Apocytochrome b_{562} as a Novel Chiral Host Molecule: The First Enantioselective Reconstitution

Yasuhiro Ishida, Katsuaki Konishi, Takuzo Aida,* and Teruyuki Nagamune

Abstract: Apocytochrome b_{562}, a naturally occurring host molecule with an exceptionally high conformational stability, recognized the structures of synthetic metalloporphyrins in reconstitution. Among manganese porphyrins containing peripheral propionate residues (**2**–**4**), the protoporphyrin IX complex (**2**), which has two proximal propionate functionalities, was preferred over the other manganese complexes by a factor of more than 100 in terms of the association constants K. In reconstitution with a chiral zinc mesoporphyrin II with a 1,4-xylylenediamide strap (**5**), the R enantiomer ((*R*)-**5**) was highly preferred over (*S*)-**5**; the ratio of the association constants K_R/K_S was as large as 30. The apoprotein also recognized the absolute configuration of an *N*,*N*′-dimethylated analogue of **5** (**6**) ($K_R/K_S = 23$), although the association constants were almost one order of magnitude lower than those of **5**. The high enantioselectivity of the apoprotein permitted the perfect separation of racemic **5** in a competitive one-pot reconstitution. In sharp contrast, apomyoglobin showed no enantioselective ability under similar conditions.

Keywords: chirality · heme proteins · molecular recognition · porphyrinoids · reconstitution

Introduction

In biological systems a variety of potential host molecules exist that are quite attractive as motifs for in vitro host–guest chemistry. Apoproteins are candidates for such host molecules, since they possess a well-defined hydrophobic pocket in which multiple coordinating and hydrogen-bonding functionalities are specifically arranged.[1] However, the rather high susceptibility of apoproteins to conformational denaturation, compared to the parent holoproteins, considerably limits their possible applications.[2]

Cytochrome b_{562} from *Escherichia coli* is an electron-transfer hemoprotein with a characteristic four-helix bundled structure, which accommodates protoheme (iron protoporphyrin IX) in a noncovalent fashion by coordination with histidine 102 and methionine 7 on the edge of the bundle (Figure 1 left).[3, 4] Compared with other apoproteins, such as apomyoglobin,[5] apocytochrome b_{562} is highly robust towards conformational denaturation over a wide range of temperature and pH,[6] and has been reported to retain most of the conformational characteristics of the holoprotein (Figure 1 right).[7] Despite such fascinating properties, the chemistry of apocytochrome b_{562} has not yet been explored because of the low biological abundance of its

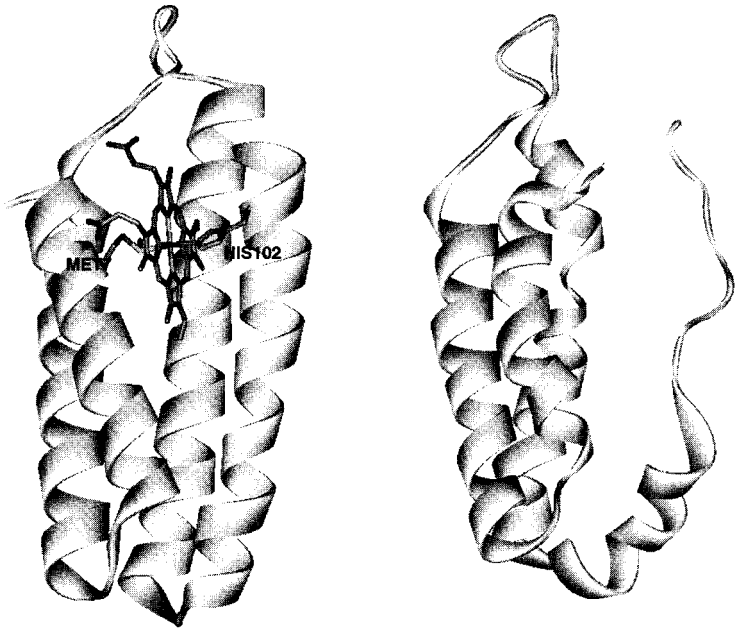


Figure 1. The schematic structure of ferricytochrome b_{562} (left) and apocytochrome b_{562} (right), as determined by X-ray crystallography[4d] and NMR spectroscopy,[7b] respectively.

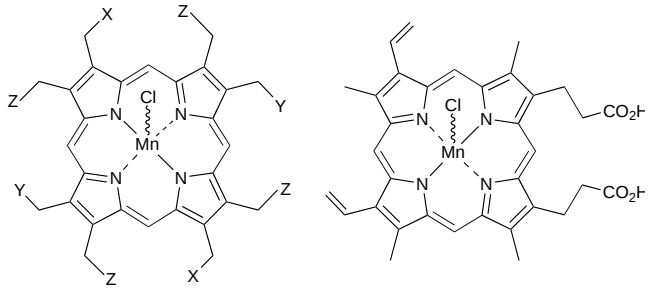
[*] Prof. T. Aida, Y. Ishida, Dr. K. Konishi, Prof. T. Nagamune
Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656 (Japan)
Fax: (+81) 3-5802-3363
E-mail: aida@macro.t.u-tokyo.ac.jp

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1998
0947-6539/98/0407-1148 $ 17.50+.50/0

holoprotein. However, cloning and efficient expression of cytochrome b_{562} have recently been successful,[8] thereby enabling its large-scale preparation and application in materials science.[9] In the present paper, we report the results of the first study on the host–guest chemistry of apocytochrome b_{562} in reconstitution with some synthetic metalloporphyrins (**1**–**6**), and highlight its extremely high ability in chiral discrimination.

