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Cytochrome *c*–Crown Ether Complexes as Supramolecular Catalysts: Cold-Active Synzymes for Asymmetric Sulfoxide Oxidation in Methanol

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A series of supramolecular complexes of various cytochrome *c* proteins with 18-crown-6 derivatives behave as cold-active synzymes in the H_2O_2 oxidation of racemic sulfoxides. This interesting behavior contrasts with native functionality, where the employed proteins act as electron transfer carriers. ESI-MS, UV, CD, and Raman spectroscopic characterizations reveal that four or five 18-crown-6 molecules strongly bind to the surface of the cytochrome *c* and also that nonnatural low-spin hexacoordinate heme structures are induced in methanol. Significantly, crown ether complexation can convert catalytically inactive biological forms to catalytically active artificial forms. Horse heart, pigeon breast, and yeast cytochromes *c* all stereoselectively oxidize (*S*)-isomers of methyl tolyl sulfoxide and related sulfoxides upon crown ether complexation. These supramolecular catalysts show the highest efficiency and enantiomer selectivity at −40 °C in the H_2O_2-dependent sulfoxide oxidation, while oxidative decomposition of the heme moieties predominantly occurs at room temperature. The oxidation reactivity of the employed sulfoxides is apparently related to steric constraints and electrochemical oxidation potentials of their S═O bonds. Among the cytochrome *c* complexes, yeast cytochrome *c* demonstrates the lowest catalytic activity and degradation reactivity. It has a significantly different protein sequence, suggesting that crown ether complexation effectively activates heme coordination but may additionally alter the native backbone structure. The proper combination of cytochrome *c* proteins, 18-crown-6 receptors, and external circumstances can be used to successfully generate "protein-based supramolecular catalysts" exhibiting nonbiological reactivities.

Biological proteins are versatile molecules for chemical modification and provide a useful basis for designing specific synzymes, which has led to the development of several chemical approaches for activating proteins in nonnative media and converting their functionalities. Methodologies include the introduction of metal coordinating sites, organization of substrate recognition pockets, use of functionalized prosthetic groups, and attachment of cofactors.[1] Some methods have successfully introduced nonbiological functionalities into the proteins which cannot be easily incorporated by genetic or other biological methods. Noncovalent complexation has received recent attention as one of the useful chemical approaches.[2,3] This method utilizes relatively small molecules which interact with specific proteins to alter their biological structures and add nonbiological functions.[2] Typically, several crown ethers bind $-NH_3^+$, $-CO_2^-M^+$, or other functional moieties exposed on the protein surface and form *n*:1 (crown ether:protein) types of supramolecular complexes.[3] Reinhoudt et al. and our group have demonstrated that the crown ether complexation remarkably enhances reactivity and enantiomer selectivity of the hydrolytic enzymes.

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Here, we report that supramolecular complexes composed of 18-crown-6 derivatives and cytochrome *c* proteins have nonbiologically activated heme structures and act as effective cold-active synzymes in asymmetric sulfoxide oxidation.[4] The cytochrome *c* proteins are one of the most popular heme proteins and mediate electron-transfer processes in mitochondrial respiratory chains.[5] They have two coordinative residues (His and Met) to form six-coordinate low-spin heme complexes though most of the catalytically active heme enzymes have highly reactive five-coordinate hemes with open sites. Some mutants and heme peptide fragments have been derived from cytochrome *c* and examined as oxidation catalysts.[6] Although they have lost the methionine ligands and generated five-coordinate hemes, their activated heme moieties readily decompose in the presence of H_2O_2 and other oxidants. The noncovalent complexation method has been applied to activate the horse heart cytochrome *c*. Hamilton and his colleagues designed a calixarene receptor having polycarboxylic acids to bind the positively charged cytochrome *c*, while Goto and Furusaki used similar calixarene derivatives for extraction of the protein.[7] Odell and Earlam have reported earlier that crown ethers solubilize cytochrome *c* into organic solvents upon supramolecular complexation.[8] Utilizing ESI-MS, Julian and Beauchamp directly observed supramolecular complexation between 18-crown-6 and cytochrome *c*.[9] More recently, we have characterized horse heart cytochrome *c* complexes with various crown ethers and revealed the uncommon heme features induced in methanol:[10] (1) The biologically inactive heme coordination structure was artificially converted. (2) The protein matrix provided an asymmetric environment around the heme center. (3) The high solubility allowed experiments to be performed in organic media. Previous observations showing that various organic asymmetric reactions proceed well in organic media at lower temperatures suggest that crown ether complexation might provide a new series of cold-active synzymes derived from cytochrome *c* proteins (Figure 1).[11]

