Porphyrin Capped with Calix[4]arene Derivative via Hydrogen Bonds

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A calix[4]arene–porphyrin duplex was prepared by mixing equimolar amounts of hydroxy or carboxy calix[4]arene derivatives and pyridyl or *o*-aminophenylporphyrin derivatives. Electrospray ionization mass spectrometry (ESI-MS) was applied to screen a suitable pair of a calix[4]arene and a porphyrin. *meso*-Tetra(2-pyridyl)porphyrin formed a duplex with tetrahydroxy or tetrahydroxymethylcalix[4]arene via triple or quadruple hydrogen bonds. ^{1}H NMR spectra showed that the tetrahydroxycalix[4]arene was symmetrically located upon the porphyrin ring, whereas trihydroxycalix[4]arene was slanted on the porphyrin ring. The duplex of *meso*-tetra(2-pyridyl)porphyrinatocobalt(II) and tetrahydroxymethylcalix[4]arene formed a complex with benzylimidazole, which was capable of reversible dioxygen-binding. The capping structure upon porphyrin provided by calix[4]arene raised the life-time of dioxygen-adduct compared with the porphyrin without calix[4]arene.

Porphyrin and calix[4]arene are multifunctional macrocyclic compounds that are good matches for each other in terms of molecular size and symmetry. There have been many reports of molecular capsules composed of calix[4]arene derivatives and porphyrin derivatives as synthetic heme-protein active site models, that is "calix[4]arene-capped-porphyrin" compounds.[1] In such a molecular capsule, a calix[4]arene is linked with a porphyrin by covalent bonds or an electrostatic interaction.[2] Less attention, however, has been paid to the formation of the molecular capsule of a porphyrin or the formation of a capping structure on a simple porphyrin via hydrogen bonds, save a few reports.[3] We have recently succeeded to form a duplex from tetra(2-pyridyl)porphyrin and tetrahydroxylcalix[4]arene through tetravalent hydrogen bonds (Fig. 1).[4] We also studied the dioxygen-adducts of various porphyrinatocobalt and porphyrinatoiron derivatives.[5] The dioxygen-binding properties of metalloporphyrin have been well-understood to depend on the capping microenvironment on the porphyrin.[6] The μ-oxo dimerization of porphyrinatocobalt(II) and the proton-driven oxidation of porphyrinatocobalt(II) to the porphyrinatocobalt(III) must be prevented to obtain stable and reversible dioxygen-adducts. The capping structure of calix[4]arene, provided by a duplex with porphyrin, would satisfy the above two prerequisites, and the measurement of dioxygen-binding properties would be a way to evaluate the calix[4]arene–porphyrin duplex.

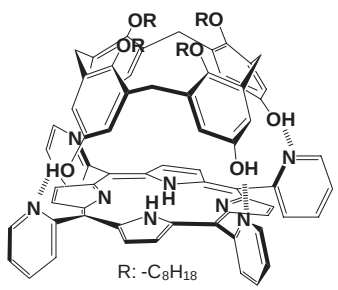
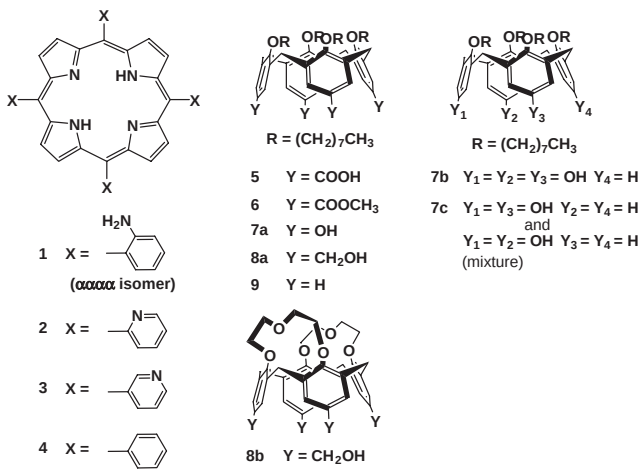


Fig. 1. A duplex of tetra(2-pyridyl)porphyrin and tetrahydroxylcalix[4]arene formed through tetravalent hydrogen bonds.

As indicated in Scheme 1, at first, a series of simple porphyrins in which meso positions were substituted with tetra-aminophenyl, -pyridyl, and -phenyl groups, and calix[4]arene, in which the upper rim was substituted with carboxyl, carboxymethyl, hydroxyl, and hydroxymethyl groups, were synthesized, and stable hydrogen-bonded pairs were investigated by careful electrospray ionization mass spectrometry (ESI-MS) screening experiments. It should be mentioned that extensive progress in mass spectrometry contributes to this field. In particular, ESI-MS is one of the most useful pragmatic tools for studying of supramolecules due to its mild ionization method.[7,8] The equilibrium constants of duplex formation were de-



Scheme 1. Various calix[4]arenes and porphyrins.

