Cobalt "Single-coronet" Porphyrin Bearing Hydroxyl Groups in Its O_2 Binding Site as a New Model for Myoglobin and Hemoglobin: Observation of Unusually Low Frequency of ν(O-O) in Resonance Raman Spectrum

Shinya Nakayama, Fumito Tani,*[†] Mikiya Matsu-ura, and Yoshinori Naruta*[†]
Department of Chemistry, Graduate School of Science, Kyushu University, Higashi-ku, Fukuoka 812-8581
[†]*Institute for Fundamental Research of Organic Chemistry, Kyushu University, Fukuoka 812-8581*

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The resonance Raman spectrum of oxy **CoSCP** with **AdIm(2-H)** showed an unusually low ν(O-O) frequency at 1123 cm^{-1}. This result is due to the cooperative effect of strong donation from **AdIm(2-H)** and hydrogen bonding between the inner hydroxyl groups and coordinated dioxygen.

Myoglobin (Mb) and hemoglobin (Hb) reversibly bind molecular oxygen (O_2) in the so-called distal site.[1] The most significant effect of the residues on the distal site, invoked in the stabilization of O_2 ligation in Hb and Mb, is the hydrogen bonding interaction between coordinated O_2 and the distal histidine.[2] Numerous experiments have provided evidence for hydrogen bonding of bound dioxygen with the distal histidine residue.[3] On the other hand, proton transfer to the heme-peroxy complex in the course of oxygen activation process in cytochrome P450 is the key of the O-O bond cleavage.[4] Thus, the evaluation of the oxy complex with a suitable hydrogen bonding is important for this chemistry.

The iron center in Mb and Hb has been replaced by cobalt, and cobalt-substituted Mb and Hb have also been shown to reversibly bind O_2.[5] The Co-substituted globins and model cobalt porphyrins have been proven to be very useful in increasing our understanding of oxy hemes.[3a,b,e] Most important differences between Co(II) and Fe(II) system are that a dioxygen adduct of Co(II) is paramagnetic, which can be characterized by ESR, and that the O-O stretching band ν(O-O) can be observed directly by resonance Raman (RR) spectroscopy.

In order to mimic the function of the distal hydrogen bond, we designed and synthesized a cobalt single-coronet porphyrin, **CoSCP** (Figure 1).[6,7] In **CoSCP**, two chiral bulky binaphthalene groups are connected to *meso* aryl groups by amide bonds on the one side of the porphyrin ring to make a hydrophobic pocket. Two naphtholic hydroxyl groups, which are oriented toward the center of the cavity, are expected to form hydrogen bonding to bound O_2. Two kinds of imidazoles with a bulky tail, **AdIm(2-H)** and **AdIm(2-Me)** were chosen as axial ligands to avoid the formation of undesired six-coordinate species.[8] The bulky group on the imidazole will also work for the prevention of undesired hydrogen bonding between the exogenous imidazoles and the naphtholic OH groups in the cavity. It allows the fair estimation of the effect of the OH groups on bound O_2.

The ESR spectrum of oxy **CoSCP** (1.18×10^{-3} M) in the presence of **AdIm(2-H)** (1.77×10^{-2} M) was obtained in a frozen toluene solution at 77 K. Resultant spectrum ($g_\perp = 2.08$, $A_\perp = 1.18$ mT, $g_{//} = 2.01$, $A_{//} = 1.68$ mT) is attributed to a complex of a six-coordinate cobalt porphyrin with one imidazole and one dioxygen molecule. A similar spectrum was also observed when **AdIm(2-Me)** was present at the same concentration ($g_\perp = 2.09$, $A_\perp = 1.30$ mT, $g_{//} = 2.01$, $A_{//} = 1.81$ mT). The splitting constants (A) and g values are similar to those for CoHb and Mb and other models.[3a,9] In the case of **CoSCP-AdIm(2-H)**, the A values were smaller than for the oxy **CoSCP-AdIm(2-Me)**. Generally, decrease of the A value is attributed to the electron transfer of the unpaired electron from the Co(II) metal onto the dioxygen $p\pi^*$ orbital, depending on the donating character of the trans axial ligand.[10] This leads to the decrease of splitting constants, because of the decrease of interaction between unpaired electron and cobalt nucleus. The UV-vis spectral data for conversion to the oxy complex also showed that **AdIm(2-H)** had higher donating character than **AdIm(2-Me)**.[11] UV-vis spectrum of **AdIm(2-H)** coordinated **CoSCP** immediately changed to that of the oxy species by addition of O_2 gas at −80 °C. The oxy **CoSCP-AdIm(2-H)** had a sharp Soret band at 438 nm. This indicates the complete conversion of the five-coordinated species to the oxy form. On the other hand, use of **AdIm(2-Me)** exhibited incomplete conversion to the corresponding oxy form at room temperature from its UV-vis spectrum, which gave a shoulder at 418 nm. These ESR and UV-vis results are in good agreement with the higher donating ability of 1-MeIm than 1,2-Me_2Im due to a steric reason.[12]