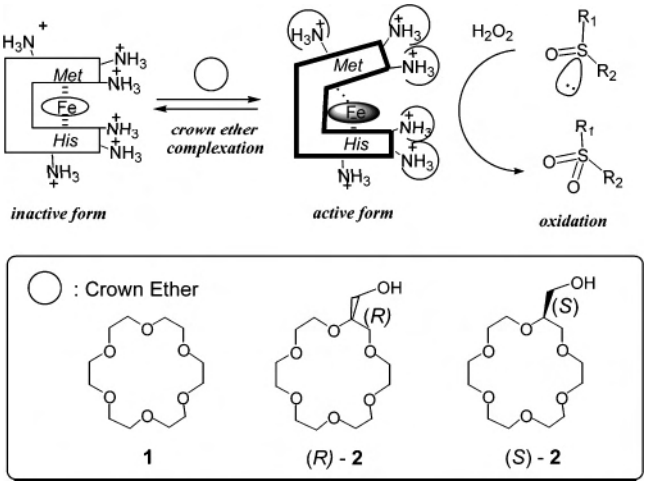

Figure 1. Supramolecular catalysts composed of cytochrome *c* proteins and 18-crown-6 derivatives effective in sulfoxide oxidation.

We present here a series of supramolecular catalysts composed of 18-crown-6 derivatives and cytochrome *c* proteins, which promote asymmetric oxidation of several sulfoxides at low temperatures. Three kinds of cytochrome *c* proteins and three different 18-crown-6 derivatives are combined in methanol solutions, and the resulting supramolecular complexes are fully characterized by resonance Raman, ESI-MS, UV, and CD spectroscopic methods. Although the supramolecular complexes readily decompose by adding H_2O_2 oxidant at room temperature, crown ether complexation generates cold-active synzyme activity in asymmetric sulfoxide oxidation at lower temperatures. Reactivity profiles for six kinds of sulfoxides are analyzed in a fashion similar to those with biological enzymes, and external factors such as crown ether additive, methanol solvent, and reaction temperature are successfully optimized to activate the cytochrome *c* proteins mediating nonbiological asymmetric catalysis.

Results and Discussion

1. Supramolecular Complexes of Cytochromes *c* with 18-Crown-6 Derivatives. Three 18-crown-6 derivatives **1** and (*R*)- and (*S*)-**2** readily solubilize not only horse heart cytochrome *c* but also pigeon breast and yeast cytochrome *c* proteins in methanol. These three proteins have similar molecular weights and unreactive six-coordinate hemes in biological forms. Typically, the preparation procedure involved mechanical stirring of horse heart cytochrome *c* powder (6.0 mg) in a methanol solution of 18-crown-6 **1** (25.4 mg/1.2 mL) for 1.5 h. Although the employed cytochrome *c* proteins are insoluble in methanol, their supramolecular complexes gave homogeneous red solutions (Figure 2).

Among them, horse heart cytochrome *c* forms the most stable complex which rarely precipitated even at −75 °C after a few days, while yeast cytochrome *c* showed relatively lower solubility and required large amounts of crown ether to offer stable supramolecular complex solution. Horse cytochrome *c* shares 89% amino acid sequence homology with pigeon cytochrome *c* and 57% with yeast,[5] suggesting

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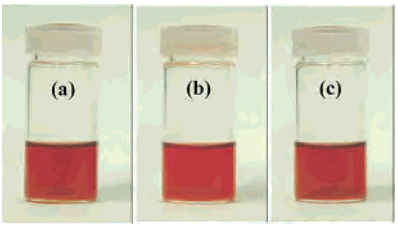

Figure 2. Supramolecular complexes of 18-crown-6 with cytochromes *c* in methanol: (a) horse heart cytochrome *c* complex; (b) pigeon breast cytochrome *c* complex; (c) yeast cytochrome *c* complex. Conditions: [cytochrome *c*] = 2.00×10^{-4} mol/L; [18-crown-6] = 4.00×10^{-2} mol/L.

