III}ClTCPP by filtration, absorption spectroscopy measurement of Mn^{III}ClTCPP–BSA at 277 and 469 nm in pH 9.3 borax buffer showed that the Mn^{III}ClTCPP to BSA binding stoichiometry is 1:1. The aqueous solution of Mn^{III}ClTCPP showed spectral changes of absorption decrease at 564 and 598

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

Catalyst	THF content [% (v/v)]	$k/10^{-5}$ s^{-1} L	D	k_L/k_D
Mn^{III}ClTCPP	30	6.25	—	—
Mn^{III}ClTCPP–BSA	10	2.97	1.96	1.52
	18	1.37	0.835	1.63
Mn^{III}ClTCPP–BSA–PEG	10	2.09	1.42	1.47
Mn^{III}ClTCPP/BSA[b]	18	2.44	1.93	1.13
Mn^{II}TPP/BSA[b]	18	1.68	1.44	1.17
Mn^{III}ClTPP/BSA[b]	18	1.89	1.64	1.15

[a] In 0.05 mol dm^{-3} $Na_2B_4O_7$ buffer (pH 9.3) under an O_2 atmosphere at 25 °C. [b] Prepared *in situ* from the Mn complex (2.5×10^{-4} mol dm^{-3}) and BSA (2.5×10^{-4} mol dm^{-3}).

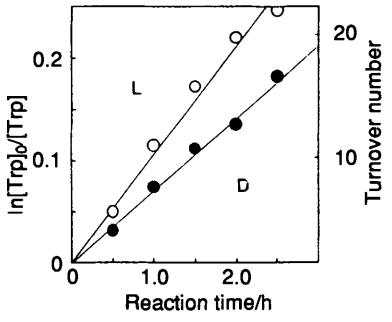


Fig. 1 Pseudo-first-order plots in the dioxygenolyses of L(+)-**1** and D(−)-**1** (2.5×10^{-3} mol dm^{-3}) by Mn^{III}ClTCPP–BSA (2.5×10^{-5} mol dm^{-3}) in 10% (v/v) THF–H_2O (borax buffer, pH 9.3) in an O_2 atmosphere at 25 °C

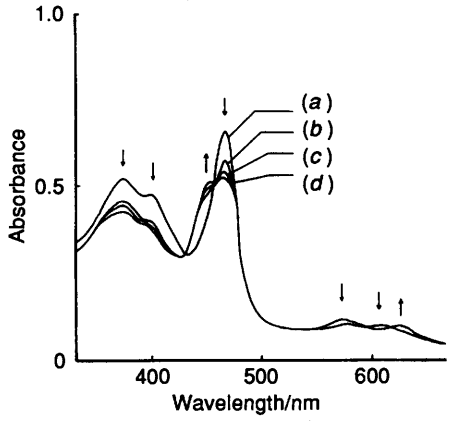
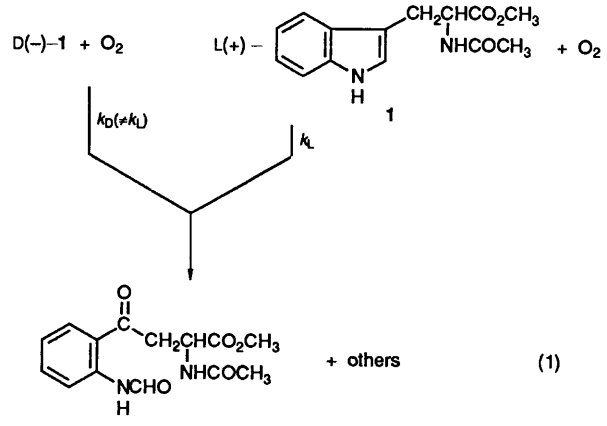


Fig. 2 Absorption spectra change of Mn^{III}ClTPP complex (1.0×10^{-5} mol dm^{-3}) in BSA [(*a*) 1.0×10^{-7}, (*b*) 5.0×10^{-7}, (*c*) 1.0×10^{-6}, (*d*), 5×10^{-6} mol dm^{-3}] in 10% (v/v) THF–H_2O ($Na_2B_4O_7$ buffer: pH 9.3)

nm, with newly appeared absorption bands at 572 and 608 nm (red-shift of Q bands) on binding to BSA. Conformational changes of BSA due to the binding of Mn^{III}ClTCPP to BSA may not occur, because no changes in the CD spectra were observed during the reaction. In order to obtain information about the binding position of Mn^{III}ClTCPP 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 and bound Mn^{III}ClTCPP was estimated to be 2.48 nm from the Forster equation which describes the relation between distance and quenching efficiency.[14] Although the crystal structure of BSA has not yet been determined, the position of the manganese porphyrin can be estimated from that of human serum albumin (HSA)[15] which has a very similar structure. Consequently, the obtained distance is consistent with the result that the manganese porphyrin complex is located in the hydrophobic region.