Results and Discussion

Recognition of peripheral substituents: To evaluate the ability of apocytochrome b_{562} as a host molecule, reconstitution with manganese(III) porphyrins with different peripheral substituents (**1**–**4**) was examined . Compared with iron(III) porphyrins, manganese(III) porphyrins have a much lower tendency to form μ-oxo dimers and have higher solubilities in aqueous media. Upon titration of a bis-Tris buffer/DMSO (3%) solution of manganese(III) octaethylporphyrin (**1**, 7.0 μM) with an aqueous solution of apocytochrome b_{562}, the UV/Vis spectrum of **1** changes to show an isosbestic point at 467 nm. Plots of the Soret absorbance (477.4 nm) versus $[\text{apoprotein}]_0/[\mathbf{1}]_0$ showed a clear inflection point at a mole ratio of unity (Figure 2a), indicating a stoichiometric interaction of **1** with the apoprotein. The association constant K, as determined from this titration curve, was $2.0 \times 10^7\,\text{M}^{-1}$ (run 1, Table 1). In sharp contrast, no reconstitution occurred with a manganese(III) porphyrin (**4**) which has four propionate functionalities (Figure 2d). On the other hand, in the reconstitution with manganese complexes containing two propionate functionalities (**2** and **3**), apocytochrome b_{562} recognized the topology of the propionates on the porphyrin periphery: The protoporphyrin IX complex (**2**), with two proximal propionate functionalities (Figure 2b), showed a comparable association constant ($K = 2.4 \times 10^7\,\text{M}^{-1}$, run 2) to that of manganese octaethylporphyrin (**1**), while the complex of mesoporphyrin II (**3**) with two diagonal propionates (Figure 2c) showed a much lower affinity ($K = 7.4 \times 10^4\,\text{M}^{-1}$, run 3).



1 X = Y = Z = CH_3
3 X = CH_3, Y = CH_2CO_2H, Z = H
4 X = Y = CH_2CO_2H, Z = H
2

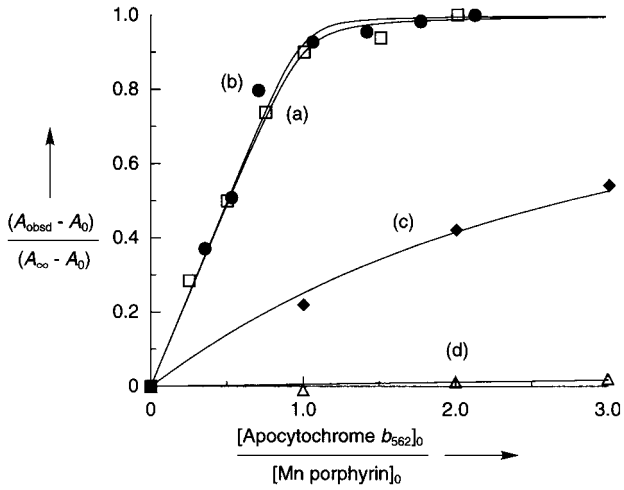


Figure 2. Spectroscopic titration (20 °C) of bis-Tris buffer (50 mM)/DMSO (3%) solutions of manganese(III) porphyrins **1** [□, (a)], **2** [●, (b)], **3** [◆, (c)], and **4** [△, (d)] (7.0 μM each) at pH 6.5 with an aqueous solution of apocytochrome b_{562} monitored at 477.4, 482.4, 476.8, and 477.0 nm, respectively. A_{obsd} represents the observed absorbance, while A_0 and A_∞ denote the absorbances of noncomplexed and complexed manganese porphyrins, respectively.

Table 1. Interactions of metalloporphyrin guests with apocytochrome-b_{562}.[a]

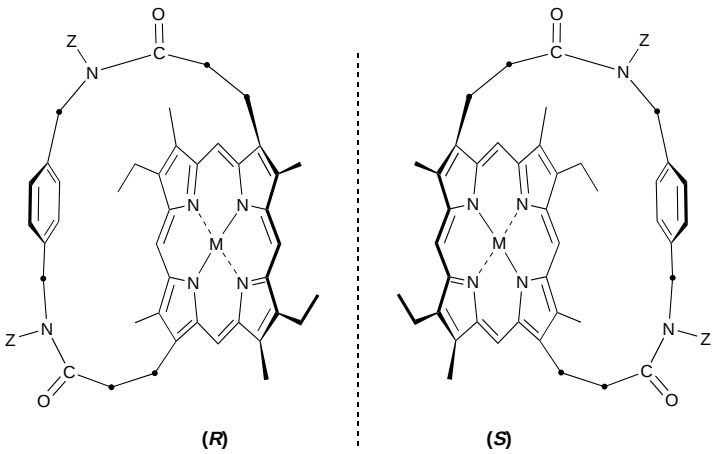
Run	Metalloporphyrin guest	Association constant [K, M^{-1}][b]
1	**1**	2.0×10^7
2	**2**	2.4×10^7
3	**3**	7.4×10^4
4	**4**	≈ 0
5	(*R*)-**5**	4.4×10^6
6	(*S*)-**5**	1.5×10^5
7	(*R*)-**6**	3.4×10^5
8	(*S*)-**6**	1.5×10^4

[a] For experimental conditions, see Figures 2 and 3. [b] Calculated from the titration profiles in Figures 2 and 3 by a nonlinear curve-fitting method.

