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Preparation and O_2 Binding Study of Myoglobin Having a Cobalt Porphycene

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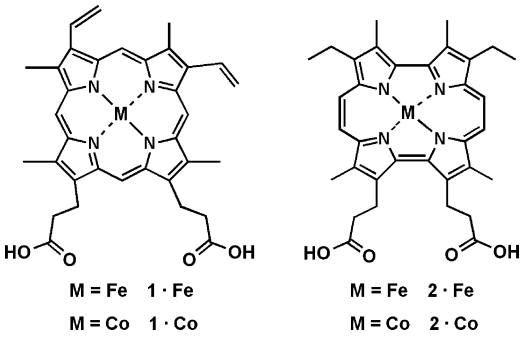
Sperm whale myoglobin, an oxygen-storage hemoprotein, was reconstituted with 2,7-diethyl-3,6,12,17-tetramethyl-13,16-bis(carboxyethyl)porphycenatocobalt(II) in order to investigate the reactivities of a cobalt porphycene in a protein matrix. Similar to the previously reported finding for the myoglobin with the iron porphycene, the reconstituted myoglobin with the cobalt porphycene was also found to have an O_2 affinity 2 orders of magnitude greater than that of the myoglobin possessing cobalt protoporphyrin IX. The EPR spectra of the deoxy and oxy myoglobins having the cobalt porphycene at 77 K also have features similar to those of the myoglobin with cobalt protoporphyrin IX. These spectra suggest that the porphycene cobalt in the deoxy form is coordinated by one nitrogenous ligand postulated to be the imidazole ring of His93, and that the bond configuration of Co^{II}–O_2 is regarded as the Co^{III}–$O_2^{\bullet-}$ species.

Introduction

Over the past few decades, a variety of porphyrin isomers and their iron complexes, in which four pyrrole rings are arranged differently from those in porphyrin, has been synthesized, and their structural features and reactivities have been investigated as an extension of the mechanistic studies on the reactivities of hemoprotein cofactors such as iron protoporphyrin IX, **1**·Fe (Chart 1).[1] With concerns about hemoprotein-engineering, some research groups have attempted to employ these iron porphyrin derivatives as an artificial prosthetic group of myoglobin (Mb), an oxygen-storage hemoprotein,[2,3] because their specific tetrapyrrolic arrangements have been expected to perturb the reactivity of the centered iron, which would drastically affect the physiological properties of the native hemoproteins. On the other hand, the substitution of the centered iron in porphyrin and porphyrin isomers with other metals is another available method to construct a unique prosthetic group with reactivities different from those of iron. In fact, the substitution of the iron of hemoproteins with Zn,[4,5] Ru,[6] Mn,[7] Rh,[8] Mg,[9]

Chart 1



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etc., has been attempted, and their unique properties in a reconstituted Mb or Hb (Hb = hemoglobin) have been demonstrated. Among them, a cobalt porphyrin complex, especially, is one of the most attractive compounds from the viewpoint of iron complex analogues, because Co^{II} porphyrins also reversibly bind O_2, as do the Fe^{II} porphyrins.[10] Co^{II} has a d^7 electronic configuration and paramagnetic character, and it works as a useful tool for spectroscopic measurements such as EPR spectroscopy, although the deoxy and oxy forms of the native hemoproteins (Fe^{II}) often produce difficulties in the spectroscopic measurements.[11] Therefore, the reconstitution with cobalt porphyrins has been recognized as a conventional approach for investigating the structural and electronic natures of Mb and Hb before and after oxygenation.[12−17] The chemical models for the cobalt Mb and Hb have also been extensively studied.[18] In addition, other than such mechanistic studies, cobalt protoporphyrin IX was used for modulating the oxygen affinity of various oxygen-binding hemoproteins without any amino acid mutations.[19]