TDO Activity and Stereoselectivity.—In our previous studies, it was found that the tryptophan dioxygenase model reactions, *i.e.* addition of molecular oxygen to 3-methylindole involving pyrrole ring cleavage, were catalysed by $FeCl_2$ (or $FeCl_3$)/bipyridine/pyridine complexes,[16] M^{II} phthalocyanines (M = Mn, Fe, Co or Cu),[17] and M^{II}TPP or M^{III}ClTPP (M = Mn, Fe or Co).[18] Among these complexes, Mn^{II}TPP and Mn^{III}ClTPP complexes, which show the highest activities for the reaction of 3-methylindole in THF, also exhibited effective activities for the dioxygenolysis of **1**; the reaction proceeded under mild conditions at 25 °C.



The protein hybrid catalyst of Mn^{III}ClTCPP–BSA catalysed the dioxygenolyses of L(+)- and D(−)-**1**, and the reaction obeyed a good pseudo-first-order rate law with the constant enantiomer rate ratio, k_L/k_D (Fig. 1). Then, catalytic stereoselectivity in the reaction could be evaluated from the value of k_L/k_D.

We first examined the catalytic abilities of *in-situ* prepared BSA hybrid complexes with Mn^{III}ClTCPP, Mn^{II}TPP, or Mn^{III}ClTPP in 18% (v/v) THF–H_2O at 25 °C (Table 1). Inclusion of Mn^{III}ClTCPP into BSA took about 4 days, because of the hydrophilic carboxyl groups of the framework. While the incorporation of Mn^{III}ClTPP to BSA in the buffer solution was so fast that changes in the absorption spectrum were observed a few minutes after mixing (Fig. 2). Although *in situ*-prepared catalytic systems (Mn^{III}ClTCPP/BSA, Mn^{II}TPP/BSA, or Mn^{III}ClTPP/BSA) also exhibited higher activities for the dioxygenolyses than that of Mn^{III}ClTCPP–BSA, these catalytic systems exhibited lower stereoselectivity than that of Mn^{III}ClTCPP–BSA, as shown in Table 1. Thus, binding of the Mn^{III}ClTCPP species to BSA *via* a covalent bond works effectively for enantiomeric molecular recognition. The reasons for differences in the catalytic behaviour between Mn^{III}ClTCPP–BSA and the *in-situ* prepared systems are still unclear.

The Michaelis–Menten parameters for the dioxygenolyses of L(+)- and D(−)-**1** by Mn^{III}ClTCPP–BSA were measured under O_2 atmosphere with excess concentration of the substrate (Table 2). When the kinetics of this reaction under O_2 conditions are treated according to the rate expression as shown in eqn. (2), the overall reaction rate ($k_{overall}$) is directly affected

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (2)$$

Table 2 Michaelis–Menten parameters for dioxygenolysis of **1**[a]

1	$k_{overall}$/ dm^3 mol^{-1} s^{-1}	K_m/mol dm^{-3}	k_2/s^{-1}
L	2.53×10^{-2}	8.58×10^{-4}	2.17×10^{-5}
D	1.32×10^{-2}	1.30×10^{-3}	1.72×10^{-5}
(L/D)	(1.91)	(1/1.52)	(1.26)

[a] Mn^{III}ClTCPP–BSA (2.5×10^{-5} mol dm^{-3}) in 10% (v/v) THF–H_2O (borax buffer, pH 9.3) at 25 °C.

Table 3 Correlation between the catalytic activity and stereoselectivity of the Mn^{III}ClTCPP–BSA catalyst and its binding number (n)[a]

	$k/10^{-5}$ s^{-1}			
n	L	D	k_L/k_D	R[b]/nm
1	2.22	1.36	1.63	2.39
2	2.97	1.96	1.52	2.48
4	2.64	2.24	1.18	4.69

[a] The reaction conditions were the same as in Fig. 1. [b] R means the distance between the Mn complex and tryptophan residue in BSA.

by the K_m (Michaelis constant) values, reflecting the extent of the catalyst–substrate complex (Michaelis complex) formation and by the reaction rate of the Michaelis complex with dioxygen.