Recognition of chirality: As described above, apocytochrome b_{562} has a characteristic helix-bundled architecture (Figure 1 right). Quite interestingly, this unique apoprotein showed a great ability for asymmetric recognition in reconstitution with a chiral 1,4-xylylenediamide-strapped metalloporphyrin with molecular asymmetry.[10, 11] In this experiment, the zinc complex (**5**) was used in place of the manganese complex (**5′**), since the spectral change of **5′** upon mixing with the apoprotein was not distinct. Upon addition of the apoprotein

Abstract in Japanese:

4本のヘリックスがバンドルしたシトクロムb_{562}は、コンフォメーションが例外的に安定なアポタンパクを与える。このアポタンパクは、ポルフィリン錯体との再構成反応において、ゲストの構造を高度に認識した。プロピオン酸残基を有する一連のマンガンポルフィリン錯体との再構成では、例えばプロピオン酸残基を隣り合う2つのピロール上に有するものと、対角のピロール上に有するものとで、錯形成定数に100倍以上の差が観察された。また、分子不斉を有するキラルなストラップポルフィリンの亜鉛錯体との再構成においては、両エナンチオマーの錯形成定数に30倍の差が生じた。さらに、両エナンチオマーの競争的な再構成においては、ラセミ体のほぼ完全な光学分割が達成された。これとは対照的に、ヘリックスの規則性が低いアポミオグロビンの再構成では、不斉選択は全く起こらなかった。



5 Z = H, M = Zn
5' Z = H, M = Mn(III)
5" Z = H, M = Cu
6 Z = CH_3, M = Zn

to an acetate buffer solution of (*R*)-**5** (1.5 μM) containing 2% DMSO, there was a distinct shift of the Soret band of (*R*)-**5** from 406.2 to 415.8 nm, and the final spectrum was quite similar to that of **5** coordinated to 1-methylimidazole in an aprotic solvent such as $CHCl_3$.[12] In contrast, use of (*S*)-**5** as the substrate in place of (*R*)-**5** for the reconstitution resulted in a similar but much smaller spectral change: At [apoprotein]$_0$/[**5**]$_0$ of 1.8, nearly 90% of (*R*)-**5** was bound to the apoprotein, whereas (*S*)-**5** was trapped in only 23% under identical conditions (Figures 3a and 3b). The association

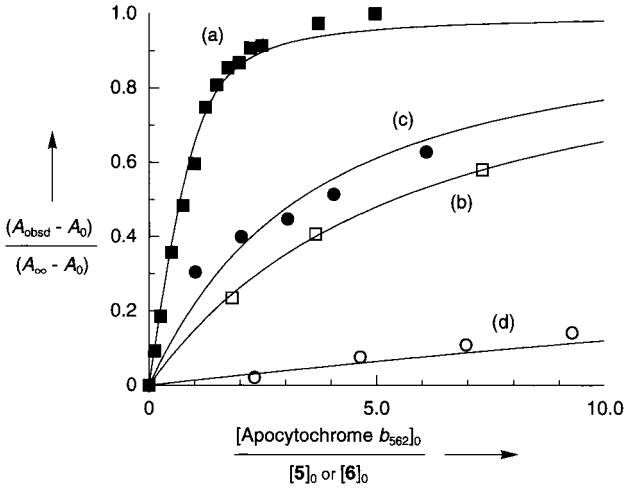


Figure 3. Spectroscopic titration (20 °C) of acetate buffer (200 mM)/DMSO (2%) solutions of (*R*)-**5** [■, (a)], (*S*)-**5** [□, (b)], (*R*)-**6** [●, (c)], and (*S*)-**6** [○, (d)] (1.5 μM) at pH 4.6 with an aqueous solution of apocytochrome b_{562} monitored at 415.8 and 416.0 nm for **5** and **6**, respectively.

constant for (*R*)-**5** (K_R), as evaluated from the spectroscopic titration, of $4.4 \times 10^6\,M^{-1}$ (run 5, Table 1), is approximately 30 times larger than that for (*S*)-**5** ($K_S = 1.5 \times 10^5\,M^{-1}$, run 6). From K_R and K_S, $\Delta\Delta G$ of the enantioselective reconstitution was calculated to be -2.0 kcal mol^{-1}, and the enantiomeric excess (*ee*) at an infinitely large [guest]/[host] mole ratio ($ee_{infinity}$) was estimated to be 93%.[13] Similarly, in reconstitution with **6**, derived from **5** by methylation of the two amide functionalities of the strap, apocytochrome b_{562} also exhibited a high enantioselectivity toward the *R* isomer (Figures 3c and 3d); the $ee_{infinity}$ value was 92%, as estimated from K_R and K_S (runs 7 and 8, Table 1).[13] However, it should be also noted that these association constants were almost one order of magnitude lower than those for **5** (runs 5 and 6).