Recently, we found that the reconstituted sperm whale myoglobin with the iron porphycene **2**·Fe (rMb(**2**·Fe), rMb = reconstituted myoglobin) shows a remarkably high O_2 affinity in which the O_2 affinity is enhanced by 2600-fold compared with that of wild type myoglobin (wtMb).[3] The high O_2 affinity is attributed to the stabilization of the Fe−O_2 σ-bonding caused by the low symmetry (D_{2h}) of the porphycene macrocycle. This finding moves us to substitute the centered iron in the porphycene ring with cobalt in order to more extensively investigate the reactivities of metalloporphycenes. Although the application of cobalt porphycene as a catalyst in organic solvents was reported,[20a,b] ligand binding for cobalt porphycene has been poorly studied.[20c] One of the most important concerns is whether the cobalt porphycene with the low symmetric framework also strongly binds O_2 as did the iron porphycene. As already described, the Co^{II} porphycene is also supposed to be a suitable complex for clarifying electronic characters of the deoxy and oxy forms mainly by EPR spectroscopy, and then the preparation of the reconstituted myoglobin with **2**·Co, rMb(**2**·Co), will provide us a new vista for the nature of a ligand-bound cobalt porphycene in a protein matrix.

In this paper, we report the preparation of reconstituted myoglobin with the cobalt porphycene and O_2 binding kinetics. The reconstituted myoglobin was found to bind O_2 as observed in the wild type protein, and the deoxy and oxy forms were characterized by EPR spectroscopy. As a result of the kinetic study on O_2 binding, the cobalt porphycene in the myoglobin also binds O_2 more strongly than the corresponding porphyrin as shown in the iron porphycene.

Experimental Section

Instruments. The NMR spectra were collected using a Bruker AVANCE500 (500 MHz) NMR spectrometer. The ^{1}H NMR chemical shift values are reported in parts per million relative to the residual solvent resonances. The UV−vis experiments were conducted using a Hitachi U-3210 double beam spectrometer. MALDI-TOF-MS spectra were recorded using an Applied Biosystems Voyager API-TOF workstation. FAB-HRMS was measured using a JEOL JMS-102A mass spectrophotometer. Kinetic measurements for the O_2 binding were carried out with a stopped-flow/laser flash photolysis system constructed by Unisoku, Co., Ltd. (Osaka, Japan). A Xe arc lamp was employed as a source of the probe light to follow the spectral changes. For the laser flash photolysis, a sample was excited with 5-ns pulses (532 nm) from a Q-switched Nd:YAG laser (Surelite I, Continuum). The EPR spectra were measured using a JEOL JES-FE1XG X-band spectrophotometer.

Materials. All reagents and chemicals were obtained from commercial sources and used as received unless otherwise noted. Protoporphyrin IX cobalt complex **1**·Co was prepared by a previously reported method.[13] The free base porphycene **2** was

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synthesized by the method described in a previous paper.[3a] The cobalt complex **2**·Co^{II} was prepared as described below. Native sperm whale myoglobin was purchased from Biozyme Laboratories Limited, and was purified by column chromatography through a CM-52 (Whatman) column. The buffer used for the reconstitution of Mb and the EPR measurements was degassed by freeze−pump−thaw cycling (five times).

Synthesis of 2,7-Diethyl-3,6,12,17-tetramethyl-13,16-bis(2′-methoxycarboxyethyl) Porphycenatocobalt(II), Dimethyl Ester of 2·Co^{II}. The dimethyl ester of the free base **2** (17.4 mg, 29.3 μmol), $Co^{II}(acac)_2$ (88.7 mg, 0.30 mmol), and phenol (20 mL) were stirred at 150 °C under a N_2 atmosphere. After being cooled, the mixture was dissolved in $CHCl_3$. It was then washed with water (1×), 5% $NaOH_{aq}$ (6×), and then water again. The organic phase was concentrated, and the residue was purified on a basic alumina column (active III). The main blue band was collected, and the solvent was evaporated. The residue was dissolved in a small amount of $CHCl_3$, and was solidified by adding hexane. The obtained powder was collected by filtration, and dried in vacuo to afford the dimethyl ester of **2**·Co^{II} (16.3 mg, 85% yield): ^{1}H NMR (500 MHz, $CHCl_3$) δ = 37.5 (6H), 33.8 (4H), 25.8 (4H), 9.47 (6H), 7.95 (6H), 6.66 (4H), 1.66 (6H), −14.0 (2H), −15.0 (2H); MALDI-TOF-MS (positive mode) 651.70 (M^+); UV−vis ($CHCl_3$) λ_{max} 331, 389, 598 nm.