According to the apparent substrate affinities ($1/K_m$) and k_2 values for the enantiomeric L(+)- and D(−)-**1**, the predominant stereoselection of the L(+)-**1** substrate by Mn^{III}ClTCPP–BSA occurs during both the substrate–catalyst binding step and the reaction step; the predominant reaction of L(+)-**1** rather than D(−)-**1** results from the lower reaction barrier ($\Delta H^{\ddagger} = 38.4$ kJ mol^{-1}, $\Delta S^{\ddagger} = -205.8$ J K^{-1} mol^{-1}) of the former as compared with that ($\Delta H^{\ddagger} = 46.0$ kJ mol^{-1}, $\Delta S^{\ddagger} = -183.2$ J K^{-1} mol^{-1}) of the latter, and on the coordination of L(+)-**1** to the catalytic Mn^{III}ClTCPP site.

The difference in the number of bonds (binding number n) formed between Mn^{III}ClTCPP and BSA also affected the catalytic properties of Mn^{III}ClTCPP–BSA (Table 3). Since the embedding did not change the CD spectra of these BSA hybrid catalysts (n = 1–4) as compared with that of BSA but did change the distance (R) between the manganese complex species and tryptophan residue of BSA of Mn^{III}ClTCPP–BSA (n = 4), the difference in the location of Mn^{III}ClTCPP in BSA (*viz.* that in the R values) must bring about the difference in the catalytic activities and stereoselectivities of Mn^{III}ClTCPP–BSA (n = 1–4). In this respect, the rotational flexibility of the Mn^{III}ClTCPP portion in the BSA hybrid catalyst, which is restricted by the increase of covalent bonds formation (n), might also affect the catalytic properties of these BSA hybrid catalysts (n = 1–4).

PEG Modification Effects on TDO Activity and Stereoselectivity.—In order to increase the solubility and stability of the hybrid Mn^{III}ClTCPP–BSA catalyst, even in organic solvents, catalytically effective Mn^{III}ClTCPP–BSA formed *via* two covalent bonds between Mn^{III}ClTCPP and BSA was then selected for the catalyst modification by PEG. PEG modification of Mn^{III}ClTCPP–BSA lowered the catalytic activity without any serious change of the stereoselectivity (Table 1), probably because PEG bound to BSA prevents the substrate diffusion to the catalytically active site in BSA by depression of the substrate incorporation into BSA. The detailed catalytic behaviour of the PEG-modified Mn^{III}ClTCPP–BSA was examined in THF–H_2O solvent (Fig. 3). PEG modification brought about a slight decline of the activity of the BSA hybrid catalyst with increasing THF content, while it kept the stereoselectivity almost constant without appreciable conformational change of BSA by THF. In contrast, the activity of Mn^{III}ClTCPP–BSA itself increased with increasing THF content, with decreasing stereoselectivity. Therefore, it is obvious that the PEG modification does not enhance the catalytic activity of Mn^{III}ClTCPP–BSA, but protects the BSA hybrid catalyst from the conformational change of BSA by organic solvents such as THF (this will be discussed later from CD and fluorescence spectroscopic results).

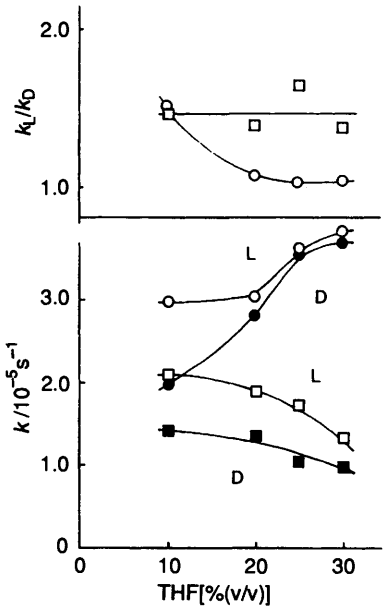


Fig. 3 Effects of THF content in an aqueous solvent on reaction rate constant (k) and stereoselectivity (k_L/k_D) in dioxygenolyses of L(+)-**1** and D(−)-**1** (2.5×10^{-3} mol dm^{-3}) by PEG-modified (□ and ■) and unmodified (○ and ●) Mn^{III}ClTCPP–BSA (2.5×10^{-5} mol dm^{-3}) at 25 °C

