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 (O_2) 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.[1] 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.[2] 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 corrphycene and porphyrin inevitably suggest the potential utilization of iron(II) corrphycene as an oxygen carrier. Although such a skeletal arrangement as found in corrphycene is unlikely to occur in nature, it is of primary interest to examine the binding ability of the iron(II) complex for O_2 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 2,7,12,17-tetraethyl-3,6,11,18-tetramethylcorrphycenatoiron(II), the iron(II) complex of etiocorrphycene (Fig. 1).

Experimental

2,7,12,17-Tetraethyl-3,6,11,18-tetramethylcorrphycene[3] and the iron(III) complex[4] were prepared according to the method reported in the literature. Sperm whale Mb (Sigma, type II) was obtained commercially. The crude 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.[5] The O_2 equilibrium curve of the Mb was recorded on the automatic recording apparatus of Imai[6] in the presence of an enzymic reduction system.[7] 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[5] afforded a 50—60% yield. Preliminary optical titration of the corrphycenatoiron(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 (shoulder), 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[7] was stable to afford reversible O_2 binding. Figure 2 shows the visible absorption spectra of the oxy and deoxy derivatives. Figure 3 illustrates the Hill plots of O_2 binding characterized with a Hill coefficient $n=1.00$ and a half-saturating pressure $P_{50}=6.7$ mmHg or $K_a=8.20\times10^4\ M^{-1}$. 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

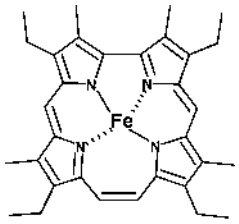

Fig. 1. Iron(II) Complex of Etiocorrphycene Employed as the Prosthetic Group of Mb

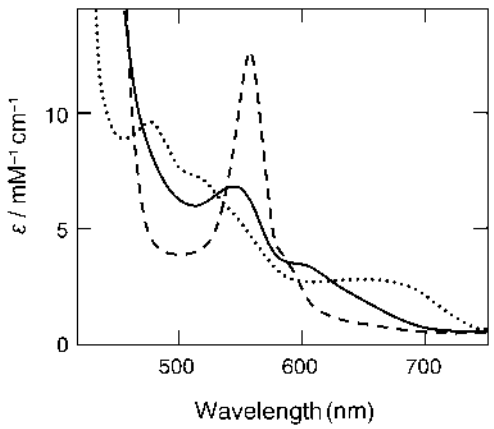

Fig. 2. Visible Absorption Spectra of the Oxy (—), Deoxy (---), and Carbonmonoxy (···) Forms of Mb Reconstituted with Etiocorrphycene in 0.1 M Tris at pH 7.4 and 20 °C

Soret bands of the oxy, deoxy, and carbonmonoxy derivatives are at 410 (80 $mM^{-1}cm^{-1}$), 434 (115), and 406 (182) nm, respectively.

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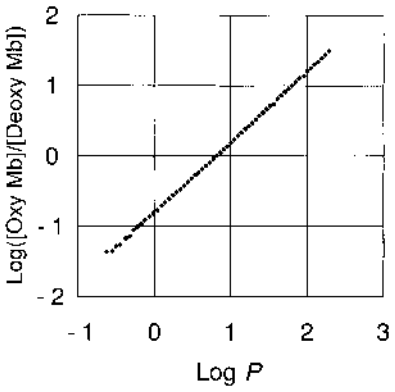

Fig. 3. Hill Plots of the Equilibrium O_2 Binding to the Deoxy Mb Containing Etiocorrphycene

Monitored at 558 nm in 0.1 M phosphate at pH 7.4 and 20 °C. *P*, partial O_2 pressure in mmHg.

with isosbestic points at 394 and 414 nm. The partition coefficient $[MbCO][O_2]/[MbO_2][CO]=2.86$ afforded $P_{50}=3.5$ mmHg or $K_a=2.1\times10^5\ M^{-1}$ for the CO ligation in 0.1 M Tris buffer at pH 7.4 and 20 °C. Briefly, the O_2 and CO affinities of etiocorrphycene Mb are reduced to 1/13 and 1/128, respectively, as compared with $K_a=1.10\times10^6\ M^{-1}$ (O_2) and $2.7\times10^7\ M^{-1}$ (CO) of native Mb.[8]

Discussion

The 1 : 1 complex formation between the iron(III) corrphycene and apoprotein suggests their successful coupling to produce reconstituted Mb. The apparently normal coordination of O_2 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 O_2 and CO. The quantitative comparison of the ligand binding profiles with native Mb reveals functional anomalies. The O_2 affinity of corrphycene Mb is only 1/13 that of native Mb[8] under comparable conditions. It could be argued that the much lower O_2 affinity may indicate the absence of propionic acid groups in the corrphycene. The possibility is ruled out because heme propionate groups only slightly affect the O_2 affinity of Mb.[5,8] 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 Mb[9,10] 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 corrphycene-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 corrphycene. The metallo cavity is trapezoidal in corrphycene,[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 $d_{x^2-y^2}$ orbital. The iron atom under these circumstances is readily displaced from the corrphycene plane. The possible large iron displacement toward the proximal histidine, similar to the T-state of hemoglobin,[11] could significantly reduce the O_2 and CO affinities.

Since the iron(II)–O_2 bond in heme has both σ and π characteristics in almost equal proportions,[12] it is not always easy to evaluate the changes in the two types of bonding interactions from the O_2 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 O_2 affinity. Since the π-type interaction dominates in iron(II)–CO interactions,[13] the significantly lower CO affinity reasonably suggests decreased iron(II) dπ–CO pπ interactions. In other words, the energy level of the iron d_{xz} and d_{yz} orbitals in corrphycene is enhanced to destabilize iron(II)–CO bonding. It is thus likely that the deformed metallo core in corrphycene is the direct cause of the remarkable reduction in the affinity for O_2 and CO. We therefore conclude that etiocorrphycene is a potential modulator of the ligand binding ability of Mb.

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