Synthesis of the Bispyridine Complex of 2,7-Diethyl-3,6,12,-17-tetramethyl-13,16-bis(carboxyethyl) Porphycenatocobalt(III), 2·$Co^{III}(Py)_2$. The dimethyl ester of **2**·Co^{II} (1 mg, 1.5 μmol) was dissolved in pyridine (2 mL), and the same volume of 0.2 M KOH_{aq} was added. The mixture was stirred at room temperature for 2 h in air, and neutralized with 0.1 M HCl. The mixture was diluted with $CHCl_3$, and washed three times with water. After the organic phase was concentrated, the addition of hexane to the residue provided **2**·$Co^{III}(Py)_2$ as a green solid: ^{1}H NMR (500 MHz, $CHCl_3$) δ = 10.2 (2H), 10.1 (2H), 5.92 (2H), 4.78 (4H), 4.46 (4H), 4.11 (4H), 3.74 (6H), 3.65 (6H), 3.12 (4H), 2.11 (6H), −0.19 (4H); FAB-HRMS 623.2067 (Int. 83%, M^+ for **2**·Co, calcd 623.2069), 781.2914 (Int. 17%, M for **2**·$Co(Py)_2$, calcd 781.2913); UV−vis ($CHCl_3$) λ_{max} 316, 398, 560, 603 nm.

Preparation of Reconstituted Deoxy Mb with Cobalt Porphycene, Deoxy-rMb(2·Co). Apomyoglobin (apo-Mb) was prepared from purified met-Mb by Teale's 2-butanone method.[21] The reconstitution of Mb with **2**·Co^{II} was carried out in a glovebox ([O_2] < 1 ppm) without air contact. For the reconstitution, **2**·Co^{II} obtained by reduction of **2**·$Co^{III}(Py)_2$ with sodium dithionite was used. The reduced **2**·Co in a 20% aqueous pyridine solution was slowly added to the buffer solution of apo-Mb. The mixture was passed through a Sephadex G-25 gel column, and concentrated by ultrafiltration to provide deoxy-rMb(**2**·Co^{II}) free from excess sodium dithionite. The concentrated solution of deoxy-rMb(**2**·Co^{II}) was kept in the glovebox at 4 °C to avoid oxygenation before use.

O_2 Binding of rMb(2·Co). Oxy-rMb(**2**·Co) was prepared by exposure of the deoxy form to air. The formation of the oxy form was confirmed by the UV−vis spectral changes. The association of O_2 was observed by the absorbance change at 400 nm after flash photolysis of the oxy form under 1 atm of O_2. The time course of the absorbance was analyzed by single-phase kinetics to give the pseudo-first-order rate constant. By dividing this value by the O_2 concentration ([O_2] = 1.26 mM), we obtained the association rate constant.[22] The dissociation of O_2 was measured by following the UV−vis spectral changes after rapidly mixing the oxy form with excess sodium dithionite using a stopped-flow apparatus.[23] The O_2 affinity was calculated from the ratio of the association and dissociation rates

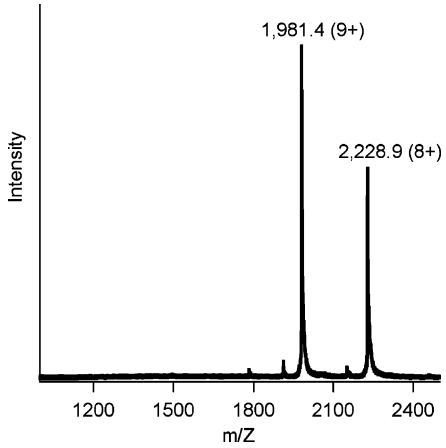

Figure 1. ESI-TOF-MS spectrum of rMb(**2**·Co). The values in parentheses denote the charge states.

