Functional Analysis of the Iron(II) Etiocorrphycene Incorporated in the Myoglobin Heme Pocket

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The iron(II) complex of 2,7,12,17-tetraethyl-3,6,11,18-tetramethylcorrphycene, an isomeric heme, was complexed with apomyoglobin to examine the ligand binding ability of the novel macrocycle under physiological conditions. The reconstituted holoprotein was found to be functionally active at pH 7.4 and 20 °C and to bind oxygen and carbon monoxide reversibly with a half-saturation pressure at 6.7 and 3.5 mmHg, respectively. Equilibrium affinities for these ligands are one to two orders of magnitude lower than those reported for native myoglobin. The functional anomaly was ascribed to the geometric and electronic strain on the iron(II) atom in the trapezoidal coordination core of corrphycene.

Key words iron(II) corrphycene; reconstituted myoglobin; oxygen binding; deformed coordination core

Aspiration is the most basic biological process in all aerobic organisms. Oxygen (O₂) from the lung is stored in muscle by myoglobin (Mb). The ability of Mb depends on the intimate linkages of the prosthetic group with the surrounding globin. The heme in Mb is the iron(II) complex of protoporphyrin IX with several types of peripheral substituents. Chemical modification of heme substituents has been carried out to analyze the heme–protein interactions in Mb. However, the fundamental structure of the porphyrin skeleton has never been altered.

Corrphycene is a novel structural isomer of porphyrin synthesized by Sessler et al. in 1994. The macrocycle is formally derived from porphyrin by removing one meso-carbon to the diagonal bridge. The structural change deforms the square metallo cavity of porphyrin into a trapezoid, although the resultant corrphycene remains flat and aromatic like the parent porphyrin. The obvious similarities between corphycene and porphyrin inevitably suggest the potential utilization of iron(II) corrphycene as an oxygen carrier. Although such a skeletal arrangement as found in corphycene is unlikely to occur in nature, it is of primary interest to examine the binding ability of the iron(II) complex for O₂ under physiological conditions. Is it possible for the irregular porphyrinoid to be placed in Mb as the prosthetic group? What type of perturbation is expected for the reconstituted Mb? We report here the first functional analyses of the artificial Mb reconstituted with etiocorrphycenatoiron(III) to apoMb, as monitored at 410 nm, further revealed a clear inflection point at a 1 : 1 binding stoichiometry. Purified ferric Mb showed light absorption maxima at 280 (34 mM⁻¹ cm⁻¹), 406 (101), 521 (6.9), 550 (550), and 654 (1.9) nm in 0.1 M Tris at pH 7.0 and 20 °C (result not shown). The ferrous Mb reduced with the enzymatic system was stable to afford reversible O₂ binding. Figure 2 shows the visible absorption spectra of the oxy and deoxy derivatives. Figure 3 illustrates the Hill plots of O₂ binding characterized with a Hill coefficient n = 1.00 and a half-saturating pressure P₅₀ = 6.7 mmHg or Kₛ = 8.20×10⁴ M⁻¹. The Mb is also capable of binding CO (Fig. 2). The CO affinity was estimated by the CO replacement of oxy Mb. Titration of CO-saturated buffer to the oxy Mb resulted in the formation of a sharp 406-nm Soret peak.

![Fig. 1. Iron(II) Complex of Etiocorrphycene Employed as the Prosthetic Group of Mb](Image)

![Fig. 2. Visible Absorption Spectra of the Oxy (—), Deoxy (—–), and Carbonmonoxy (···) Forms of Mb Reconstituted with Etiocorrphycene in 0.1 M Tris at pH 7.4 and 20 °C](Image)

Mb reconstituted with etiocorrphycenatoiron(III) was purified by column chromatography with a linear gradient of Tris buffer, 20—120 mM, on a carboxymethylated-cellulose (Whatman, CM52) column after the preparation of the Mb-bearing etiohemin. The O₂ equilibrium curve of the Mb was recorded on the automatic recording apparatus of Imai in the presence of an enzymatic reduction system. The carbon monoxide (CO) affinity was optically determined by titrating CO-saturated 0.1 M Tris buffer to the oxy Mb solution at pH 7.4 and 20 °C. Visible absorption spectra were recorded on a Shimadzu MPS-2000 spectrophotometer with a cell compartment equipped with a circulating water bath.