Table 1. ESI-MS Distribution Profiles of Supramolecular Complexes of Chiral 18-Crown-6 **2** with Horse Heart Cytochrome *c*

charge state of cytochrome *c*	no. of adducts for (*R*)-**2**	no. of adducts for (*S*)-**2**
18	*a*	*a*
17	*a*	*a*
16	*a*	*a*
15	*a*	*a*
14	*a*	*a*
13	2	2
12	4	4
11	5	5
10	5	4
9	5	5
8	4	4
7	2	2

[a] Not Detected

that sequence variations are reflected in the supramolecular complexation behavior of a protein. Polyethylene-glycolated horse heart cytochrome *c* derivatives were widely prepared for use in organic media.[12] They form insoluble gel materials in methanol at <0 °C and, therefore, have severe limitation for use at low temperatures. Furthermore, its preparation requires more than 3 days: activation of poly(ethylene glycol) (1 day); reaction with poly(ethylene glycol) and cytochrome *c* (3 h); dialysis (1 day); lyophilization (1 day). A time of 2 h is sufficient for crown ether complexation.[10] Therefore, the present method provides a simplified approach for the preparation of cytochrome *c*-based catalysts in organic solvents.

ESI-MS measurements were employed to provide direct information about the manner in which 18-crown-6 forms complexes with cytochrome *c*. When a methanol/water (80:20) solution containing the horse heart cytochrome *c* with (*R*)- or (*S*)-**2** was electrosprayed, the spectra revealed that up to four or five 18-crown-6 molecules of each enantiomer (respectively) attach to cytochrome *c*. Table 1 indicates that most of the exposed lysine residues are protonated under the employed ESI-MS conditions, yet the number of attached crown ethers is lower and corresponds well with the number of lysine moieties located near the entrance of the heme crevice. Both enantiomers of chiral 18-crown-6 **2** exhibit similar ESI-MS signal distributions, suggesting that the chirality of crown ethers does not significantly influence the supramolecular complexation of cytochrome *c* at room temperature (see Supporting Information, Figure S1). The

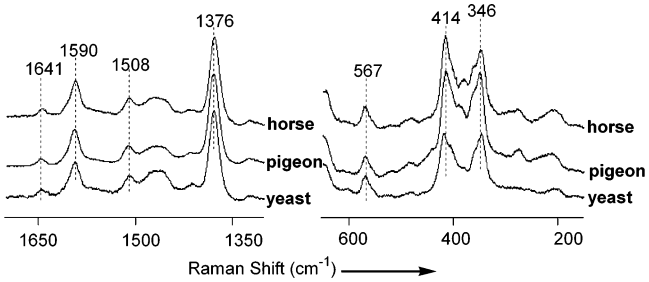

Figure 3. Resonance Raman spectra of three cytochrome *c*−crown ether **1** complexes at −40 °C. Conditions: [cytochrome *c*] = 5.00×10^{-5} mol/L; [18-crown-6] = 1.00×10^{-2} mol/L; in methanol; λ_{ex} = 406.7 nm.

spectrum of 18-crown-6 **1** and cytochrome *c* obtained previously under similar conditions also resulted in the attachment of approximately four crown ethers.[9] Thus, the 18-crown-6 ring can provide effective coordination of lysine moieties which are exposed on the protein surface. This complexation leads to greatly enhanced solubility for cytochrome *c* proteins in methanol.

UV, Raman, and circular dichroism (CD) spectroscopic characterizations demonstrate that each cytochrome *c* complex has a nonbiological six-coordinate heme structure in methanol. The three cytochrome *c* proteins have unreactive six-coordinate heme groups in the natural form, in which methionine acts as the second axial ligand. This is clearly supported by LMCT bands observed at 695 nm due to methionine coordination.[13] When the three cytochrome *c* proteins form supramolecular complexes with 18-crown-6 derivatives **1** and **2** in methanol, no LMCT band appears, indicating that the coordinative methionine was displaced from the sixth coordination position of the heme center. Resonance Raman spectroscopic studies further reveal that all of the cytochrome *c* complexes have heme coordination structures at room temperature similar to those reported with 18-crown-6 **1**.[11] The data in Figure 3 indicate that the supramolecular complexes still maintain the artificially activated heme coordination structures in methanol even at −40 °C. The Raman bands observed in the high-frequency region (1300−1700 cm^{-1}) were assigned to porphyrin in-plane vibrational modes that are sensitive to the electron density in the porphyrin ring and to the coordination and spin state of the iron atom. The bands ν_2 and ν_{10} were recorded at 1590 and 1641 cm^{-1}, and other bands due to the six-coordinate low-spin iron(III) structures appeared at 1376 cm^{-1} (electron density marker) and 1508 cm^{-1} (spin and coordination sensitive band). The presence of three characteristic bands in the low-frequency region (200−600 cm^{-1}) suggests that the examined cytochrome *c* complexes have nonbiological, six-coordinate low-spin heme groups at −40 °C.[14] The crown ether−cytochrome *c* complexes exhibited characteristic CD signals at Soret band regions (ca. 407 nm). Their shapes are similar to those observed at pH 10.3, though their intensity was larger. The native cytochrome *c* is known