EPR Measurements. The EPR measurements were performed at the X-band (9.22 GHz) microwave frequency with (3300 ± 1000) Gauss field modulation (1 mT) at 77 K. The range of the microwave power was 2 mW. The EPR parameters (g_x, g_y, g_z, $|A_x|$, $|A_y|$, $|A_z|$, and $|a^N|$ values) were calculated by fitting the observed EPR spectrum by the global simulation program provided by the Illinois EPR Research Center (IERC), University of Illinois at Urbana-Champaign.

Results and Discussion

Characterization of rMb(2·Co). The cobaltous **2**, **2**·Co, was isolated as the cobalt bispyridine complex, **2**·$Co^{III}(Py)_2$, after alkali hydrolysis of the dimethyl ester of **2**·Co^{II} in aqueous pyridine under aerobic conditions. The reduced form, **2**·Co^{II}, was not obtained because of autoxidation during the hydrolysis. The reconstitution with the **2**·Co^{III} species was unsuccessful under both aerobic and anaerobic conditions. The unsuccessful reconstitution with cobalt(III) porphyrin was also reported by Yonetani and co-workers.[13] After **2**·$Co^{III}(Py)_2$ was reduced to **2**·Co^{II} by sodium dithionite in a glovebox, deoxy-rMb(**2**·Co) was then prepared by adding **2**·Co^{II} to the buffer solution of apo-Mb, followed by a Sephadex G-25 gel filtration under anaerobic conditions.

The ESI-TOF-MS spectrum of rMb(**2**·Co) displayed the two peaks assigned as the multiple charged species (8+ and 9+), which was deconvoluted to the mass number of 17 823 (Figure 1). This number is equivalent to the mass number of the complex composed of apo-Mb with **2**·Co.

The UV−vis spectrum of deoxy-rMb(**2**·Co) shows bands at 387, 577, and 615 nm. After exposure to air, these bands shifted to 396, 579, and 623 nm, respectively, indicative of

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(22) There is the relationship $k_{obs} = k_{on}[O_2] + k_{off}$, where k_{obs} is the apparent pseudo-first-order rate constant, k_{on} is the association rate constant, and k_{off} is the dissociation rate constant. As described in the text, k_{off} is small enough to be approximated as $k_{obs} \approx k_{on}[O_2]$.

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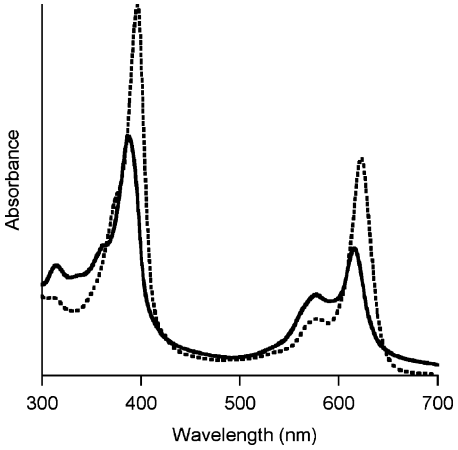

Figure 2. UV−vis spectra of deoxy- and oxy-rMb(**2**·Co): solid line for the deoxy form, dotted line for the oxy form; 100 mM phosphate buffer, at 25 °C.

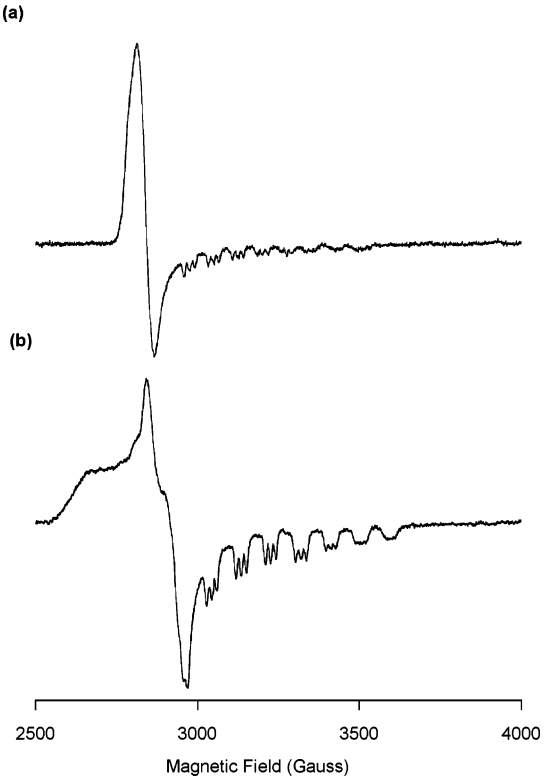

Figure 3. EPR spectra of reconstituted deoxy myoglobins at 77 K: (a) deoxy-rMb(**1**·Co), (b) deoxy-rMb(**2**·Co).