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termined by a NMR dilution experiment. The resulting constants were compared with ESI-MS data, and the stability for a duplex was considered in terms of both gas and solution phases. Also, we measured the dioxygen-binding properties of the calix[4]arene-capped porphyrinatocobalt(II) to characterize the duplex by UV–vis spectroscopy and a laser flash photolysis apparatus.

Experimental

General Method. All solvents were purchased from Kanto Chemical Industry Co. CH_2Cl_2 and $CHCl_3$ were distilled from P_2O_5. THF and toluene were distilled using sodium wire. DMF was treated with silica gel and distilled under reduced pressure. Other solvents, including deuterated solvents, were used as received. Pyrrole (Kanto Chemical Industry Co.) and 2-pyridinecarbaldehyde (Tokyo Kasei Kogyo Co., Ltd., TCI) were used after vacuum distillation. Lewis acids ($TiCl_4$, $AlCl_3$, and $SnCl_4$) were used as received and while fresh. All organic reagents were purchased from TCI and used without further purifications. All synthesized compounds were characterized by ^{1}H NMR and mass spectrometry (FAB or ESI); if necessary, further detailed characterization was performed using COSY and NOESY. NMR spectra were recorded on a JEOL JNM-LA500 (500 MHz for ^{1}H) with chemical shifts (δ) downfield from tetramethylsilane as the internal standard. Infrared analyses were performed as a film on a NaCl crystal plate, using a JASCO FT-IR 5300 spectrometer. Plastic sheets coated with 0.2 mm silica gel 60 without a fluorescent indicator (Merck) were used for thin-layer chromatography. $\alpha,\alpha,\alpha,\alpha$-*meso*-Tetra(*o*-aminophenyl)porphyrin,[6] *meso*-tetra(2-pyridyl)porphyrin,[9] cone-conformer 5,11,17,23-tetraformyl-25,26,27,28-tetraoctyloxycalix[4]arene,[10] 5,11,17-triformyl-25,26,27,28-tetraoctyloxycalix[4]arene,[11] 5,11- or 5,17-diformyl-25,26,27,28-tetraoctyloxycalix[4]arene,[11] 5,11,17,23-tetracarboxy-25,26,27,28-tetraoctyloxycalix[4]arene,[10] 5,11,17,23-tetracarboxymethyl-25,26,27,28-tetraoctyloxycalix[4]arene, 5,11,17,23-tetrahydroxy-25,26,27,28-tetraoctyloxycalix[4]arene, and 5,11,17,23-tetraformyl-25,26:27,28-biscrown-3-calix[4]arene[12] were synthesized via reported procedures.

Synthesis of *meso*-Tetra(2-pyridyl)porphyrin (2). *meso*-Tetra(2-pyridyl)porphyrin was synthesized using pyrrole and pyridine-2-carbaldehyde as starting materials. They were dissolved in propionic acid and refluxed for 2 h. The resulting black solution was evaporated and triturated with a NaOH solution until the black residue became solid. The black solid was dried in vacuo, and dispersed in CH_2Cl_2, and ultrasonicated in order to extract porphyrin. The dispersion was filtered through Celite™, and evaporated to yield a black powder. This powder was dissolved in $CHCl_3$ and loaded on the top of a short silica-gel column. After the impurities were eluted by $CHCl_3$, $CHCl_3$/acetone (30/1, v/v), and $CHCl_3$/acetone (20/1, v/v), the porphyrin was eluted using $CHCl_3$/acetone (10/1, v/v). The crude product was washed extensively with MeOH to yield *meso*-tetra(*o*-pyridyl)porphyrin in ~8% yield.

Synthesis of 5,11,17,23-Tetrahydroxymethyl-25,26,27,28-tetraoctyloxycalix[4]arene (8a). 5,11,17,23-Tetraformyl-25,26,27,28-tetraoctyloxycalix[4]arene (100 mg, 0.101 mmol) was dissolved in 30 mL of 2-propanol and CH_2Cl_2 (1/1, v/v). To the solution deoxygenated by nitrogen, $NaBH_4$ (152 mg, 4.04 mmol) was added, and the mixture was stirred for 3 h at room temperature, followed by the addition of 10 mL water. After evaporation of organic solvents, calix[4]arene was extracted by ethyl acetate and washed with water. After evaporation, the residue was triturated in hexane, yielding the product as a white powder (95% crude; purified by column chromatography, 63 mg, 63%). ^{1}H NMR (δ in $CDCl_3$) 6.66 (s, 8H, Ph), 4.43 (d, 4H, *endo*-Ar-CH), 4.36 (s, 8H, $PhCH_2OH$), 3.77 (t, 8H, $PhOCH_2$), 3.14 (d, 4H, *exo*-Ar-CH), 1.91 (m, 8H, $PhOCH_2CH_2$), 1.33 (m, 40H), 0.88 (t, 12H, CH_3); ESI-MS, m/z 1015.5 (found $M + Na^+$), 1016 (calcd); Anal. Calcd for $C_{64}H_{96}O_8$: C, 77.48; H, 9.64%. Found: C, 77.38; H, 9.74%.