Results

Etiocorrphycenatoiron(III) is an all-alkyl compound essentially insoluble in water. Insolubility in water, however, did not prevent the Mb reconstitution because the procedure used for etiohemin afforded a 50—60% yield. Preliminary optical titration of the corphycenatoiron(III) to apoMb, as monitored at 410 nm, further revealed a clear inflection point at a 1 : 1 binding stoichiometry. Purified ferric Mb showed light absorption maxima at 280 (34 mM⁻¹ cm⁻¹), 406 (101), 521 (6.9), 550 (550), and 654 (1.9) nm in 0.1 M Tris at pH 7.0 and 20 °C (result not shown). The ferrous Mb reduced with the enzymatic system was stable to afford reversible O₂ binding. Figure 2 shows the visible absorption spectra of the oxy and deoxy derivatives. Figure 3 illustrates the Hill plots of O₂ binding characterized with a Hill coefficient n = 1.00 and a half-saturating pressure P₅₀ = 6.7 mmHg or Kₛ = 8.20×10⁴ M⁻¹. The Mb is also capable of binding CO (Fig. 2). The CO affinity was estimated by the CO replacement of oxy Mb. Titration of CO-saturated buffer to the oxy Mb resulted in the formation of a sharp 406-nm Soret peak.
with isosbestic points at 394 and 414 nm. The partition coefficient \([\text{MbCO}]/[\text{MbO}_2]/[\text{CO}]\) = 2.86 afforded \(P_{50} = 3.5\) mmHg or \(K_p = 2.1 \times 10^5 \text{M}^{-1}\) for the CO ligation in 0.1 M Tris buffer at pH 7.4 and 20 °C. Briefly, the O₂ and CO affinities of etiocorrphycene Mb are reduced to 1/13 and 1/128, respectively, as compared with \(K_p = 1.10 \times 10^6 \text{M}^{-1}\) (O₂) and 2.7×10⁸ M⁻¹ (CO) of native Mb.⁸

**Discussion**

The 1:1 complex formation between the iron(III) corphycene and apoprotein suggests their successful coupling to produce reconstituted Mb. The apparently normal coordination of O₂ and CO provides support to the structural integrity of the Mb. It is now evident that the iron(II) etiocorrphycene serves as the prosthetic group of Mb. To our knowledge, this is the first demonstration of etiocorrphycenatoiron(II) as a carrier of O₂ and CO. The quantitative comparison of the ligand binding profiles with native Mb reveals functional anomalies. The O₂ affinity of corphycene Mb is only 1/13 that of native Mb⁹ under comparable conditions. It could be argued that the much lower O₂ affinity may indicate the absence of propionic acid groups in the corphycene. The possibility is ruled out because heme propionate groups only slightly affect the O₂ affinity of Mb.⁸ It could also be argued that the insertion of the isomeric heme may induce conformational transition of the heme pocket to disturb ligand binding. This is also unlikely because crystallographic analyses of the Mb⁹,¹⁰ reconstituted with synthetic hemins revealed that the globin conformation is fairly insensitive to the molecular shape of the prosthetic group. In view of the results,⁹,¹⁰ the corphycene-Mb is likely to retain the native conformation, although some minor conformational transition of the distal histidine cannot be ruled out.

The functional anomaly most likely arises from the peculiar molecular shape of corphycene. The metallo cavity is trapezoidal in corphycene,² in remarkable contrast with the square cavity in porphyrin. The deformed equatorial ligand field could cause unfavorable overlap of the electron lobes of the four pyrrole-nitrogens with the iron \(d_{yz}\) orbital. The iron atom under these circumstances is readily displaced from the corphycene plane. The possible large iron displacement toward the proximal histidine, similar to the T-state of hemoglobin,¹¹ could significantly reduce the O₂ and CO affinities.

Since the iron(II)–O₂ bond in heme has both \(\sigma\) and \(\pi\) characteristics in almost equal proportions,¹² it is not always easy to evaluate the changes in the two types of bonding interactions from the O₂ equilibrium curve alone. Consideration of the CO binding result allows us to elucidate the bonding effects. It is noteworthy that the reduction (1/128) in CO affinity is much larger than the decrease (1/13) in O₂ affinity. Since the \(\pi\)-type interaction dominates in iron(II)–CO interactions,¹³ the significantly lower CO affinity reasonably suggests decreased iron(II) dx₂–CO pπ interactions. In other words, the energy level of the iron \(d_{xz}\) and \(d_{yz}\) orbitals in corphycene is enhanced to destabilize iron(II)–CO bonding. It is thus likely that the deformed metallo core in corphycene is the direct cause of the remarkable reduction in the affinity for O₂ and CO. We therefore conclude that etiocorrphycene is a potential modulator of the ligand binding ability of Mb.

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**References**