the oxy-rMb(**2**·Co) formation (Figure 2). Very similar spectral changes were reported in the oxygenation of tetrapropylporphycenatocobalt(II) in CH_2Cl_2 at low temperature.[20c] Although the oxygenated porphycene in CH_2Cl_2 is unstable at higher than 263 K, oxy-rMb(**2**·Co) is sufficiently stable even at room temperature for at least 1 day.

The deoxy- and oxy-rMb(**2**·Co) were also characterized by EPR spectroscopy at 77 K. Figures 3 and 4 show the EPR spectra of these two forms along with those of the corresponding cobalt porphyrin-containing rMb, rMb(**1**·Co), for comparison. The EPR parameters from the global simulations (for deoxy-rMbs and oxy-rMb(**1**·Co), see the Supporting Information) are listed in Table 1. Unfortunately, the calculation for oxy-rMb(**2**·Co) was unsuccessful because

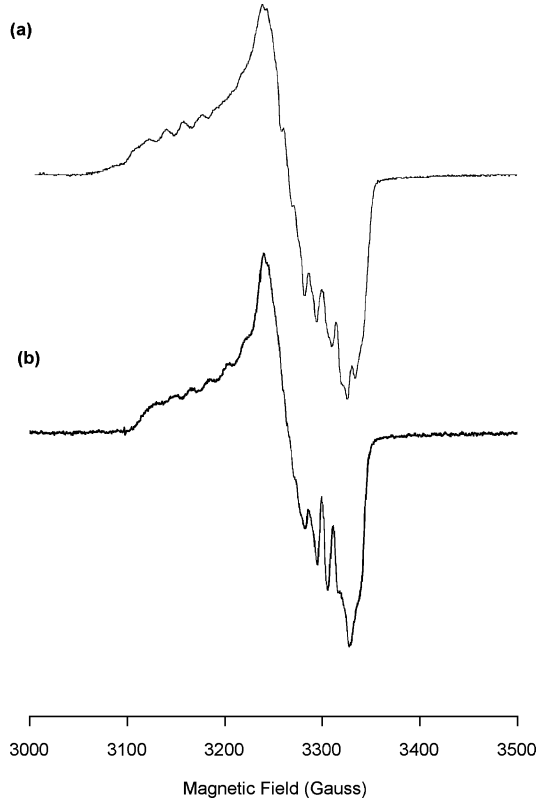

Figure 4. EPR spectra of reconstituted oxy myoglobins at 77 K: (a) oxy-rMb(**1**·Co), (b) oxy-rMb(**2**·Co).

of its complicated signal shape; therefore, only the g values are shown in the table.

The EPR spectrum of deoxy-rMb(**2**·Co) (Figure 3b) is typical of a pentacoordinated low-spin d^7 configuration of Co^{II}, and g_z having a value larger than those of g_x and g_y indicates that the unpaired electron is predominantly located in the d_{z^2} orbital.[16] The eight hyperfine split signals in the high-field region are attributed to the coupling between the unpaired electron and the ^{59}Co nucleus ($I = 7/2$) (one signal around 2900 G overlaps with a signal in the lower-field region), and three resolved superhyperfine splittings of each signal is indicative of one axial coordination of the ^{14}N ligand that is supposed to be the imidazole of His93.[16] The spectrum of deoxy-rMb(**2**·Co) is rhombic in the lower-field region because of the low symmetry of the porphycene ring. Such a rhombic spectrum was also observed in a low symmetric porphyrin isomer, such as cobalt corrolazine, reported by Goldberg et al.[24]