One-pot optical resolution of chiral metalloporphyrins: The excellent enantioselectivity of apocytochrome b_{562} towards **5** enabled one-pot optical resolution of racemic **5**. Thus, racemic **5** ([**5**]$_0 = 14.4$ μM) was incubated for 0.5 h with 0.5 equivalent of the apoprotein[14] in an acetate buffer at pH 4.6, and the mixture, after centrifugation and dialysis, was treated with $CHCl_3$ to allow complete extraction of **5** into the organic phase (35% yield[15] of isolated product based on the initial amount of the apoprotein). The chiral complex (**5**), thus isolated, displayed clear circular dichroism (CD) bands with virtually the same intensities as those of enantiomerically pure (*R*)-**5** (Figure 4). Even when a substrate (**5**) enriched

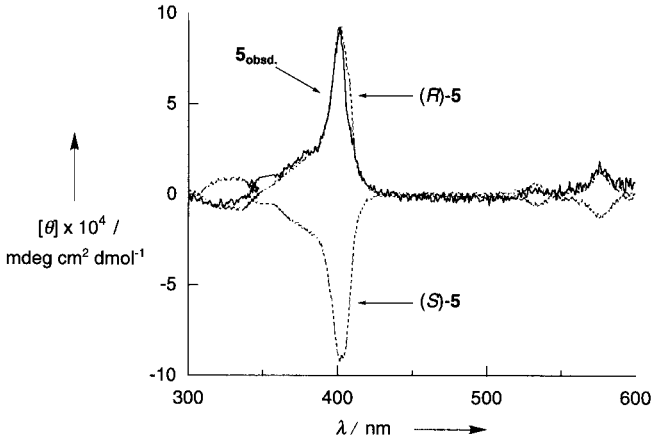


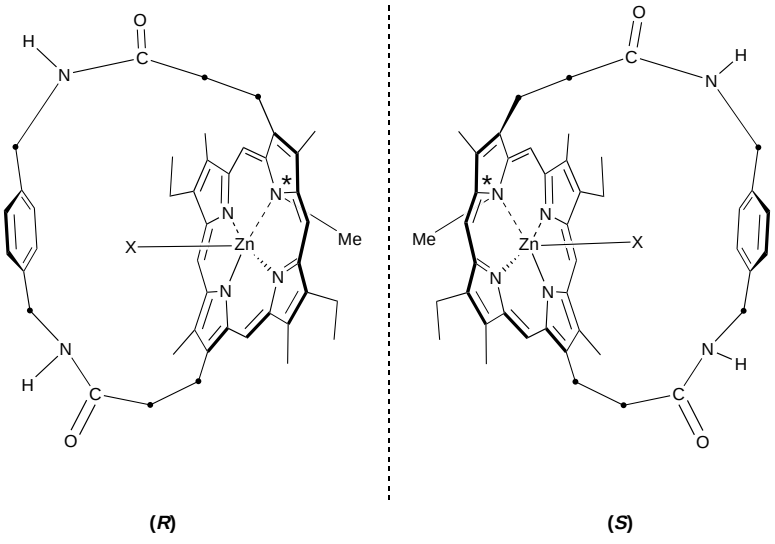
Figure 4. CD spectrum ($CHCl_3$, 20 °C) of **5** extracted from cytochrome b_{562} reconstituted with racemic **5** at a mole ratio [apoprotein]/[**5**] of 0.5 (**5**$_{obsd}$, solid curve), and those of (*R*)- and (*S*)-**5** (dotted curves) as optically pure authentic references. The concentration of **5**, for the calculation of [Θ], was determined from the absorbance at 407.5 nm with $\varepsilon_{407.5} = 2.05 \times 10^5\,cm^{-1}M^{-1}$.

with the unfavorable *S* enantiomer ([(*R*)-**5**]$_0$/[(*S*)-**5**]$_0 = 1/5.4$) was employed for the reconstitution ([**5**]$_0$/[apoprotein]$_0 =$ 16.6/3.5 μM), the selectivity towards (*R*)-**5** still remained high ([(*R*)-**5**]/[(*S*)-**5**] = 71/29). On the other hand, a competitive reconstitution of racemic **6** with the apoprotein, under identical conditions to the above, resulted in a recovery of **6** in only 18% yield. Although the reaction was again enantioselective, the observed *R*/*S* ratio (70:30) was lower than that with **5**. Nevertheless, these two examples demonstrate the high potential of apocytochrome b_{562} in chiral discrimination. In sharp contrast, apomyoglobin, one of the most extensively studied apoproteins, showed no enantioselective capability for **5** under identical conditions: Reconstitution of apomyoglobin with racemic **5** under conditions similar to those with apocytochrome b_{562}, (centrifugation and dialysis, followed by extraction with $CHCl_3$) allowed the isolation of **5** in 32% yield. However, the CD spectrum of **5** thus recovered did not show any sign of optical activity.

© WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1998
0947-6539/98/0407-1150 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 7

Mechanistic aspects: As a control experiment, reconstitution with (*R*)-**5** was attempted for cytochrome b_{562} whose binding site is occupied by the native guest (iron(III) protoporphyrin IX). However, no spectral change associated with the reconstitution with (*R*)-**5** was observed. Furthermore, upon addition of the native guest to the (*R*)-**5**–apoprotein assembly, the (*R*)-**5** bound to the apoprotein was completely liberated, while the holoprotein was formed. From these observations, the binding of **5** to the apoprotein is most likely to occur at the heme pocket. In the reconstitution of apocytochrome b_{562} with **5**, we believe that axial coordination at the zinc atom of **5,** presumably with the imidazole group of a histidine unit in the protein, and a hydrogen-bonding interaction involving the strap-amide moieties[16–18] operate simultaneously in addition to a hydrophobic interaction. In fact, there was no indication of any reconstitution if a copper analogue of **5** without axial coordination ability (**5″**) or zinc octaethylporphyrin without hydrogen-bonding capability was used as the guest. With respect to the hydrogen-bonding profile of **5**, our previous studies have shown that the strap-amide functionalities serve both as proton donor (NH) and acceptor (C=O) in interaction with *Z*-amino acids[16] and synthetic polypeptides.[17] In the reconstitution with apocytochrome b_{562}, the strap amides of **5** are likely to operate, at least, as proton acceptors to form hydrogen bonds with the apoprotein, since the *N*,*N*′-dimethylated analogue (**6**), which is devoid of any proton-donating character, is interactive, though more weakly than **5**, with the apoprotein (runs 7 and 8, Table 1).

As already described, the enantiomers of **5** bound to apocytochrome b_{562} all display virtually identical absorption spectra. However, their CD profiles were found to be essentially different. For example, (*R*)-**5** in the reconstituted cytochrome b_{562}[19] showed a monosignated CD band at 416.5 nm (Figure 5a), similarly to the enantiomers of free **5** in $CHCl_3$ (Figure 4). In sharp contrast, (*S*)-**5** upon reconstitution[19] clearly exhibited split Cotton effects at 419 and 429 nm with a considerable enhancement of the CD intensity (Figure 5b). Such a split CD pattern is quite similar to that of the strapped zinc porphyrin with a methylated core nitrogen atom (**7**) .[16] On the basis of NMR and CPK model studies, it has been suggested that the strap phenylene moiety of **5** adopts a parallel orientation to the porphyrin ring,[10] whereas that of **7** is tilted,[16] thereby allowing intramolecular exciton coupling between these two chromophores. Thus, the marked difference in the CD profile of unfavorable (*S*)-**5** from that of favorable (*R*)-**5** upon reconstitution with apocytochrome b_{562} (Figure 5) suggests a conformational deformation of (*S*)-**5** within the heme pocket, possibly due to a steric requirement from shape mismatching.



7

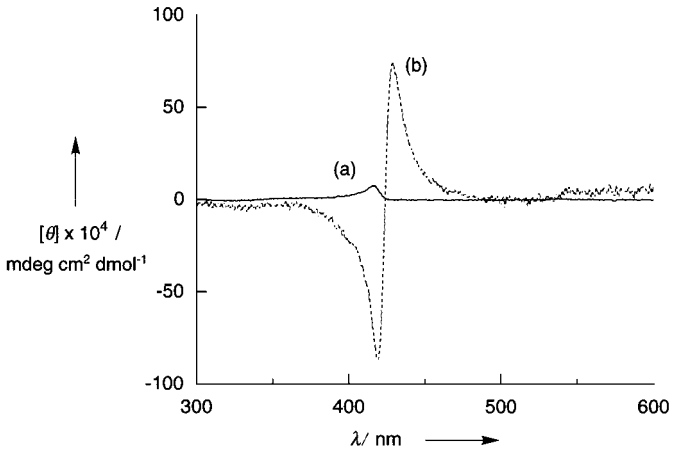


Figure 5. CD spectra (acetate buffer [200 mM], 20 °C) of (*R*)-**5** (a; solid curve) and (*S*)-**5** (b; dotted curves) bound to apocytochrome b_{562}. The concentration of reconstituted cytochrome b_{562}, for the calculation of $[\theta]$, was determined from the absorbance at 415.5 nm with $\varepsilon_{415.5} = 1.94 \times 10^5\ cm^{-1}M^{-1}$.

Conclusion

We have demonstrated that the apoprotein of cytochrome b_{562} is a new chiral host molecule that is capable of recognizing the absolute configurations of chiral metalloporphyrins in reconstitution. The perfect, one-pot separation of the enantiomers of **5** is quite interesting,[2] since it could not be achieved by the use of apomyoglobin, the most extensively studied apoprotein for reconstitution. Considering the potential of apoproteins to trap small molecules,[20] the utilization of this unique helix-bundled apoprotein as chiral host and catalyst is worthy of further investigation.

Experimental Section

Materials: Cytochrome b_{562} was obtained from *Escherichia coli* (TB-1 harboring pNS 207) and purified as reported previously.[8] The chloromanganese complex of protoporphyrin IX (**2**) was purchased from Sigma. Octaethylporphyrin, mesoporphyrin II dimethyl ester, and coproporphyrin

tetramethyl ester were prepared according to the literature.[21] Racemic mesoporphyrin II with a 1,4-xylylenediamide strap was prepared by a procedure reported previously.[16] THF was distilled over sodium benzophenone ketyl prior to use. All other chemicals and reagents were used as received.