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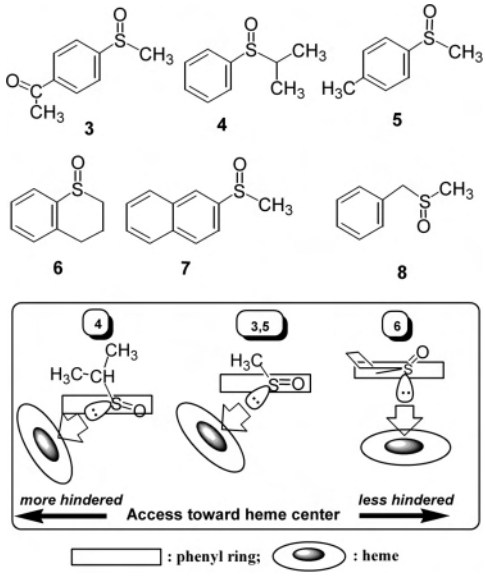

Figure 4. Sulfoxide substrates and their favorable conformations.

to undergo a conformational change at such high pH, in which methionine-80 is displaced by lysine-79 at an axial ligand of heme and the heme crevice is opened. The obtained CD spectra suggest that the crown ether complexation of the cytochrome *c* was accompanied by similar conformational changes. The cytochrome *c* complex further exhibited the enhanced CD signals around 209 nm, indicating the stabilized α-helix structure of the cytochrome *c*.

2. Sulfoxide Substrates for Asymmetric Oxidation. Horse heart cytochrome *c* was reported previously to catalyze the oxidation of aromatic hydrocarbons and organosulfides in aqueous methanol, but it did not work well in nonaqueous methanol.[15] We have shown that complexation with 18-crown-6 **1** leads to uncommon catalytic activities in the H_2O_2 oxidation of pinacynol chloride.[10] Previous results yielded a series of cytochrome *c*−crown ether complexes with activated heme groups in chiral protein matrixes as described above; therefore, various sulfoxides **3**−**8** were examined as oxidative substrates. They are tetrahedral compounds with chiral sulfur centers, in which various aromatic and aliphatic substituents were attached to the sulfur atoms of the S═O bonds. When phenyl-substituted sulfoxides **3**−**6** are compared, they have somewhat different conformations due to steric constraints (Figure 4). Although the aromatic planes prefer (energetically) to be parallel to S═O bonds in sulfoxides **3** and **5**, sulfoxide **4** is forced into a twisted conformation due to steric repulsion between isopropyl substituent and aromatic ring. Sulfoxide **6** has a conformation different from that of **3**−**5**, in which the S═O bond is incorporated in a rigid six-membered ring. Several sulfoxides were reported to act as ambident ligands for heme-iron centers,[16] suggesting that the steric hindrance around the sulfur atom may control the accessibility of the sulfoxide substrate toward the cytochrome *c* heme center.

Table 2 summarizes first oxidation potentials recorded by cyclic voltammetry and log *P* values calculated by the PALLAS program (PrologP, version 6.0, Compu Drug Chemistry Ltd.) for various sulfoxides. Sulfoxide **6** exhibits electrochemical properties different from those of other phenyl-substituted sulfoxides **3**−**5**, or naphthyl methyl sulfoxide **7**, though direct comparison with benzyl methyl sulfoxide **8** is difficult.