The EPR parameters for deoxy-rMb(**2**·Co) show a g_z value lower than and an A_z value higher than those for deoxy-rMb(**1**·Co), suggesting a significant electronic perturbation along the axial direction by the change in the ring framework. Upon oxygenation, the EPR pattern drastically changes (Figure 4b). Both the rhombic signals in the low-field region and the resolved splitting in the high-field region disappear, whereas the typical EPR absorption of a free radical centered around $g = 2$ appears. Therefore, the electronic configuration of the $Co^{II}-O_2$ bonding in oxy-rMb(**2**·Co) is very close to

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Table 1. EPR Parameters for Deoxy- and Oxy-Reconstituted Myoglobins[a]

rMb	g_x	g_y	g_z	$\|A_x\|$ (G)	$\|A_y\|$ (G)	$\|A_z\|$ (G)	$\|a^N\|$ (G)
deoxy-rMb(**1**·Co)[b]	2.323	2.323	2.302	7	7	69.9	17
deoxy-rMb(**2**·Co)[b]	2.434	2.267	2.018	19	10	92	16.5
oxy-rMb(**1**·Co)[b]	2.007	2.007	2.080	9	9	16	[d]
oxy-rMb(**2**·Co)[c]	2.008	2.008	2.090	[d]	[d]	[d]	[d]

[a] Phosphate buffer (100 mM), pH = 7.0, 77 K. [b] EPR parameters were obtained by the global simulations (see the Supporting Information). [c] g values are determined from the middle of the peaks in the raw spectrum. [d] Not determined.

that of $Co^{III}-O_2^{\bullet-}$ as observed in the oxy Co-substituted Mb, rMb(**1**·Co).[16] However, the splitting on the edge of the signal in the low-field region is not very well resolved in the spectrum of oxy-rMb(**2**·Co), probably due to somewhat wide line width in each splitting. The broad EPR signal may suggest that there are several conformations of the oxygenated cobalt complex in oxy-rMb(**2**·Co).[16f]

Kinetic Measurements for O_2 Binding. The O_2 affinity of rMb(**2**·Co) was calculated from the ratio of the association and dissociation rates. After the flash photolysis of oxy-rMb(**2**·Co) with a 5-ns pulse laser, the absorbance change at 400 nm showed a single pseudo-first-order curve (Figure 5), indicating the recombination of O_2 to the porphycene cobalt atom. By analyzing the observed reaction curve, we determined the O_2 association rate constant to be 95 μM^{-1} s^{-1}. The O_2 dissociation rate was measured by the O_2 trap with excess sodium dithionite using a stopped-flow apparatus (Scheme 1). The spectral change as shown in Figure 6 indicates the smooth conversion of the oxy-rMb(**2**·Co) into the corresponding deoxy form without any significant intermediates with a dissociation rate constant of 82 s^{-1}. As observed in the EPR spectrum of oxy-rMb(**2**·Co) with somewhat broad bands, the plausible existence of several conformations (vide supra) could affect the kinetics of O_2 dissociation, but the observed reaction curve was able to be analyzed by a simple first-order kinetic mechanism. The several conformations are probably in rapid equilibrium.

The kinetic parameters for the O_2 binding of rMbs are summarized in Table 2 together with those for the porphyrin-containing rMb, rMb(**1**·Co), reported by Yonetani and coworkers. The kinetic parameters for the iron analogues measured in our hands are also listed for the comparisons. The porphycene-containing Mb, rMb(**2**·Co), has an O_2 affinity 2 orders of magnitude greater than the corresponding porphyrin Mb, rMb(**1**·Co), which mainly stems from the

Table 2. Kinetic Parameters of O_2 Bindings for Myoglobins

Mb	k_{on}[a] (μM^{-1} s^{-1})	k_{off}[b] (s^{-1})	K_{O_2}[c] (M^{-1})	ref
rMb(**1**·Co)	40 ± 4[d]	2800 ± 400[d]	1.4×10^4[d]	15
rMb(**2**·Co)	95 ± 10[e]	82 ± 20[e]	1.2×10^6[e]	this work
wtMb	17 ± 1[e]	28 ± 2[e]	6.1×10^5[e]	3b
rMb(**2**·Fe)	91 ± 10[e]	0.057 ± 0.005[e]	1.6×10^9[e]	3b

[a] Association rate constants. [b] Dissociation rate constants. [c] $K_{O2} = k_{on}/k_{off}$. [d] phosphate buffer (100 mM), pH = 7.0, 22 °C. [e] phosphate buffer (100 mM), pH = 7.0, 25 °C.