Synthesis of 5,11,17,23-Tetrahydroxymethyl-25,26,27,28-biscrown-3-calix[4]arene (8b). 5,11,17,23-Tetraformyl-25,26:27,28-biscrown-3-calix[4]arene (40 mg, 0.059 mmol) was dissolved in 15 mL of 2-propanol. The solution was deoxygenated with nitrogen, $NaBH_4$ (152 mg, 4.04 mmol) was added, and the mixture stirred for 3 h at room temperature. To the dispersion, 10 mL of water was added. After evaporation of organic solvents, the calix[4]arene was taken up by ethyl acetate and washed with water. After evaporation, the residue was triturated in hexane, yielding **8b** as a white powder (28 mg, 78%). ^{1}H NMR (δ in acetone) 7.66 (s, 8H, Ph), 5.23 (d, 2H, *endo*-Ar-CH), 4.23 (d, 2H, *endo*-Ar-CH), 3.75–4.59 (m, 24H, $PhOCH_2$, $PhCH_2OH$), 3.40–3.60 (2d, 4H, *exo*-Ar-CH); ESI-MS, m/z 685 (found $M + Na^+$), 684 (calcd); Anal. Calcd for $C_{40}H_{44}O_{10}$: C, 70.16; H, 6.48%. Found: C, 70.96; H, 6.58%.

Preparation of *meso*-Tetra(2-pyridyl)porphyrinatocobalt(II) (2(CoII)). Cobalt(II) acetate was dissolved in 10 mL of DMF, and the solution was stirred under an argon atmosphere. To the solution, *meso*-tetra(2-pyridyl)porphyrin was added. During stirring of the mixture at 60 °C for 2 h, an aliquot of the solution was removed and monitored with UV–vis spectroscopy. After the free-base porphyrin was completely consumed, the DMF was evaporated and the residue successively extracted with ethyl acetate. After the organic layer was washed with water several times, the solution was evaporated. The residue was dispersed in water and ultrasonicated for 3 min, and then the dispersion was filtered to yield *meso*-tetra(2-pyridyl)porphyrinatocobalt. This porphyrin was produced as a mixture of two compounds, *meso*-tetra(2-pyridyl)porphyrinatocobalt(II) and the *meso*-tetra(2-pyridyl)porphyrinatocobalt(III) μ-oxo dimer, as suggested by a splitting in the Soret band. Therefore, before precise analyses, the porphyrinatocobalt(II) was purified by GPC using Sephadex LH-20 with a $CHCl_3$/EtOH (1/1, v/v) eluent.

^{1}H NMR Experiments (Dilution Experiments). Porphyrins and calix[4]arenes were freeze-dried from benzene in advance, in order to weigh easily. The typical preparation procedure was as follows. To a 10 mL vial charged with equimolar porphyrin and calix[4]arene powder, 3 mL of $CDCl_3$ was added (3 mM). Under airtight and shaded conditions, the dispersion was warmed at 50 °C for 30 min, and ultrasonicated for 15 min to be a transparent solution. (Extended ultrasonication and heating caused the formation of porphyrin-dication due to the degradation of chloroform). Thus, a stock solution was obtained. Using the stock solution, diluted solutions (0.25–2 mM) were prepared. Then, the molar ratio of calix[4]arene and porphyrin was adjusted to 1:1 by referring to the integral values of the ^{1}H NMR signal assigned to the inner proton (2H) or pyrrole β-proton (8H) of the porphyrin, and the terminal methyl proton (12H) or phenyl proton (8H) of calix[4]arene. The concentration of the porphyrin was determined by the calibration curve utilizing the Soret band in the UV–vis spectra, after dilution to an appropriate concentration.