The first oxidation potential for **6** was lower than those of phenyl-substituted ones **4** and **5** having alkyl substituents: 2.06 V (**3**) > 1.97 V (**4**) ≃ 1.93 V (**5**) > 1.86 V (**6**). Under the electrochemical conditions, sulfoxide **6** has the most easily oxidative S═O group.[17] We also calculated the log *P* value for each sulfoxide, which can be considered a measure of hydrophobicity: log P = 1.9 (**7**) > 1.8 (**8**) > 1.6 (**4**) > 1.2 (**6**) > 1.1 (**5**) > 0.3 (**3**). These numbers may be related to hydrophobic interactions between the sulfoxides and cytochrome *c* complexes and support the idea that sulfoxide **3** is more hydrophilic in nature than the other sulfoxides.

3. H_2O_2 Degradation of Supramolecular Complexes. The cytochrome *c* proteins are known to display minor peroxidase activities in aqueous solution from previous experiments,[15] but in these experiments the hemes were rapidly decomposed by H_2O_2, which was followed by bleaching and catalyst degradation.[18] The activated cytochrome *c* complexes with crown ethers also rapidly decompose upon H_2O_2 addition in methanol. The degradation process can be followed by monitoring the Soret bands at 407 nm. The half-life of horse heart cytochrome *c* complexed with crown ether **1** is estimated to be 0.3 min at 20 °C, which is much shorter than results obtained previously in water (20 min). As reported earlier,[11] lowering the temperature can effectively suppress the degradation process: half-life = 6 min at 0 °C, 52 min at −20 °C, 150 min at −40 °C, and > 2500 min at −60 °C. Other cytochrome *c*−crown ether **1** complexes exhibit further long-term activities at low temperatures: half-life = 700 min (−40 °C) for pigeon breast cytochrome *c* and 2400 min (−40 °C) for yeast cytochrome *c*. Although our Raman studies indicate that the cytochrome *c* complexes have similarly activated hemes, the reduced degradation reactivity for yeast cytochrome *c* indicates that it has an effectively protected heme center.[19]

The degradation of the cytochrome *c* complexes with H_2O_2 is largely suppressed in the presence of sulfoxide, despite a

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Table 2. Selected Characteristics of Sulfoxide Substrates and Their Degradation and Oxidation Profiles

				oxidn[b]	
sulfoxide	oxidn potential (V vs SCE)	log *P*	degradatn[a] half-life (min)	% conversn (preferred isomer[c])	ee %[d]
3	2.06	0.3	486	55 (*S*)	37
4	1.97	1.6	451	93 (*S*)	24
5	1.93	1.1	344	56 (*S*)	47
6	1.86	1.2	1067	18 (*S*)	21
7	2.00	1.9	1057	73 (*S*)	47
8	1.83	1.8	*e*	15 (*S*)	8

[a] Conditions: horse heart cytochrome *c*, 3.30×10^{-5} mol/L; 18-crown-6 **1**, 6.60×10^{-3} mol/L; H_2O_2, 2.00×10^{-3} mol/L; in methanol, at −40 °C; monitored by decrease in absorbance at 407 nm. [b] Conditions: horse heart cytochrome *c*, 2.00×10^{-4} mol/L; sulfoxide, 4.00×10^{-4} mol/L; 18-crown-6 **1**, 4.00×10^{-2} mol/L; H_2O_2, 6.00×10^{-3} mol/L; for 15 h in methanol, at −40 °C. [c] (*S*)-Isomer of the sulfoxide was more rapidly oxidized. [d] Enantiomer excess % was calculated at 80% conversion. [e] Not determined.

negligible amount of the sulfoxide reacting with H_2O_2 in the absence of cytochrome *c* complex (Table 2). When 4 equiv of sulfoxide is added to the horse heart cytochrome *c* complex with crown ether **1**, the half-life increases remarkably (depending on the nature of sulfoxide): half-life at −40 °C = 1067 min for **6**, 1057 min for **7**, 486 min for **3**, 451 min for **4**, 344 min for **5**, and 150 min for no sulfoxide. Although no UV or CD spectral change is induced for the cytochrome *c* complex by addition of these sulfoxides, less sterically hindered sulfoxide **6** yields a longer half-life than the more sterically hindered sulfoxides **3**−**5** (see Figure 4). As sulfur coordination has been repeatedly reported to occur in heme complexes,[16,20] the employed sulfoxides also appear to prevent access of the H_2O_2 oxidant to the heme center.

