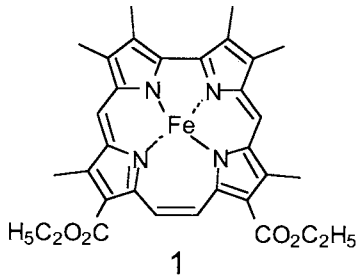
Functional Regulation of Myoglobin by Iron Corrphycene

Saburo Neya,* Noriaki Funasaki, Hiroshi Hori,† Kiyohiro Imai,††
Shigenori Nagatomo,††† Tadashi Iwase,††† and Takashi Yonetani††††
Department of Physical Chemistry, Kyoto Pharmaceutical University, Yamashina, Kyoto 607-8414
†*Division of Biophysical Engineering Science, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531*
††*Department of Physiology and Biosignaling, Graduate School of Medicine, Osaka University, Yamadaoka, Suita, Osaka 565-0871*
†††*Institute for Molecular Science, Okazaki National Research Institutes, Okazaki, Aichi 444-8585*
††††*Department of Biochemistry and Biophysics, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania 19104-6089, U.S.A.*

(Received June 21, 1999; CL-990537)

Iron corrphycene, a novel isomeric heme, was incorporated into myoglobin to examine the influence of heme deformation on the function. The reconstituted protein, although functionally active, exhibits extremely lower affinities for O_2 and CO as compared with the native protein. The functional anomaly was explained in terms of modified iron reactivity in the trapezoidal coordination core.

The chemistry of hemes and hemoproteins has been described within the symmetric molecular framework of porphyrin. Corrphycene,[1] a novel isomeric [18]porphyrin-(2.1.0.1) contains a direct pyrrole-pyrrole link and the diagonal ethene bridge. The coordination hole of corrphycene is trapezoidal, in remarkable contrast with the square core of regular porphyrin. The trapezoidal core is expected to impose geometric strain on the chelating metal ions to induce peculiar reactivity. Although the physical properties of corrphycene have been intensively characterized and reviewed,[2] no successful application of iron corrphycene to hemoproteins has been reported. We report here the first analysis for corrphycene-substituted myoglobin (Mb) to reveal the functional consequence upon deforming the basic porphyrin skeleton.



1

We employed the iron corrphycene (**1**)[3] for Mb reconstitution. This is an isomeric compound of 1,2,3,4,5,8-hexamethyl-6,7-dicarboxyheme[4] that serves as the prosthetic group for Mb. Optical titration of apoMb with **1** revealed a 1:1 binding stoichiometry. The Soret absorbance of the ferric corrphycene Mb[5] remarkably changes between pH 7 and 9 with well-defined isosbestic points at 362 and 428 nm. The transition of $pK_a = 7.9 \pm 0.1$ accompanied with a single proton equilibrium indicates the aquomet form. The resonance Raman spectrum of the ferrous deoxy Mb exhibits the iron-histidine stretching band at 223 cm^{-1}, comparable with 220 cm^{-1} for native Mb,[6] suggesting the five-coordination state for the iron atom. The reduced Mb[7] is capable of reversible binding O_2 and CO. The electronic spectra of the Mb derivatives are summarized in Table 1.

The quantitative analysis of the O_2 equilibrium curve, recorded over a 1-92% saturation range, afforded a Hill coefficient $n = 1.01$ and an equilibrium constant $P_{50} = 37.2$ mmHg (1 mmHg = 133.322 Pa), i.e. $K = 0.015$

Table 1. Visible absorption spectra of the corrphycene-substituted Mb in 0.1 M Tris at pH 7.0 and 20 °C

Ligand	λ/nm (ε/$mM^{-1}cm^{-1}$)				
H_2O	411(87)	490(6.5)	525(6.3)	562(9.1)	620(2.4)
Deoxy	436(101)	520(9.2)	558(12.9)	594(14.0)	
O_2	409(67)	557(10.9)	595(8.7)		
CO	411(111)	417(114)	488(15.0)	523(12.9)	620(5.5)