Screening Experiments by ESI-MS. A mixed solution of porphyrin and calix[4]arene was prepared as follows. A porphyrin derivative was mixed with a calix[4]arene derivative in CH_2Cl_2 (1

mM each) and sonicated with a bath-type sonicator for 15 min at room temperature. To the mixed solution, 50 equivalents of $NaClO_4$ were added and stirred carefully for 18 h. Then, the excess salt was filtered off. Mass spectrometric studies were performed in the positive-ion mode using an ion-trap, Thermo Quest FINNIGAN LCQ DECA. The capillary temperature was kept at 180 °C. A sample solution was injected into the mass-spectrometer source with a syringe pump at a flow rate of 30 μL min^{-1}. The spray voltage was 4 kV. The ESI-MS spectra were measured with 50 times accumulation for high reproducibility.

Sample Preparation of the Benzylimidazole-Complex of the Duplex for Dioxygen-Binding. Stock solutions of 20 μM *meso*-tetra(2-pyridyl)porphyrinatocobalt(II) and 50 mM benzylimidazol in absolute CH_2Cl_2 were prepared in a grove box. To a 1.0-cm path length cell, 4.2 mL of the porphyrinatocobalt solution and 9.4 mg of calix[4]arene **8a** (110 equiv)[13] were charged and cooled to −18 °C, and then 12 μL of the benzylimidazol solution (7 equiv) was added. The dioxygen-binding affinity of the benzylimidazol complex for the calix[4]arene–porphyrin duplex was measured by increasing the partial pressure of dioxygen (bubbling in 30 s). After bubbling 100% dioxygen through the solution for 1 min, the time-course of the oxidized product was monitored for absorbance at 434 nm, and then the lifetime of the dioxygen adduct was determined.[24] The dioxygen association and disassociation rate constants (k_{on} and k_{off}) were measured using a Unisoku TSP-600 laser-flash photolysis apparatus.[20] UV–vis absorption spectra were recorded on a JASCO V-570 spectrophotometer.

Results and Discussion

ESI-MS Screening Experiment. We carried out ESI-MS experiments after mixing dichloromethane solutions of calix[4]arene and porphyrin derivatives, as shown in Scheme 1. In a simply mixed solution of **2** and **7a**, not only a heterodimer (**2**·**7a**, 1576 *m/z*), but also homodimers **2**·**2** and **7a**·**7a** were detected in addition to the monomers.[4] The broadened Soret band in the UV–vis spectrum of the porphyrin–dichloromethane solution indicated the homodimerization of the porphyrin caused by π–π stacking. The low solubility of the calix[4]arene **7a** in dichloromethane would result in homodimerization via hydrogen bonds.[14] However, when the calix[4]arene was added to the solution of the porphyrin, followed by ultrasonication and heating, the heterodimer consisting of calix[4]arene and porphyrin was strongly detected as shown in Fig. 2. This suggested that the irradiation energy should dissociate the homodimers of calix[4]arene and porphyrin, and the more thermodynamically stable heterodimer should be formed predominantly over homodimerization.

Solutions of the other pairs of porphyrins and calix[4]arenes were prepared as described above and investigated by ESI-MS. In the matrix of Table 1, "S" means that the intensity of the molecular ion peak of the heterodimer was more than 50% of the calix[4]arene, "W" means that the peak intensity of the heterodimer was less than 10% of the calix[4]arene, and "dashed mark" indicates no molecular ion peak for the heterodimer under any conditions. For the stock solution of tetracarboxycalix[4]arene **5** with *meso*-tetra(*o*-aminophenyl)porphyrin **1**, *meso*-tetra(2-pyridyl)porphyrin **2**, and *meso*-tetra(3-pyridyl)porphyrin **3**, the homodimer **5**·**5** was strongly detected,[15] while the intensity of the heterodimer ion peak was not detected at all, or weakly. The carboxyl group of **5** as a donor and the pyridyl group of **2** or **3** as an acceptor would not be situated in a linear position to permit the formation of a hydrogen bond. Also, for the mixed solution of **1** and **5**, the carboxyl groups of **5** as acceptors would not be linearly oriented with the aminophenyl group of **1**. Thus, **5** predominantly formed the homodimer.

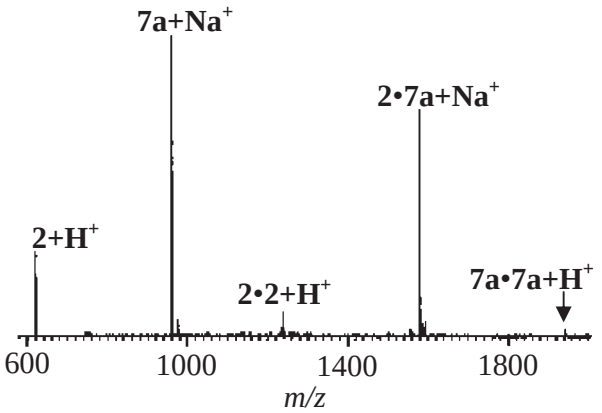

Fig. 2. ESI-MS spectrum of a 1:1 mixture of the porphyrin **2** and the calix[4]arene **7a** in 2 mM CH_2Cl_2 solution after ultrasonication.