4. Enantiomer-Selective Oxidation with Supramolecular Complexes. Cytochrome *c* complexes with 18-crown-6 derivatives promote oxidation reactions for several organic sulfoxides, sulfides, and anthracene with H_2O_2 in methanol, though styrene, ethylbenzene, and benzyl alcohol rarely react.[21] The asymmetric catalytic activities of these synzymes were systematically characterized in the oxidation of sulfoxides because sulfides yield mixtures of corresponding sulfoxides and sulfones too rapidly for careful evaluation. When racemic sulfoxide **4** is oxidized with H_2O_2, the reaction mixture is readily analyzed by chiral HPLC method (DAICEL, Chiracel OB, 92:8 hexane/ethanol) after the cytochrome *c* complex is removed by centrifugal filtration (Millipore Corp. Microcon YM-3 membrane). Three HPLC peaks are observed for each reaction mixture, indicating that clean oxidation of the sulfoxide occurs (i.e. only two peaks due to the sulfoxide enantiomers and one peak due to the oxidized sulfone were detected). Blank experiments in the absence of either the cytochrome *c* complex or the H_2O_2 oxidant confirm that the sulfoxide is adsorbed slightly on the filtration membrane (<5%) and rarely oxidized (<1%). When naphthyl methyl sulfoxide (**7**) is treated with $[^{18}O]H_2O_2$,[22] the recovered sulfone includes > 92% of the oxygen atom labeled. Although the active intermediate cannot be detected, this supports the idea that the oxygen atom of the reactive heme is directly added to the sulfoxide substrate as proposed in microperoxidase-catalyzed oxidations.[23]

On the basis of chiral HPLC analysis of the remaining sulfoxide substrates, the enantiomer-selective oxidation profiles were quantitatively analyzed in terms of "enantiomeric ratio", *E* value, as done in many enzymatic reactions (see Experimental Section).[24] The ee % values at 80% conversion were calculated for comparison and are summarized with conversions after 15 h oxidation in Table 2. As we communicated earlier,[11] the horse heart cytochrome *c*−crown ether **1** complex acts as a cold-active synzyme in oxidation of naphthyl methyl sulfoxide (**7**), which exhibits the highest reactivity and enantiomer selectivity at −40 °C. The asymmetric oxidation of other sulfoxides **3**−**6** and **8** is effectively promoted at −40 °C by various cytochrome *c* complexes (see Table 2). In each oxidation, (*S*)-sulfoxide is more rapidly converted to the sulfone than (*R*)-sulfoxide. The oxidation efficiencies increase in the order of **6** (18%) < **3** (55%) ∼ **5** (56%) < **7** (73%) < **4** (93%), while the enantiomer selectivity, ee % at 80% conversion, exhibits substrate dependency of **6** (21%) ∼ **4** (24%) < **3** (37%) < **5** (47%) = **7** (47%). The observed oxidation profiles are largely different from those recorded in degradation. When the oxo−heme intermediate is formed in the sulfoxide oxidation, the substrate may have very loose sterical requirements for Fe=O−sulfoxide coordination, compared with those in the degradation inhibition (based on Fe−sulfoxide coordination). Although sulfoxide **6** is easily oxidized under electrochemical conditions, it is very slowly oxidized by the cytochrome *c* complex and effectively depresses heme degradation (Table 2). Thus, sulfoxide oxidation and cytochrome *c* degradation appear to occur through different intermediates.

When the amount of crown ether **1** added to the naphthyl methyl sulfoxide (**7**) solution is increased, both the oxidation conversion and enantiomer selectivity increase slightly: for conversion after 15 h, 63% (100 equiv) < 73% (200 equiv) < 77% (400 equiv) < 81% (800 equiv); for ee % at 80% conversion, 40% (100 equiv) < 47% (200 equiv) = 47%

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(400 equiv) ∼ 48% (800 equiv). Although the ESI-MS studies reveal that four or five crown ether molecules strongly bind to cytochrome *c*, additional crown ethers might also interact with cytochrome *c* in solution and accelerate the sulfoxide oxidation. The different enantiomers of 18-crown-6 derivative **2** were compared, because the chirality of the interacting species is often involved in biological recognition processes. However, both enantiomers (*R*)- and (*S*)-**2** promote the asymmetric oxidation of sulfoxides, yielding almost the same results. Nevertheless, it should be noted that lower concentrations were required to solubilize cytochrome *c* with (*R*)- and (*S*)-**2** (see Experimental Section). For conversion after 15 h, 61% (**1**) > 41% ((*R*)-**2**) ∼ 44% ((*S*)-**2**); for ee % at 80% conversion, 58% (**1**) = 58% ((*R*)-**2**) ∼ 61% ((*S*)-**2**). Complexation with crown ethers effectively activates cytochrome *c*, but the chirality of the crown ethers results in only a slight influence on heme reactivity. These observations clearly demonstrate that external circumstances such as the addition of crown ether, the use of methanol as a solvent, and lower temperatures can be used to finely tune cytochrome *c* activity in a nonbiological reaction system.