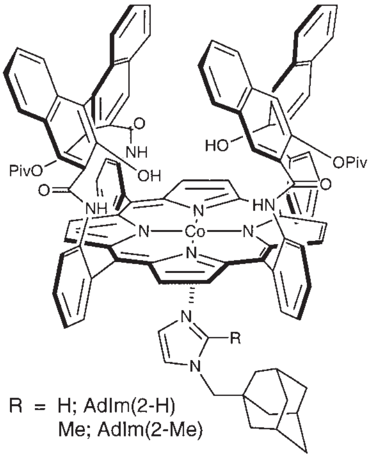

Figure 1. **CoSCP** coordinated by bulky imidazoles.

The ν(O-O) band for oxy **CoSCP-AdIm(2-H)** in RR spectra appeared at 1123 cm^{-1} [$\Delta_{obs}(^{16}O_2/^{18}O_2) = 68\ cm^{-1}$; $\Delta_{calcd} = 64\ cm^{-1}$] (Figure 2). On the other hand, oxy **CoSCP-AdIm(2-Me)** showed its ν(O-O) band at 1145 cm^{-1} [$\Delta_{obs}(^{16}O_2/^{18}O_2) = 61\ cm^{-1}$]. The ν(O-O) frequency of oxy **CoSCP-AdIm(2-H)** shifted by 22 cm^{-1} to the lower region than that for oxy **CoSCP-AdIm(2-Me)**.[13] The value of 1123 cm^{-1} is unu-

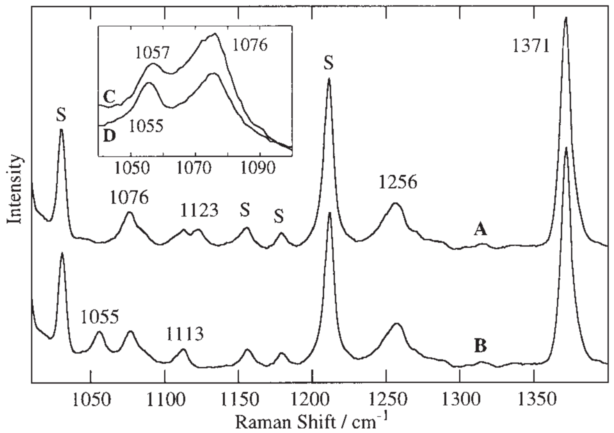

Figure 2. RR spectra of oxy **CoSCP** (1.23×10^{-4} M) with **AdIm(2-H)** (1.85×10^{-3} M), toluene, −80 °C, 441.6 nm excitation, 15 mW: trace **A**, $^{16}O_2$; trace **B**, $^{18}O_2$. Inset: $^{18}O_2$ adduct of **CoSCP** on H/D exchange experiment. trace **C**, -OD; trace **D**, -OH. S denote solvent peak.

sually low in comparison with those (1140–1150 cm^{-1}) of the oxy Co-porphyrins.[14] Therefore, the 1123 cm^{-1} band is characteristic to oxy **CoSCP**-**AdIm(2-H)**.

From a molecular modeling of the oxy **CoSCP**-**AdIm(2-H)**, the distances between the four amide N atoms and both the two O atoms of the ligated O_2 are estimated to be over 4.0 Å, and those between the two hydroxyl O atoms and the bound O_2 are estimated to be 2.8–3.2 Å. The distances of the bound O_2 to the amide protons are almost the same as those of picket fence model.[15] However, the two naphtholic hydroxyl groups are considered to be much closer to the ligated O_2. The partially negative charge accumulated on the terminal oxygen atom in the O_2 adduct would be stabilized by a hydrogen bonding with hydroxyl groups in the cavity. The hydrogen bonding in the oxy form enhances the electron donation from cobalt *d* orbitals to O_2 $p\pi^*$, significantly decreasing the O-O bond order. To confirm the hydrogen bonding interaction in this complex, ν(O-O) was observed after deuterium substitution of the exchangeable protons of **CoSCP(OH)**.[16] In the RR spectrum of oxy **CoSCP(OD)**-**AdIm(2-H)**, the $\nu(^{18}O\text{-}^{18}O)$ peak upshifted by 2 cm^{-1}.[17,18] Therefore, the bound dioxygen interacts with the adjacent exchangeable protons, that is, the inner hydroxyl groups in the cavity.[19,20]

In summary, we concluded that the lower ESR splitting values and the unusually low ν(O-O) of oxy **CoSCP**-**AdIm(2-H)** are due to the cooperative effect of strong donation from **AdIm(2-H)** and hydrogen bonding with the inner hydroxyl groups. To the best our knowledge, the 1123 cm^{-1} band is the lowest ν(O-O) frequency of oxy Co-porphyrinato systems.