Table 1. Suitable Combination of a Porphyrin and a Calix[4]arene for Duplex Formation as Determined by ESI-MS Screening Experiments[a)]

Porphyrin	Calix[4]arene							
	5	**6**	**7a**	**7b**	**7c**	**8a**	**8b**	**9**
1	W	W	—	—	—	—	—	—
2	—	—	S	S	W	S	S	—
3	—	—	—	—	—	W	—	—
4	—	—	—	—	—	—	—	—

a) "S" means that the intensity of the molecular ion peak of the heterodimer was more than 50% of the calix[4]arene. "W" means that the peak intensity of the heterodimer was less than 10% of the calix[4]arene, and "dashed mark" means no heterodimer.

meso-Tetra(2-pyridyl)porphyrin **2** and tetrahydroxycalix[4]arenes **7a**, **8a**, and **8b** were shown to be suitable pairs for a duplex formation (S block). In hydroxycalix[4]arene **7a–c** and **2** systems, tetra-substituted (**7a**) and tri-substituted (**7b**) calix[4]arene formed duplexes with **2**, while di-substituted calix[4]arene (**7c**)[16] did only scarcely. These results indicate that at least three hydrogen bonds are required for stable heterodimer formation.

One piece of interesting information obtained from the ESI-MS screening experiments is that tetrahydroxycalix[4]arene **7a** did not form a heterodimer with *meso*-tetra(3-pyridyl)porphyrin **3**, and tetrahydroxymethylcalix[4]arene **8a** did to a limited degree, whereas two calix[4]arenes (**7a** and **8a**) formed a stable heterodimer with *meso*-tetra(2-pyridyl)porphyrin **2**. We presume that the lone-pair of the 3-pyridyl group should be oriented to the outside of the porphyrin ring, and could not linearly link to the hydroxy proton of **7a** by hydrogen bonding. The tetrahydroxymethylcalix[4]arene **8a**, which has a flexible methylene spacer at the upper rim and flexible alkyl chains in-

stead of the rigid crown clip at the lower rim of calix[4]arene **8b**, could form a heterodimer with porphyrin **3** by weak hydrogen bonding (W block). Of course, the heterodimer was not observed for the pair of tetraphenylporphyrin **4** and calix[4]arene **9** with no hydrogen-bonding substituent groups, and for the pair of calix[4]arenes and porphyrins having an acceptor site only in each other, such as with **2**–**6** and **3**–**6**. These results reveal that heterodimer is not formed by intermolecular forces but, rather, by hydrogen bonds.

ESI-MS screening experiments reflected the characterization of a hydrogen bond which oriented linearly, and indicated that the exact suitability for each other in terms of molecular size and symmetry was required for the formation of a suitable porphyrin–calix[4]arene duplex. As a result, *meso*-tetra(2-pyridyl)porphyrin **2** formed a stable duplex with tetrahydroxy or tetrahydroxymethylcalix[4]arene **7** or **8**, and that at least three hydrogen bonds were required for stable duplex formation. Since the screening was made in the gas phase, duplex formation in the liquid phase was analyzed by NMR.

NMR of Duplex-Formation Equilibrium. The equilibrium constants of duplex formation (K_{dim}) at 25 °C were determined using a Scatchard (Foster–Fyfe) method,[17] as summarized in Table 2.

The K_{dim} of the other pairs could not be determined by the Foster–Fyfe method, because their values were too small. In solution, hydroxy or hydroxymethylcalix[4]arene, **7a** and **7b** and **8a** and **8b**, formed a stable duplex with porphyrin **2** compared with the other pairs. These results were reflected in the ESI-MS screening experiments. When duplexes formed stably in a solution, the molecular ion peaks of the duplex were strongly detected by ESI-MS. On the other hand, the peak was not detected at all, or weakly, by ESI-MS if duplexes did not form at all or only to a limited extent. Although the methodology of obtaining solution-phase information to bolster the gas-phase measurements regarding the stability of the non-covalent aggregate was not fully established,[18] the differences between the S block and the W or N block in the ESI-MS study for the gas phase were clearly reflected in the NMR results in the solution phase. The values of K_{dim} for the tetrahydroxycalix[4]arene **7a** and tetra(2-pyridyl)porphyrin **2** were approximately 10^3, which is almost the same as that of other examples[10] of hydrogen-bonded duplexes using calix[4]arene, whereas the K_{dim} of tetrahydroxymethylcalix[4]arene **8a** and porphyrin **2** was 10^2, a full order of magnitude smaller. This variation is the result of two differences. One is the acidity of the phenol group of **7a**, which is stronger than the hydroxymethyl group of **8a**. Another is the structure of **7a**, which is more rigid than **8a**, having methylene spacers. The larger K_{dim} value of **2**·**8b** (10^3), as compared to that of **2**·**8a** (10^2), can be ascribed to the pre-organized structure of the rigidified calix[4]arene **8b**.