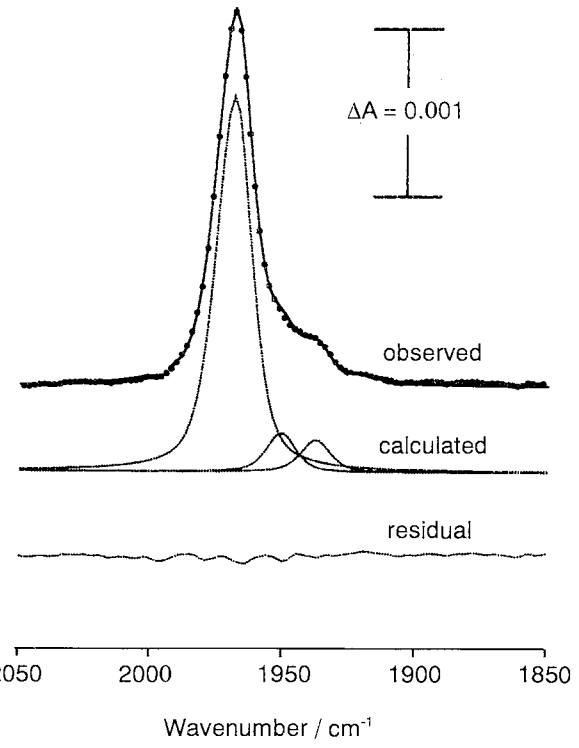


Figure 1. Infrared absorption of the iron-bound CO in the corrphycene Mb in 50 mM phosphate buffer at pH 7.0 and 20 °C. The curve analysis was carried out assuming Gaussian absorption peaks. Mb concentration, 1.4 mM; light path length, 7.3 μm. Recorded on a Shimadzu infrared spectrometer 8100-A.

μM (1 M = 1 $mol^{-1}dm^3$) at 20 °C. The observed K is significantly smaller than 1.1 and 0.37 μM^{-1} for native Mb[8] and 6,7-dicarboxyheme-substituted Mb,[4] respectively, under comparable conditions. A more pronounced anomaly was found in CO ligation. The kinetic analysis for CO binding at 20° C and pH 7 revealed k_{on} = 0.12 $\mu M^{-1}s^{-1}$ and k_{off} = 2.0 s^{-1}, corresponding to K = 0.060 μM^{-1} or P_{50} = 12.3 mmHg. The equilibrium affinity for CO is reduced to 1/450 as compared with K = 27 μM^{-1} for native Mb.[8] The CO stretching band of the carbonyl Mb in the IR spectrum (Figure 1) was resolved into one strong peak and two minor signals at 1970, 1952, and 1937 cm^{-1} with an intensity ratio of 86:8:6.

The remarkable functional profile for the deoxy Mb is the significantly low affinity for exogenous ligands. Since corrphycene **1** contains two methyl groups and two carboxylates instead of two vinyl groups and two propionates, it might be asked that the altered ligand affinity reflects the steric difference in heme-globin contacts. However, such a possibility is excluded because the isomeric 1,2,3,4,5,8-hexamethyl-6,7-dicarboxy-heme exhibits a normal function in Mb[4] despite the absence of the vinyl and propionate groups. In addition, we have previously demonstrated that the total disruption of the specific heme-globin contacts by rotating heme[9] affects only slightly the O_2 binding of Mb. It could be still asked that the lowered O_2 affinity is due to breaking up of the original salt-bridges between heme propionates and apoprotein. This possibility is also less important because esterification,[10] contraction,[4] or removal[9] of heme propionates only slightly affects the Mb function. The resonance Raman observation further demonstrates that the unstrained iron-histidine bond, similar to that in native Mb, is retained in the corrphycene-bound Mb.