5. Supramolecular Catalysts of Pigeon and Yeast Cytochromes *c*. The present crown ether complexation method is effective for the chemical activation of pigeon breast and yeast cytochromes *c* as supramolecular catalysts. Although Raman spectra recorded at −40 °C indicate that crown ether complexes have similarly activated hemes compared to horse heart cytochrome *c* (see Figure 2), subtle structural differences cause marked differences in the synzymatic activities. The pigeon breast cytochrome *c* complex with crown ether **1** exhibits comparable activities in the sulfoxide oxidation to horse heart cytochrome *c*, while the yeast complex only modestly promotes oxidation. When naphthyl methyl sulfoxide (**7**) is oxidized for 15 h at −40 °C, the conversion and ee % values at 80% conversion are estimated as 79%/49% for pigeon breast and 31%/31% for yeast. Careful analysis of the amino acid sequence[5] reveals that yeast cytochrome *c* incorporates more than 30 different amino acids from horse heart and pigeon breast cytochromes c. As described above in the degradation studies, the protein matrix of yeast cytochrome *c* impedes access to the heme even in the crown ether complex form. These observations reveal that the crown ether complexation can effectively modify the heme coordination structures in cytochrome *c*, but modification of the protein backbone structures is rarely observed.

Conclusion

We have demonstrated that an optimized combination of cytochrome *c* protein, crown ether, and other circumstances can effectively promote asymmetric catalysis for the sulfoxide oxidation. This supramolecular complexation method has several advantages, including facile sample preparation, structural versatility, and high catalytic activity. A series of cytochrome *c* proteins can be structurally activated and functionally converted from catalytically inactive proteins to cold-active synzymes. Among the examined cytochrome *c* proteins, horse heart and pigeon breast cytochromes *c* exhibit excellent cold-activity and enantiomer selectivity in the oxidation of aromatic sulfoxides. Further applications of synthetic receptors could provide an effective activation method to convert various biological proteins into a new series of biocatalysts exhibiting nonbiological functions.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were recorded on JEOL LA-300 and 400 spectrometers. IR, mass, and CD spectra were obtained on Jasco FT/IR-420, JEOL AX500, and Jasco J-720 spectrometers, respectively. Raman scattering was excited by a 406.7 nm Kr ion laser (Spectra Physics, model 2580). Resonance Raman light was dispersed with a JEOL 400D spectrometer equipped with a photomultiplier.

Materials. Racemic sulfoxides **3**−**8**[25] were prepared and characterized as reported earlier, though chiral sulfoxide **5** was purchased from Aldrich. Sulfone derivatives of **4**, **6**, and **7** were also prepared in the literature,[26] and others were commercially available. Chiral crown ethers (*R*)-**2** and (*S*)-**2** were synthesized according to the literature,[27] and their optical purities were estimated >95% ee by Mosher's NMR measurement. Cytochromes *c* from horse heart (Wako, M_r = 12 384), pigeon breast muscle (Sigma-Aldrich, M_r = 12 173), and *Saccharomyces cerevisiae* yeast (Sigma-Aldrich, M_r = 12 588) are commercially available. Since their catalytic activities were slightly enhanced by oxidation with K_3-$[Fe(CN)_6]$ before use, we usually used them as received.

Preparation of Supramolecular Complexes. The supramolecular complexation was carried out by adding a methanol solution of crown ether (1 mL, 80 μmol) to cytochrome *c* powder (0.4 μmol). After the mixture had been stirred for 1.5 h, the methanol phase was separated by centrifugal filtration and characterized using the UV method. The obtained methanol solutions of supramolecular complexes with horse heart cytochrome *c* were particularly stable at room temperature and exhibited exactly the same UV and CD spectra after 10 days.

CD and Raman Spectroscopic Experiments. For measurements of Soret region CD spectra (350−500 nm): cytochrome *c*, 0.4 mmol; crown ether, 80 mmol; MeOH, 4 mL. For measurements of α-helix region (200−250 nm) CD spectra: cytochrome *c*, 0.04 mmol; crown ether, 8 mmol; MeOH, 4 mL. For measurements of Raman spectra: cytochrome *c*, 0.2 mmol; crown ether, 40 mmol; MeOH, 2 mL.