The detailed analysis of the stability of the oxy form as well as its equilibrium with the deoxy form is now under way.

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6 Synthetic details of **CoSCP** will be reported elsewhere. **CoSCP**: UV-vis (CH_2Cl_2): λ_{max} ($10^{-3}\varepsilon$, $dm^3\ mol^{-1}\ cm^{-1}$) = 414 (Soret, 269), 528 nm (14.4). IR (neat): 3540, 3401, 3354, 3058, 2973, 2934, 2871, 1678, 1582, 1526, 1479, 1462, 1442, 1349, 1309, 1256, 1196, 1153, 1085, 1024, 999, 905, 883, 798, 752 cm^{-1}. ESR (1.18×10^{-3} M, frozen toluene at 77 K): $g_\perp = 2.90$, $A_\perp{}^{Co} = 15.97$ mT, $g_\parallel = 2.04$, $A_\parallel{}^{Co} = 16.92$ mT. HR-FAB-MS *m/z* Found: 1576.4484, Calcd for $C_{98}H_{69}N_8O_{10}Co$ $(M+H)^+$: 1576.4469. Anal. Found: C 68.90, H 4.16, N 6.75%. Calcd for $C_{98}H_{68}N_8O_{10}Co\cdot 2CH_2Cl_2$: C 69.08, H 3.95, N 6.86%.

7 The relating MeO-substituted single-coronet porphyrin has been reported as an efficient epoxidation catalyst. See, Y. Naruta and K. Maruyama, *Tetrahedron Lett.*, **28**, 4553 (1987).

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9 ESR parameters of five-coordinated **CoSCP-AdIm(2-H)**, $g_\perp = 2.31$, $g_\parallel = 2.03$, $A_\parallel{}^{Co} = 7.96$ mT, $A_\parallel{}^{N} = 1.80$ mT, and **AdIm(2-Me)**, $g_\perp = 2.31$, $g_\parallel = 2.01$, $A_\parallel{}^{Co} = 9.90$ mT, $A_\parallel{}^{N} = 1.70$ mT.

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11 UV-vis spectrum (light-pass length 2 mm) of **CoSCP** (1.23×10^{-4} M) in the presence of the imidazole (1.85×10^{-3} M) was obtained in a toluene solution at −80 °C. Five-coordinate form; with **AdIm(2-H)**, $\lambda_{max}(\%) = 416(100)$, 437 (27), 532 (0.6) nm; with **AdIm(2-Me)**, $\lambda_{max}(\%) = 419$ (100) nm. The Q band did not appear as a clear peak. O_2 ligated six-coordinate form; with **AdIm(2-H)**, $\lambda_{max}(\%) = 438$ (100), 549 (5.5) nm; with **AdIm(2-Me)**, $\lambda_{max}(\%) =$ 418sh (48), 454 (100), 572 (14) nm.

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13 ν(O-O) of oxy **CoSCP** with 1-MeIm and 1,2-Me_2Im appeared at 1124 (1055) and 1146 (1082) cm^{-1} [$^{16}O_2$($^{18}O_2$)], respectively.

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16 In the IR spectrum of **CoSCP(OH)**, O-H and N-H signals (3540, 3401 and 3354 cm^{-1}) were replaced with lower O-D and N-D bands (2616 and 2523 cm^{-1}) upon H/D exchange treatment.

17 Similar shifts for ν(O-O) in CoMb and CoHb were reported. See References 3e–g.

18 No shift on ν(O-O) was observed after H/D exchange treatment in the case of oxy **CoSCP**-**AdIm(2-Me)**. Based on only this RR result, the hydrogen bonding cannot be completely ruled out. At least, it is considered that the decrease of the imidazole donation appreciably weakened the hydrogen bonding interaction between the OH groups and bound O_2.

19 Weak hydrogen bonding with an amide NH groups in picket-fence porphyrins showed their ν(O-O) in the ordinary region [1148–53 cm^{-1} for oxy CoTpivPP(1-MeIm)]. See References 14d, e.

20 Similar hydrogen bonding interaction was also observed in our TCP models. See, M. Matsu-ura, F. Tani, S. Nakayama, N. Nakamura, and Y. Naruta, *Angew. Chem., Int. Ed.*, **39**, 1989 (2000); F. Tani, M. Matsu-ura, S. Nakayama, M. Ichimura, N. Nakamura, and Y. Naruta, *J. Am. Chem. Soc.*, **123**, 1133 (2001).