The PEG-modification effects obtained for the present dioxygenolyses with PEG-modified and unmodified Mn^{III}ClTCPP–BSA were also reflected by Michaelis–Menten parameters (Tables 2 and 4). Interestingly, in the Mn^{III}ClTCPP–BSA–PEG-catalysed reaction in 30% (v/v) THF–H_2O, the value of the Michaelis constant, K_m(L), for the L(+)-substrate, was higher than the K_m(D) value for the D(−)-substrate, though the K_m(D) value was higher than K_m(L) in the Mn^{III}ClTCPP–BSA-catalysed reaction in 10% (v/v) THF–H_2O. The change of the favoured conformation of the substrate from L(+)-**1** to D(−)-**1** in the binding step by the use of Mn^{III}ClTCPP–BSA–PEG instead of Mn^{III}ClTCPP–BSA was not a result of the increased THF content in the solvent, but of the PEG attachment to the BSA hybrid catalyst; however, Mn^{III}ClTCPP–BSA–PEG, as well as Mn^{III}ClTCPP–BSA, reacts with the L-substrate more easily than the D-substrate, and hence the preferred stereoselection of the L-form substrate by the PEG-modified catalyst was recognized in the overall reaction (see Table 4) similarly to the case of that by the unmodified one. This is ascribed to the slight conformation change of the BSA portion in the catalyst by the PEG modification, as is indicated by the CD and fluorescence spectroscopic analyses mentioned later.

However, the catalytic activity and stereoselectivity of these PEG-modified or unmodified catalysts appeared in a different fashion by the change of solvent system; the use of CH_3CN instead of THF in the organic–H_2O system enhanced the catalytic activity of Mn^{III}ClTCPP–BSA–PEG with the predominant stereoselection of the D(−)-substrate instead of the L(+)-one (Table 5). It is also notable that the PEG-modified Mn^{III}ClTCPP–BSA afforded a lower k_D/k_L value of 1.18 as compared with $k_D/k_L = 1.59$ by the unmodified catalyst. These phenomena seem to be due to the deformation of BSA in such a

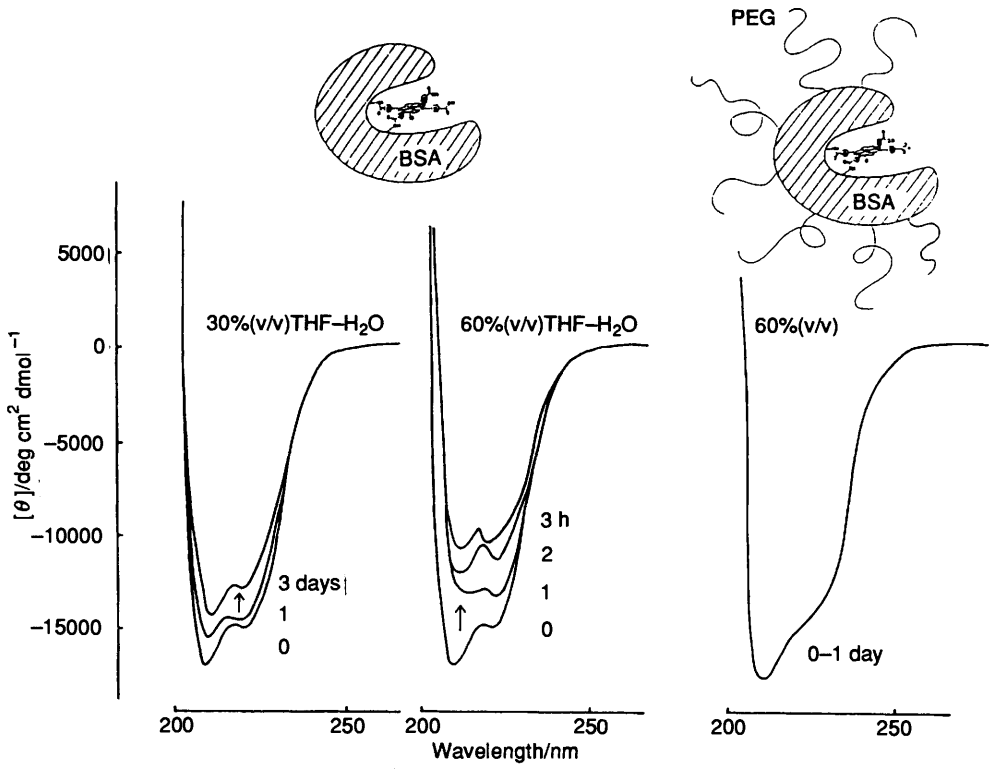


Fig. 4 CD spectra of Mn^{III}ClTCPP–BSA and Mn^{III}ClTCPP–BSA–PEG in THF–H_2O (pH 9.3)