Table 2. The Equilibrium Constants of Duplex Formation between Porphyrin and Calix[4]arene[a)]

Porphyrin	Calix[4]arene	log K_{dim}/—
2	**7a**	3.18
2	**7b**	2.15
2	**8a**	2.25
2	**8b**	3.23

a) ^{1}H NMR dilution experiments for equilibrium constants were performed with concentrations [**2**] = [calix[4]arene] = 0.25–2.0 mM in $CHCl_3$ at 25 °C.

NMR experiments indicate that the duplex is considered not to be formed by unspecific interaction, but by a complemental hydrogen-bonding. For $CDCl_3$ mixed with **2** and **7a**, the chemical shift of the equatorial methylene-bridge proton showed an extraordinarily high-field shift (≈1 ppm) caused by a ring-current effect of the porphyrin, whereas that of the terminal methyl protons shifted slightly (≈0.03 ppm).[4] The high-field shifts correlate to the distance from the centre of the porphyrin, supporting that the porphyrin should be located beneath the calix[4]arene to take a capping structure.

The asymmetricity of the structure of **7b** presented a quite intricate splitting of the signals in the ^{1}H NMR spectrum of the duplex **2**·**7b** (Fig. 3). The different high-field shifts of the phenyl (signals a–e) and methylene-bridge protons (signals f–i) mean a different environment in terms of the porphyrin's ring-current effect, whereas the proton signals are shifted symmetrically for the duplex **2**·**7a**. This asymmetrical high-field shift suggests a distorted capping structure for **2**·**7b**, as shown in Fig. 4.

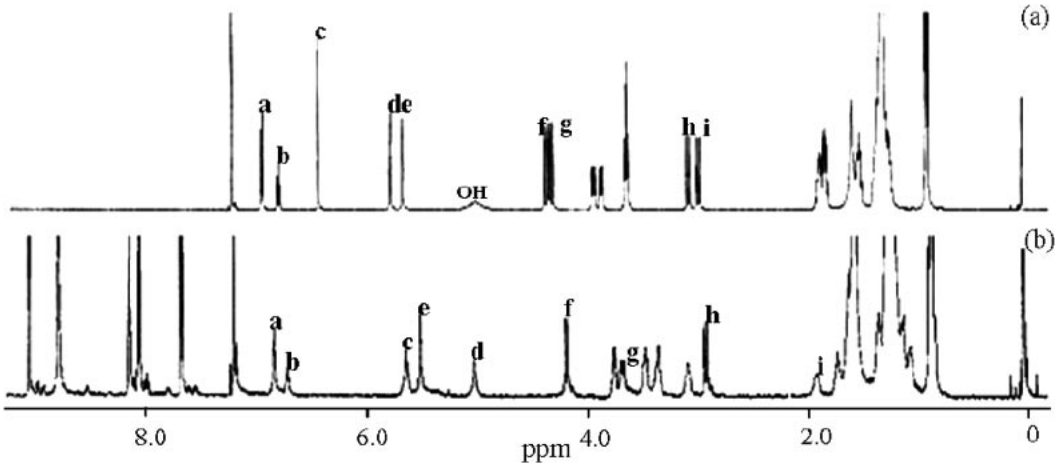

Fig. 3. ^{1}H NMR chart of the mixture of **2** and **7b**; (a) **7b** and (b) the mixture of **2** and **7b**. The signal of a–e and f–i were assigned to the phenyl and the methylene bridge protons, respectively.

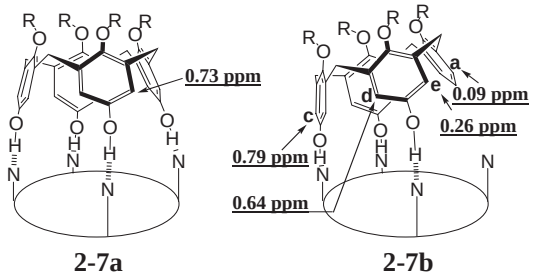

Fig. 4. Schematic representation of the tetra-(**7a**) and the tri-(**7b**) substituted calix[4]arene–porphyrin (**2**) duplex.