Measurements: Absorption (UV/Vis) and CD spectra were recorded on a JASCO V-560 spectrometer and a JASCO J-720 spectropolarimeter, respectively. ^{1}H NMR spectra were recorded on a JEOL GSX-270 spectrometer operating at 270 MHz. FAB-MS spectra were recorded on a JEOL JMS-HX110 spectrometer in a 3-nitrobenzyl alcohol matrix. HPLC experiments were performed at room temperature on a JASCO 880-PU equipped with a JASCO 875-UV variable wavelength detector at a flow rate of 0.5 mL min^{-1} and monitored at 400 nm.

Preparation of apocytochrome b_{562}:[22] 1.0 N Hydrochloric acid was added at 0 °C to an aqueous solution of cytochrome b_{562} until the pH of the solution reached 2.0. An equal volume of 2-butanone chilled at 0 °C was added to this reaction mixture to extract protoheme until the butanone phase showed no UV/Vis absorptions due to the heme. The aqueous phase was then separated and subjected to repeated dialysis against water, followed by centrifugation at 0 °C for 10 min (18000 g), to give an aqueous solution of apocytochrome b_{562}, the concentration of which was determined on the basis of the absorbance at 276 nm[6] or by titration with manganese octaethylporphyrin (**1**).

Manganese(III) porphyrins (**1**, **3**, and **4**): A free-base porphyrin (octaethylporphyrin, mesoporphyrin II dimethyl ester, or coproporphyrin I tetramethyl ester) was refluxed with $MnCl_2$ in AcOH/Ac_2O, and the reaction mixture was washed twice with water. The organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated under reduced pressure and the residue recrystallized from $CHCl_3$/hexane to give the corresponding chloromanganese(III) complexes **1** and the methyl esters of **3** and **4**.[23] The latter two were then subjected to acid hydrolysis of the methyl ester functionalities in a mixture of concentrated hydrogen chloride and 88% aqueous HCO_2H,[24] followed by recrystallization of the crude products from EtOH/hexane to give **3** and **4**.

1: UV/Vis (CH_3OH): $\lambda_{max} = 365.5$, 458.0, 545.0 nm; FAB-MS: m/z: 587 $[M-Cl]^+$.

3: UV/Vis (CH_3OH): $\lambda_{max} = 366.0$, 458.0, 542.5 nm; FAB-MS: m/z: 619 $[M-Cl]^+$.

4: UV/Vis (CH_3OH): $\lambda_{max} = 366.5$, 458.5, 543.0 nm; FAB-MS: m/z: 707 $[M-Cl]^+$.

Zinc complex of mesoporphyrin II with a 1,4-xylylenediamide strap (**5**): To a $CHCl_3$ solution (10 mL) of racemic 1,4-xylylenediamide-strapped mesoporphyrin II (67 mg, 0.1 mmol) was added a saturated methanolic solution of $Zn(OAc)_2$ (2 mL), and the mixture was stirred for 2 h. The reaction mixture was washed twice with water, the organic layer dried over anhydrous Na_2SO_4, and then filtered. The filtrate was evaporated under reduced pressure, and the residue recrystallized from CH_2Cl_2/cyclohexane to give racemic **5** as purple crystals in quantitative yield. ^{1}H NMR ($CDCl_3$, 25 °C): $\delta = 9.52$ and 9.48 (2 × s, 4H; *meso*-H), 4.53 and 4.13 (2 × m, 4H; diastereotopic porph-CH_2CH$_2$CO), 3.87 (q, 4H; porph-CH_2CH$_3$), 3.52 and 3.44 (2 × s, 12H; porph-CH$_3$), 2.78 (m, 2H; diastereotopic porph-CH$_2$CH_2CO), 2.60 (t, 2H; CONH), 2.42 and 2.41 (2 × d, 4H; C_6H_4CH_2), 2.3–2.1 (m, overlapped, 6H; C_6H_4 (4H) and diastereotopic porph-CH$_2$CH_2CO (2H)), 1.79 (t, 6H; porph-CH$_2$CH_3); UV/Vis ($CHCl_3$): $\lambda_{max} = 407.5$, 537.5, 574.5 nm; FAB-HRMS: calcd for $C_{42}H_{44}N_6O_2Zn$ ($[M]^+$): 728.2817, found 728.2810.

Optical resolution of racemic 5 by chiral HPLC: Separation of the enantiomers of **5** was performed on a 4.6 × 153 mm HPLC column packed with silica gel coated with cellulose tris(3,5-dimethylphenylcarbamate) as the chiral stationary phase (Daicel Chiralcel OD-H). In a typical experiment, a 20 μL portion of a CCl_4 solution of racemic **5** (3 mg in 2 mL) was loaded on the column with hexane/ethanol (92:8 v/v) as the eluent, and the compounds with retention times of 11.7 and 13.4 min were fractionated. This procedure was repeated several times, and the eluates were then purified by column chromatography on silica gel ($CHCl_3$) to give the enantiomers of **5**. For the determination of the absolute configurations of the enantiomers, optically pure **5** was demetallated to give the free base, which was methylated at the core nitrogen atom with CH_3I, followed by metallation of the resulting N-methylated porphyrin with $Zn(OAc)_2$, to give a zinc acetate complex (**7**). By reference to the CD spectral profiles of configurationally defined analogous compounds,[16, 18] the first- and second-eluted enantiomers of **5** in chiral HPLC had the *R* and *S* configurations, respectively.