Table 4 Michaelis–Menten parameters for dioxygenolysis of **1**[a]

1	$k_{overall}$/ dm³ mol⁻¹ s⁻¹	K_m/10⁻³ mol dm⁻³	k_2/s⁻¹
L	6.03 × 10⁻³	1.79 × 10⁻²	1.08 × 10⁻⁴
D	4.58 × 10⁻³	1.38 × 10⁻²	0.632 × 10⁻⁴
(D/D)	(1.32)	(1.30)	(1.71)

[a] Mn^{III}ClTCPP–BSA–PEG (2.5 × 10⁻⁵ mol dm⁻³) in 30% (v/v) THF–H_2O (borax buffer, pH 9.3) at 25 °C.

Table 5 Solvent effects on catalytic activities and stereoselectivities of Mn^{III}ClTCPP–BSA and Mn^{III}ClTCPP–BSA–PEG[a]

Solvent[b]	Catalyst	k/10⁻⁵ s⁻¹ L	D	k_L/k_D
THF–H_2O	Mn^{III}ClTCPP–BSA	3.83	3.67	1.04
	Mn^{III}ClTCPP–BSA–PEG	1.34	0.97	1.38
CH_3CN–H_2O	Mn^{III}ClTCPP–BSA	2.49	3.94	0.63
	Mn^{III}ClTCPP–BSA–PEG	2.77	3.24	0.85

[a] The reaction conditions were the same as in Fig. 3. [b] In 30% (v/v) organic–aqueous solvents.

highly polar solvent as CH_3CN, and this was confirmed by the appreciable CD spectral change of the BSA conformation by the use of CH_3CN instead of THF.

PEG Modification Effects on BSA Conformation.—In order to elucidate the effects of PEG attachment to BSA on the catalytic properties of Mn^{III}ClTCPP–BSA in the THF–H_2O solvent system, we have examined the conformational change of BSA in the Mn^{III}ClTCPP–BSA catalyst by the use of organic solvents, and this is reflected by the intensity change in the CD spectra of the BSA hybrid catalyst (Fig. 4). In 30% (v/v) THF–H_2O, the CD spectrum intensity of the PEG-unmodified catalyst decreased from day to day, and the increase of THF

Table 6 Distance (R) between the tryptophan residue and the Mn porphyrin part in the BSA hybrid catalysts[a]

Catalyst	THF–H_2O [% (v/v)]	R/nm
Mn^{III}ClTCPP–BSA	0	2.48
	30	2.63
	60	3.55
Mn^{III}ClTCPP–BSA–PEG	0	2.73
	30	2.76
	60	2.91

[a] Distance were calculated by fluorescence and absorption spectra of BSA (4 × 10⁻⁶ mol dm⁻³) and Mn^{III}ClTCPP (4 × 10⁻⁶ mol dm⁻³) in THF–H_2O (0.05 mol dm⁻³ $Na_2B_4O_7$) at 25 °C.

content to 60% (v/v) resulted in faster decrease (hour by hour) of the CD intensity. However, the CD spectrum of the PEG-modified Mn^{III}ClTCPP–BSA remained unchanged even in 60% (v/v) THF–H_2O. Thus, the PEG modification prevents deformation of BSA and keeps it stable even at a high content of organic solvent in H_2O. It is also noteworthy from Fig. 4 that the PEG modification does not change the CD peak intensity (at 208 nm) of the α-helix structure in the BSA framework of the catalyst, but weakened that (at 214 nm) of the BSA β-sheet in Mn^{III}ClTCPP–BSA–PEG, probably through its partial deformation effect on the secondary β-sheet structure in BSA.

The PEG-modification effects on the conformational change of BSA by organic solvents could also be discussed on the basis of the distance (R) between the Mn^{III}ClTCPP part and the tryptophan residues of BSA in the BSA hybrid catalyst (Table 6). In the case of PEG-unmodified Mn^{III}ClTCPP–BSA, the R value of 2.48 nm in the borax buffer solution was extended to 3.55 nm in 60% (v/v) THF, while the distance (R) in the PEG-modified catalyst was kept constant in less than 30% (v/v) THF and was slightly enlarged in 60% (v/v) THF. These results are consistent with the above observations in the CD spectra. It is also seen from Table 6 that the distance for Mn^{III}ClTCPP–BSA

is slightly longer than that for the PEG–modified BSA complex. The PEG modification thus gives rise to a slight change of the BSA conformation (β-sheet), as already discussed.