The above consideration suggests that the functional anomaly comes from the deformed coordination core in corrphycene. It is notable in the corrphycene Mb that the reduction in affinity is more significant for CO (1/450) than O_2 (1/74). Since CO is an excellent π acceptor,[11] a remarkable decrease in CO affinity suggests unfavorable iron(II) dπ-ligand pπ interactions. This interpretation is consistent with the IR result in Figure 1 that the vibrational frequency of CO is enhanced by 26 cm^{-1} as compared with 1944 cm^{-1} in native Mb.[12] Increase in the C-O stretching frequency indicates weaker iron-CO interactions because the iron-CO bond is formed through charge transfer from the iron d orbitals to the anti-bonding orbitals in CO.[11] It is notable that the four nitrogen atoms in corrphycene core are in trapezoidal arrangement. The geometric situation could deform the iron d_{xz} and d_{yz} orbitals through the modified iron dπ and corrphycene pπ interactions, thereby diminishing overlap between the iron d orbitals and CO π* orbitals to weaken the iron-CO bond. It is also possible that the four σ orbitals of pyrrole nitrogens at the trapezoidal corner overlap to a less extent with the orthogonal lobes of iron $d_{x^2-y^2}$ orbitals. This distortion in the iron-N(pyrrole) bonds causes the iron atom to be accommodated less favorably into the corrphycene plane. In other words, the iron atom is more easily displaced from the central cavity of corrphycene. This interpretation is fully consistent with the CO binding kinetics showing increase in k_{on} and decrease in k_{off} as compared with those for native Mb.

In summary, corrphycene-bound Mb exhibits a peculiar ligand-binding profile that is hidden behind the symmetric framework of regular porphyrin. Corrphycene-Mb, therefore, is a unique system in the search of relationship between heme deformation and fine tuning of protein function.[13]

We would like to thank Professor Teizo Kitagawa (Institute for Molecular Science) for providing facility of resonance Raman measurements and Dr. K. S. Reddy (University of Pennsylvania) for preliminary IR observation. This work was supported by grants-in-aid from the Japan Private School Promotion Foundation, the Ministry of Education, Science, Sports and Culture, Japan (#10672031 and Frontier Research Program), and Ministry of Health and Welfare, Japan (H10-Ketsueki-003).

References and Notes

1 J. L. Sessler, E. A. Brucker, S. J. Weghorn, M. Kisters, M. Schäfer, J. Lex, and E. Vogel, *Angew. Chem., Int. Ed. Engl.*, **33**, 2308 (1994).
2 E. Vogel, *J. Heterocycl. Chem.*, **33**, 1461 (1996).
3 S. Neya, K. Nishinaga, K. Ohyama, and N. Funasaki, *Tetrahedron Lett.*, **39**, 5217 (1998).
4 S. Neya, N. Funasaki, N. Igarashi, A. Ikezaki, T. Sato, K. Imai, and N. Tanaka, *Biochemistry*, **37**, 5487 (1998).
5 We used **1** with the ester groups because alkaline hydrolysis decomposed the macrocycle. Mb (sperm whale, Sigma) with **1** was prepared and purified to $A_{Soret}/A_{280\ nm}$ = 2.2 according to the reported method[9] in a 60% yield. The ferric Mb was determined with ε_{411} = 87 $mM^{-1}cm^{-1}$ based on the pyridine hemochromogen spectrum with ε_{561} = 19.3 $mM^{-1}cm^{-1}$.
6 J. Teraoka and T. Kitagawa, *J. Biol. Chem.*, **256**, 3969 (1981).
7 A. Hayashi, T. Suzuki, and M. Shin, *Biochim Biophys. Acta*, **310**, 309 (1973).
8 B. A. Springer, S. G. Sliger, J. S. Olson, and G. N. Phillips, Jr., *Chem. Rev.*, **94**, 699 (1994).
9 S. Neya, N. Funasaki, Y. Shiro, T. Iizuka, and K. Imai, *Biochim. Biophys. Acta*, **1208**, 31 (1994).
10 M. Tamura, G. V. Woodrow III, and T. Yonetani, *Biochim. Biophys. Acta*, **317**, 34 (1973).
11 D. F. Shriver, P. W. Atkins, and C. H. Langford, "*Inorganic Chemistry*", Oxford University Press, Oxford (1994), 2nd ed, Chap. 16, p. 667.
12 W. T. Potter, J. H. Hazzard, M. G. Choc, M. P. Tucker, and W. S. Caughey, *Biochemistry*, **29**, 6283 (1990).
13 M. Ravikanth, and T. K. Chandrashekar, *Struct. Bonding*, **82**, 105 (1995).