Zinc complex of mesoporphyrin II with a 1,4-xylylene-*N,N′*-dimethylamide strap (**6**): A solution of $(Me_3Si)_2NLi$ (30 μmol) in THF (0.03 mL) at −78 °C was added under nitrogen to a solution of (*R*)- or (*S*)-**5** (1.5 μmol) in THF (2.0 mL), and the mixture stirred at 20 °C for 30 min. Subsequently, CH_3I (3.2 mmol, 0.2 mL) was added, and the mixture stirred at 20 °C for 2.5 h. Volatile fractions were removed from the reaction mixture under reduced pressure, and the residue was washed with water, dried over anhydrous Na_2SO_4, and evaporated to give **6** as a purple powder in quantitative yield: [18] ^{1}H NMR ([D$_6$]DMSO, 180 °C): $\delta = 10.02$, 9.86 (2 × s, 4H; *meso-H*), 4.67 (m, 2H; diastereotopic porph-CH_2CH$_2$CO), 4.3–3.9 (m, overlapped, 6H; diastereotopic porph-CH_2CH$_2$CO (2H) and porph-CH_2CH$_3$ (4H)), 3.53 and 3.51 (2 × s, 12H; porph-CH$_3$), 2.83 (br., 4H; porph-CH$_2$CH_2CO), 1.75 (m, 6H; porph-CH$_2$CH_3), 0.80 (br., 6H; CONCH$_3$); UV/Vis ($CHCl_3$): $\lambda_{max} = 405.0$, 537.5, 574.5 nm; FAB-HRMS: calcd for $C_{44}H_{48}N_6O_2Zn$ ($[M]^+$): 756.3130, found 756.3122.

Spectroscopic titrations:

Figures 2a–d: A DMSO solution (230 μM) of manganese porphyrin was diluted with bis-Tris buffer (50 mM, pH 6.5), and mixed with an aqueous solution of apocytochrome b_{562} to give sample solutions with designated molar ratios of protein:manganese porphyrin (= 7.0 μM), which were subjected to UV/Vis spectroscopy.

Figures 3a–d: A DMSO solution (470 μM, 40 μL) of zinc porphyrin was diluted with acetate buffer (200 mM, pH 4.6, 0.196 mL), and the mixture centrifuged (8000 g) for 10 min at 20 °C to remove insoluble fractions. The resulting supernatant solution was diluted with acetate buffer (200 mM, pH 4.6) containing 2% DMSO ([zinc porphyrin] = 1.5 μM), and titrated with an aqueous solution of apocytochrome b_{562} (90 μM) by UV/Vis spectroscopy, whereby each spectrum was corrected upon consideration of a decrease in concentration of the guest (100 → 83%) by titration. The association constants *K* were obtained from the spectral changes by a nonlinear curve-fitting analysis.

One-pot optical resolution of racemic 5 and 6 by apoproteins: A DMSO solution of racemic **5** or **6** (1.8 mM, 360 μL) was added to an acetate buffer solution of apocytochrome b_{562} or apomyoglobin (3.75 μM, 8.64 mL), and the mixture with a [host]/[guest] mole ratio of 0.5 was allowed to stand for 0.5 h at 0 °C. The reaction mixture was then centrifuged (8000 g) for 5 min at 0 °C, dialyzed for 3 h against acetate buffer (200 mM, pH 4.6) at 0 °C, and centrifuged again. Extraction of the resulting aqueous solution with $CHCl_3$ (2 × 2 mL) allowed the isolation of the guest. The yield and the optical purity of the isolated guest were determined by absorption and CD spectroscopy, respectively, with reference to those of the optically pure authentic samples.[8]

Acknowledgements: K.K. thanks the Izumi Science and Technology Foundation and the Yazaki Memorial Foundation for Science and Technology for financial support. We are grateful to Prof. S. G. Sligar for his kind gift of the TB-1 cells harboring pNS 207 plasmid.

Received: January 5, 1998 [F948]

[1] C. Branden, J. Tooze, *Introduction to Protein Structure*, Garland Publishing, Inc., New York, **1991**.

[2] Very recently, apoprotein-immobilized membranes have been reported to be useful for the enantiomeric separation of amino acids: B. B. Lakshmi, C. R. Martin, *Nature* **1997**, *388*, 758–760.

[3] a) E. Itagaki, L. P. Hager, *J. Biol. Chem*. **1966**, *241*, 3687–3695; b) E. Itagaki, G. Palmer, L. P. Hager, *ibid*. **1967**, *242*, 2272–2277.

[4] a) F. S. Mathews, P. H. Bethge, E. W. Czerwinski, *Biochemistry* **1979**, *254*, 1699–1706; b) F. Lederer, A. Glatigny, P. H. Bethge, H. D. Bellamy, F. S. Mathews, *J. Mol. Biol.* **1981**, *148*, 427–448; c) J. Wu, G. N. La Mar, L. P. Yu, K. Lee, F. A. Walker, M. L. Chu, S. G. Sligar, *Biochemistry* **1991**, *30*, 2156–2165; d) K. Hamada, P. H. Bethge, F. S. Mathews, *J. Mol. Biol*. **1995**, *247*, 947–962.