ESI-MS Measurements. All spectra were obtained using a Finnigan LCQ ion trap quardrupole mass spectrometer. The critical instrument settings that yield adduct formation included capillary temperature 200 °C and tube lens offset −39 V. The tube lens voltage was minimized to avoid collision with the He buffer gas. All samples were electrosprayed in a mixture of 80:20 methanol/water and were electrosprayed with a flow of 3−5 μL/min from a

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500 μL Hamilton syringe for optimal signal. Silica tubing with an inner diameter of 0.005 in. was used as the electrospray tip. No acid was added to any of the samples.

Electrochemical Measurements. Cyclic voltammograms of a methanol solution containing 2.00×10^{-3} mol/L sulfoxide were measured in the presence of 1.00×10^{-1} mol/L Bu_4NPF_6 as a supporting electrolyte with a Hokuto HZ-3000 polarizationmeter. The employed sulfoxides having redox-active S═O groups showed irreversible oxidation processes, and their first oxidation potentials (V) were recorded vs SCE (see Table 2).

Degradation of Cytochrome *c* Complexes with Hydrogen Peroxide. The degradation process of the cytochrome *c* complex was monitored by absorbance changes at 407 nm at several different temperatures. When 2.00×10^{-3} mol/L hydrogen peroxide was added to the methanol solution of cytochrome *c*−crown ether complex, its Soret band disappeared. We similarly followed spectral changes of the Soret band in the presence of sulfoxide. The details of half-life determination conditions are indicated in Table 2.

Sulfoxide Oxidation with Cytochrome *c* Complexes. The reaction mixture (1.00 mL) contained 4.00×10^{-4} mol/L racemic sulfoxide and 2.00×10^{-4} mol/L cytochrome *c*−crown ether complex (1:200 cytochrome *c*/crown ether) in methanol. The oxidation was started by adding 6.00×10^{-3} mol/L hydrogen peroxide at the specific temperature. After the cytochrome *c* complex was removed after several hours by centrifugal filtration (Millipore Corp. Microcon YM-3 membrane), the reaction solution was analyzed by the chiral HPLC method: for reaction of **3**, DAICEL, Chiracel OJ, 80:20 hexane/ethanol; for reaction of **4**, DAICEL, Chiracel OB, 92:8 hexane/ethanol; for reaction of **5**, DAICEL, Chiracel OB, 95:5 hexane/ethanol, and Mightysil Si60, 90:10 hexane/2-propanol; for reaction of **6**, DAICEL, Chiracel OJ, 90:10 hexane/ethanol; for reaction of **7**, DAICEL, Chiracel OB, 70:30 hexane/ethanol; for reaction of **8,** DAICEL, Chiracel OB, 90:10 hexane/ethanol, and Mightysil Si60, 80:20 hexane/2-propanol. The absolute configuration of each sulfoxide remaining was determined with a HPLC polarimeter detector.[25f,28] When chiral crown ethers **2** were employed, different oxidation conditions were required due to the low solubilities of their complexes: cytochrome *c*, 1.00×10^{-4} mol/L; crown ether, 2.00×10^{-2} mol/L; sulfoxide, 4.00×10^{-4} mol/L; H_2O_2, 6.00×10^{-3} mol/L.

On the basis of the chiral HPLC analysis, ee of the remaining sulfoxide and oxidation conversion were calculated. The enantiomer-selective oxidation profiles were quantitatively analyzed in term of "enantiomeric ratio", *E* value, as done in many enzymatic reactions.[24] Four combinations of ee of the remaining sulfoxide and oxidation conversion were measured in each reaction to obtain the *E* value using the following equation:

$$E = \ln[(1 - \text{ee})(1 - \text{conversion})]/\ln[(1 + \text{ee})(1 - \text{conversion})]$$

The *E* values indicated in Table 2 were obtained by the curve-fitting method as illustrated in the Supporting Information (Figure S2). The ee % values at 80% conversion were calculated for comparison and are summarized with the conversion after 15 h of oxidation in Table 2.

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Supporting Information Available: ESI-MS spectra of horse heart cytochrome *c*−crown ether **2** complexes (Figure S1) and plots for *E*-value calculations (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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