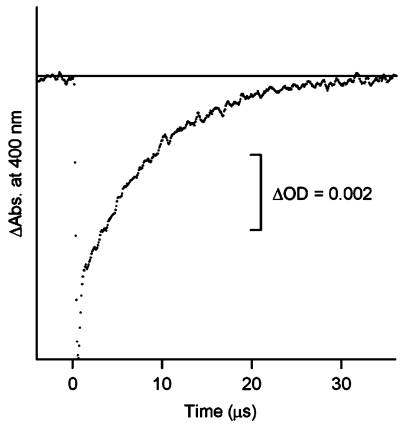
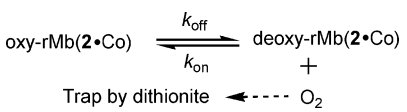


Figure 5. Time course of the absorbance at 400 nm after flash photolysis of oxy-rMb(**2**·Co) under 1 atm of O_2; 100 mM phosphate buffer, pH = 7.0, 25 °C; [rMb] = 53 μM.

Scheme 1



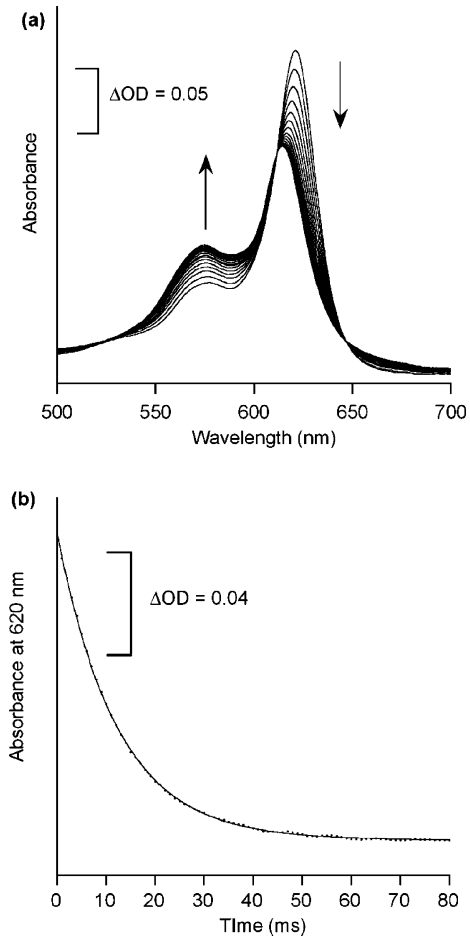


Figure 6. Reaction of oxy-rMb(**2**·Co) with sodium dithionite. (a) Transient spectra observed after mixing oxy-rMb(**2**·Co) with sodium dithionite; every 2 ms over 30 ms. (b) Time course of the absorbance at 620 nm over 80 ms. The solid line represents the simulated curve with k_{obs} = 82 s^{-1}; 100 mM phosphate buffer, pH = 7.0, 25 °C; $[oxy\text{-}rMb(\mathbf{2}\cdot Co)]_0$ = 53 μM; $[Na_2S_2O_4]_0$ = 8 mM.