[5] a) Y. V. Griko, P. L. Privalov, S. Y. Venyamiov, *J. Mol. Biol.* **1988**, *202*, 127–138; b) F. M. Hughson, P. E. Wright, R. L. Baldwin, *Science* **1990**,

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 1998 0947-6539/98/0407-1152 $ 17.50+.50/0

249, 1544–1548; c) D. D. Barrick, F. M. Hughson, R. L. Baldwin, *J. Mol. Biol.* **1994**, *237*, 588–601, and references therein.
[6] Y. Q. Feng, S. G. Sligar, *Biochemistry* **1991**, *30*, 10150–10155.
[7] a) Y. Q. Feng, S. G. Sligar, *Biochemistry* **1991**, *30*, 7711–7717; b) Y. Q. Feng, S. G. Sligar, A. J. Wand, *Nat. Struct. Biol*. **1994**, *1*, 30–35.
[8] a) H. Nikkila, R. B. Gennis, S. G. Sligar, *Eur. J. Biochem.* **1991**, *202*, 309–313; b) T. G. M. Schmidt, A. Skerra, *J. Chromatogr. A* **1994**, *676*, 337–345.
[9] a) H. Sasabe, T. Furuno, J. Otomo, H. Tomioka, Y. Urabe, T. Nagamune, K. H. Kim, K. Kobayashi, *Thin Solid Films* **1992**, *216*, 99–104; b) H. Sasabe, T. Furuno, K. Kobayashi, Y. Kobayashi, T. Nagamune, *Mol. Cryst. Liq. Cryst*. **1994**, *255*, 271–278; c) I. Hamachi, S. Tanaka, S. Tsukiji, S. Shinkai, M. Shimizu, T. Nagamune, *Chem. Commun*. **1997**, 1735–1736.
[10] K. Konishi, K. Oda, K. Nishida, T. Aida, S. Inoue, *J. Am. Chem. Soc.* **1992**, *114*, 1313–1317.
[11] The chirality of **5**–**7** originates from the enantiotopic structure of the precursor **3**.
[12] UV/Vis ($CHCl_3$, 20 °C) for **5**/1-methylimidazole (2.1/126 μM): λ_{max} = 416.5, 544.0, 582.0 nm.
[13] Enantiomeric excess $(ee) = (K_R[(R)\text{-guest}] - K_S[(S)\text{-guest}])/(K_R[(R)\text{-guest}] + K_S[(S)\text{-guest}])$. When $[\text{guest}]_0/[\text{host}]_0$ is infinitely large, $[(R)\text{-guest}]/[(S)\text{-guest}]$ can be regarded as constant, and *ee* ($= ee_{\text{infinity}}$) is given by $(K_R - K_S)/(K_R + K_S)$.
[14] In order to keep the reaction mixture homogeneous, a low [guest]/[host] mole ratio, such as 2.0, was employed.
[15] A partial loss of **5** from the reconstituted protein was inevitable during dialysis and centrifugation.
[16] K. Konishi, K. Yahara, H. Toshishige, T. Aida, S. Inoue, *J. Am. Chem. Soc.* **1994**, *116*, 1337–1344.
[17] K. Konishi, S. Kimata, K. Yoshida, M. Tanaka, T. Aida, *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 2823–2825.
[18] A. Ito, K. Konishi, T. Aida, *Tetrahedron Lett*. **1996**, *37*, 2585–2588.
[19] The samples for Figures 5a and 5b were prepared by incubation of the apoprotein with 2 equivalents of the guest, followed by centrifugation and dialysis to remove unreacted guest. The samples contained non-reconstituted apoprotein in 37 and 93%, respectively, as judged from the isolated yields of **5** from the assemblies.
[20] For interaction of apomyoglobin with small organic molecules, see: a) L. Stryer, *J. Mol. Biol.* **1965**, *13*, 482–495; b) K. E. Lind, J. V. Möller, *Biochem. J.* **1976**, *155*, 669–678; c) R. B. Macgregor, G. Wever, *Nature* **1986**, *319*, 70–73; d) U. G. Wagner, N. Müller, W. Schmitzberger, H. Falk, C. Kratky, *J. Mol. Biol*. **1995**, *247*, 326–337.
[21] a) J. L. Sessler, A. Mozaffari, M. R. Johnson, *Organic Synthesis*, *Vol. 70* (Ed.: A. I. Meyers), Wiley, New York, **1992**, p. 68; b) J. P. Collman, P. Denisevich, Y. Konai, M. Marrocco, C. Koval, F. C. Anson, *J. Am. Chem. Soc.* **1980**, *102*, 6027–6035; c) K. M. Smith, *Porphyrins and Metalloporphyrins* (Ed.: K. M. Smith), Elsevier, Amsterdam, **1975**, p. 33.
[22] F. W. Teale, J. *Biochem. Biophys. Acta*. **1959**, *35*, 543.
[23] R. D. Jones, D. A. Summerville, F. Basolo, *J. Am. Chem. Soc.* **1978**, 100, 4416–4424.
[24] S. S. Eaton, G. R. Eaton, C. K. Chang, *J. Am. Chem. Soc.* **1985**, *107*, 3177–3184.