In the duplex of the porphyrin **2** and calix[4]arene **8a** or **8b**, the maximum high-field shift was approximately 0.1 ppm. This result suggests that **8a** and **8b** should be located more distant from **2** than **7a** because of the methylene spacer of the calix[4]-arene.

The Stability of a Dioxygen-Adduct of the Benzylimidazole Complex of the Calix[4]arene–Porphyrinatocobalt(II) Duplex. We chose the duplex with *meso*-tetra(2-pyridyl)porphyrinatocobalt(II) **2(CoII)** and **8a** as the most suitable duplex to study the characterization of the hydrogen-bonded duplex of calix[4]arene and porphyrinatocobalt(II) for the following reasons. From the above ESI-MS and NMR experiments, the pair of porphyrin **2** and calix[4]arene **7a** resulted in the most stable duplex. However, the hydroxyl of the phenol groups of **7a** and **7b** strongly coordinate to the cobalt metal of the porphyrin, preventing reversible dioxygen-binding, whereas there was no cobalt cooridination for **8a** and **8b**. Although **2**·**8b** also gave a stable duplex, the ion peak of the duplex was not detected in the mixed solution of **2(CoII)** and **8b** by ESI-MS, whereas it was detected for **2(CoII)** and **8a**. These results suggest that after the insertion of cobalt into porphyrin **2**, the skeleton of the porphyrin would become more rigid,[19] and the resulting **2(CoII)** would not fit well with the rigid calix[4]arene **8b** compared with the flexible **8a**. Accordingly, we decided to use the combination of **2(CoII)** and **8a**.

A porphyrinatocobalt(II) preferentially forms its five-coordinate amine complex,[20] and the calix[4]arene is presumed not to be accessible sterically to the face complexed with benzylimidazole,[2] but to the other open face of the porphyrinatocobalt(II). Therefore, dioxygen binding would take place on the face capped with calix[4]arene. When dioxygen gas was bubbled to a CH_2Cl_2 solution of **2(CoII)**, **8a**, and benzylimidazole, the Soret band at 411 nm in the deoxy state reversibly shifted to 431 nm, which was attributed to the oxy state in the UV–vis absorption spectrum (Fig. 5). However, under dioxygen bubbling, the *meso*-(2-pyridyl)porphyrinatocobalt(II) **2(CoII)** was slowly oxidized to porphyrinatocobalt(III) **2(CoIII)**, of which the Soret band was located at 434 nm in the CH_2Cl_2 solution, and the addition of benzylimidazole facilitated the oxidation extraordinarily. Therefore, we had to prepare the duplex in an airtight glove box. The lifetime of the dioxygen-adduct was measured for the benzylimidazole complex of the duplex **2(CoII)**·**8a** to investigate the capping effect of calix[4]arene **8a**. Under a 100% oxygen atmosphere at −18 °C, the **2(Co)** existed in the mixture of a deoxy and oxy states, and then the deoxy state slowly decreased with an increase of **Co(III)**. From the timeline of the oxidation

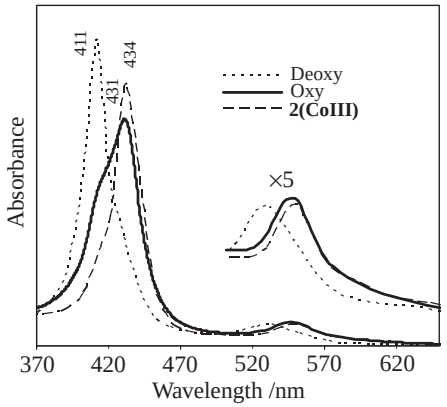

Fig. 5. Visible absorption spectral changes of **2(Co)**, **8a**, and benzylimidazole in CH_2Cl_2 solution at −18 °C.

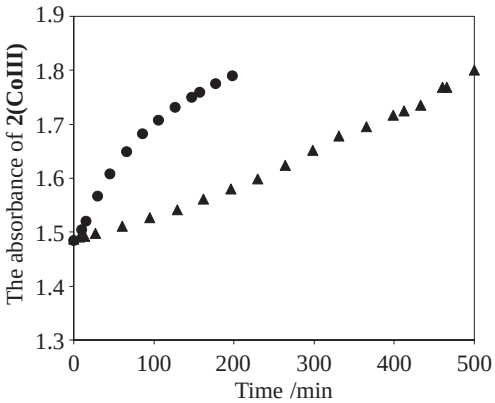

Fig. 6. The timeline of the absorbance of **2(CoIII)** under 100% dioxygen at −18 °C. ●: The mixture of **2** and benzylimidazole, ▲: the mixture of **2**, **8a**, and benzylimidazole.

shown in Fig. 6, it took only 200 min for the naked porphyrinatocobalt(II) **2(CoII)** to be completely oxidized without **8a**, whereas it took over 500 min for the porphyrinatocobalt(II) capped with the calix[4]arene **8a**. The difference supports the capping effect of the the calix[4]arene. That is, calix[4]arene **8a** capping of the dioxygen-adduct of **2(Co)** prevented the other porphyrin from approaching the adduct and participating in μ-oxo dimerization.