PEG modification of the BSA hybrid catalyst protects the stereoselective ability of the Mn^{III}ClTCPP–BSA catalyst from the drastic conformational change of the BSA part induced by organic solvents, but it results in an appreciable lowering of the catalytic activity through the steric hindrance of the PEG moiety bound to the catalyst against the approach of the substrate to the active Mn^{III}ClTCPP site in the hydrophobic BSA hole.

Experimental

Materials.—Tetrahydrofuran and other solvents were dried and distilled before use. Mn^{III}ClTCPP was prepared and purified by a literature method.[19] BSA was obtained from Sigma Chemicals (Fraction V) and purified by gel filtration on a Sephadex G-50 column and successive ultrafiltration (cut-off less than molecular weight of 50 000) with the borax buffer solution (0.05 mol dm^{-3} $Na_2B_4O_7$ and 0.025 mol dm^{-3} NaCl). The substrate **1** was prepared according to the literature.[20] All other chemical reagents used were of reagent grade.

Preparation of Manganese Porphyrin bound to Bovine Serum Albumin (Mn^{III}ClTCPP–BSA).—Introduction of the manganese porphyrin (Mn^{III}ClTCPP) to BSA was carried out by coupling the succinimidyl ester of Mn^{III}ClTCPP (Mn^{III}ClTCPP–succinate) to BSA in pH 9.3 borax buffer. Mn^{III}ClTCPP–succinate was prepared from Mn^{III}ClTCPP (0.34 g, 0.37 mmol) and *N*-hydroxysuccinimide (0.085 g, 0.74 mmol) in DMF (5 cm^3) by using DCC (0.15 g, 0.74 mmol); the reaction mixture was stirred at 0 °C for 1 h, and stirred again at room temperature for 1 day affording Mn^{III}ClTCPP-succinate (0.10 g, 24%) which was then purified by washing with 1,4-dioxane. Mn^{III}ClTCPP-succinate (1.0×10^{-5} mol) and BSA (5.0×10^{-6} mol) were dissolved in 0.05 mol dm^{-3} $Na_2B_4O_7$ buffer (20 cm^3) and allowed to stand for 4 days at room temperature. The product, Mn^{III}ClTCPP–BSA, was obtained by removing the unreacted Mn^{III}ClTCPP by gel filtration chromatography and successive ultrafiltration as described above.

PEG Attachment.—According to the method of Abuchowski *et al.*,[21] the activated monomethoxy-PEG (molecular weight of 5000) of *N*-succinimidyl succinate ester was synthesized *via* esterification with succinic anhydride and then with *N*-hydroxysuccinimide by using DCC. Coupling the PEG to the BSA hybrid Mn porphyrin (Mn^{III}ClTCPP–BSA) was carried out in pH 9.3 borax buffer (0.05 mol dm^{-3}). The extent of PEG modification of amino groups (mainly Lys residue) in the BSA part was 33%.

Physical Measurements.—The amount of Mn^{III}ClTCPP bound to BSA was determined by means of UV spectrophotometric analysis at 277 and 469 nm with a Hitachi 150-20 spectrophotometer. CD spectroscopic measurements for the conformational changes of BSA were performed with a JASCO data processor DP-500 attached to the J-500 spectropolarimeter. The spectral determination of the distance between Trp-134, 212 and the bound manganese complex was carried out by means of fluorescence-energy transfer measurements with a Hitachi F-3010 fluorescence spectrophotometer.

General Procedure for the Dioxygenolyses of Tryptophan Derivatives.—The dioxygenolyses of the tryptophan derivatives, which include no dioxygenated products before reaction, by the manganese porphyrins under atmospheric O_2 were carried out at 25 °C in a mixture of aqueous $Na_2B_4O_7$ buffer and THF for 2.5 h. The ring-opening products were identified with authentic samples by ^1H NMR, IR, MS and UV spectroscopy.[3] The amounts of unreacted substrates (retention time of 15.9 min) and the products (retention time of 10.7 min) were determined by HPLC (JASCO Finepack SIL C_{18}, UV 280 nm) eluted with 40% (v/v) MeOH–H_2O.

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