small k_{off}. This tendency was also observed in our previous report on the iron version, in which the very small k_{off} for rMb(**2**·Fe) brings about the remarkable enhancement of the O_2 affinity.[3] Therefore, the porphycene ring obviously shows the nature to bind O_2 strongly. We previously reported that the stabilization of Fe−O_2 σ-bonding caused by the low symmetric porphycene ring significantly contributes to the slow O_2 dissociation in oxy-rMb(**2**·Fe). Therefore, Co−O_2 σ-bonding in oxy-rMb(**2**·Co) would also be stabilized, leading to the k_{off} value smaller than that of oxy-rMb(**1**·Co). This is the same trend as the previous result of the resonance Raman measurement for the oxygenated cobalt tetrapropylporphycene in CH_2Cl_2, in which the strong Co−O_2 bonding was suggested.[20c] However, in the case of rMb(**2**·Co), the enhancement of the O_2 affinity upon the change in the cofactor framework is moderate when comparing the O_2 affinities for a set of iron-containing Mbs (86-fold for rMb(**2**·Co) vs rMb(**1**·Co) and 2600-fold for rMb(**2**·Fe) vs wtMb). This is a result of the small retardation of the O_2 dissociation in rMb(**2**·Co).

In the case of rMb(**2**·Fe), the strong coordination of the proximal His has been proposed to facilitate the π-back-donation from the metal to the oxygen, which partially contributes to the formation of the stable oxygenated complex,[25] although the predominant factor of a very slow O_2 dissociation for rMb(**2**·Fe) is the stabilization of the Fe−O_2 σ-bonding.[3] However, it has been reported that the coordination of a π-donor ligand to Co^{II} porphycene is weaker than that to the corresponding porphyrin complex,[26] whereas both the Fe^{II} and Fe^{III} atoms in porphycenes are more strongly coordinated by a π-donor ligand than those in porphyrins.[27] Therefore, the coordination to the reduced cobalt of rMb(**2**·Co) by the imidazole of proximal His93 would not be very strong, and the contribution of the π-back-donation from the metal to the oxygen is not significant. This is the most likely reason for the moderate effects on the O_2 dissociation in rMb(**2**·Co).[28]

The effects of the difference in the framework on the O_2 association rate are also small between the cobalt-containing rMbs. In the deoxy form of wtMb, the Fe^{II} atom in the porphyrin takes the out-of-plane position, and the movement of the iron is required in the process of O_2 association. However, in the case of the deoxy-rMb(**2**·Fe), the iron is considered to be in the plane of the porphycene ring. This is because the $d_{x^2-y^2}$ orbital, which contributes to the σ*-antibonding orbital between the iron and a pyrrole nitrogen, is vacant because of the destabilization of this orbital caused by the low symmetry of the porphycene. Therefore, the O_2 association to deoxy-rMb(**2**·Fe) is accelerated. On the other hand, both deoxy cobalt Mbs, rMb(**1**·Co) and rMb(**2**·Co), have a low-spin Co^{II} electronic configuration as indicated by their EPR spectra, suggesting that the $d_{x^2-y^2}$ orbital is already vacant. This electronic configuration is favorable for O_2 association. In fact, the refined X-ray structure of rMb(**1**·Co) proves that the Co^{II} is not as significantly deviated from the porphyrin plane as the iron-containing Mb.[14] Therefore, replacing the framework of the prosthetic group causes no significant effect on the O_2 association rates.

Conclusions

The reconstituted myoglobin with a cobalt porphycene was successfully prepared and characterized by several spectroscopic methods. The UV−vis spectra indicated a reversible O_2 binding. The EPR spectrum of the reconstituted deoxy myoglobin suggested that the cobalt porphycene is coordinated to a nitrogenous ligand similar to the myoglobin with the corresponding cobalt porphyrin, and is in a state in which axial perturbation is significant. In the oxy form, the bond configuration of Co−O_2 can be regarded as a Co(III)−$O_2^{\bullet-}$-like species. The kinetics for O_2 binding revealed that the reconstituted myoglobin with the cobalt porphycene has a high O_2 affinity caused by its slow O_2 dissociation, as observed in the myoglobin with the iron porphycene. To the best of our knowledge, this is the highest O_2 affinity for a series of cobalt-containing myoglobins and hemoglobins, and interestingly, the value of the O_2 affinity exceeds that for the wild type myoglobin. These results clarify the nature of the porphycene ring for O_2 binding, and a more precise investigation on the characteristics of porphycene is now in progress.

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Supporting Information Available: The global simulations for EPR spectra for deoxy-rMb(**1**·Co) and deoxy-rMb(**2**·Co). This material is available free of charge via the Internet at http://pubs.acs.org.

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