Dioxygen-Binding Equilibrium and Kinetic Parameters. The oxygen-binding equilibrium constant (K) is expressed by

$$K = k_{on}/k_{off}. \quad (1)$$

The dioxygen-binding affinity ($P_{50} = 1/K$) can be determined according to Drago's equation[21] (Eq. 2), where P, b, $[\mathbf{2(CoII)}]_0$, $\Delta\varepsilon$, and ΔA are the partial pressure of dioxygen, the path length of cuvette, the initial concentration of the porphyrinatocobalt(II), the difference of the molar absorption coefficient, and the difference of the absorbance from the deoxy form, respectively.

$$P = b[\mathbf{2(CoII)}]_0\Delta\varepsilon \times (P/\Delta A) - P_{50}. \quad (2)$$

The P_{50} value obtained using Drago's equation shows that the dioxygen-binding affinity of the calix[4]arene-capped porphy-

Table 3. Kinetic Parameters of Dioxygen-Binding[a)]

	$k_{on}/10^8$ M^{-1} s^{-1}	$k_{off}/10^5$ s^{-1}	$K/10^2$ M^{-1}	P_{50}/Torr
2(Co)·8a[b)]	1.5	6.2	2.4	240
2(Co)[c)]	1.6	2.5	6.4	90

a) Measurements were performed in CH_2Cl_2 at -18 °C. b) [**2(Co)**] $= 2.0 \times 10^{-5}$ M, [**8a**] $= 2.2 \times 10^{-3}$ M, and [Benzylimidazole] $= 1.4 \times 10^{-4}$ M. c) [**2Co**] $= 2.0 \times 10^{-5}$ M and [Benzylimidazole] $= 1.4 \times 10^{-4}$ M.

rinatocobalt(II) **2(CoII)·8a** was much lower than that of the **2(CoII)** without **8a**, as listed in Table 3. This low binding affinity would be caused by the capping structure. The dioxygen-binding and -dissociating rate constants (k_{on} and k_{off}) were measured with a laser-flash photolysis apparatus.[22] As shown in Table 3, the k_{on} was almost the same for **2(CoII)** and **2(CoII)·8a**. k_{on} was measured after rapid photo dissociation of the dioxygen coordinated to the **2(CoII)**. The recombination of the dioxygen to the **2(CoII)** of the duplex **2(CoII)·8a** would be carried out under the capping calix[4]arene **8a**. Therefore, **8a** would not behave like an obstruction for the dioxygen-binding, resulting in a similar k_{on} **2(CoII)**. From the larger k_{off} rate, it is suggested that the frequent decapping of the calix[4]arene would not make the calix[4]arene as an obstruction of dioxygen dissociation,[23] or such a dynamic atmosphere above the dioxygen-binding side of the porphyrin would facilitate the dissociation of dioxygen. However, the dioxygen-binding mechanism is very complicated in this system and a more detailed analysis would be required to clarify this capping effect.

A capping structure upon *meso*-(2-pyridyl)porphyrinatocobalt(II) provided by calix[4]arene via hydrogen bonding raised the stability of a dioxygen adduct, which never acts as an oxygen carrier alone.

Conclusion

Pyridyl or *o*-aminophenylporphyrin derivatives **1**–**4** and hydroxyl or carboxycalix[4]arene derivatives **5**–**8** were mixed to prepare a duplex, and a suitable duplex pair was determined by a convenient ESI-MS screening experiment. We found that *meso*-tetra(2-pyridyl)porphyrin **2** formed a duplex with tetrahydroxy or tetrahydroxymethylcalix[4]arene **7**, **8** via triple or quadruple hydrogen bonds. We recommend such a convenient screening experiment to determine the appropriate combination of the host–guest complexes. ^{1}H NMR spectra revealed that tetrahydroxycalix[4]arene was symmetrically located upon the *meso*-tetra(2-pyridyl)porphyrin ring, whereas trihydroxycalix[4]arene was slanted upon the porphyrin ring. The duplex of *meso*-tetra(2-pyridyl)porphyrinatocobalt(II) and tetrahydroxymethylcalix[4]arene formed a complex with benzylimidazole, and the higher stability for the complex of the dioxygen adducts indicates the capping effect of the calix[4]arene. Fabricating the duplex should contribute to designing synthetic heme protein-models based on non-covalent bonds.

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