Electrochemistry of Myoglobins Reconstituted with Symmetric Protoheme-III and Protoheme-XIII

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In nature, asymmetric protoheme, protoheme-IX (Fig. 1), is a cofactor molecule of a heme protein such as myoglobin (Mb), of which function is known to be oxygen storage in its reduced state in mammalian muscles. The asymmetric protoheme-IX generally takes two (normal and inverted) orientations that differ by 180˚ rotation to the □-□ meso carbon axis in Mb. On the difference in function between Mbs with protoheme-IX in normal and inverted orientations, opposite conclusions have been given: on the one hand, Mb with inverted orientation was reported to exhibit 10-fold higher O_2 affinity than the normal Mb [1], and the other is that such a difference is negligibly small [2]. To solve such a problem, use of symmetric heme would give us a useful insight. Recently, we have succeeded to synthesize the two symmetrical regioisomers of protoheme-IX, i. e., protoheme-III and –XIII [3]. These symmetric hemes have only one orientation in Mb because of which inverted orientation is the same as the normal one.

In the present study, protoheme-IX was replaced by the symmetric protoheme, protoheme-III or –XIII, and structural, functional and electrochemical properties of reconstituted Mbs have been examined.

The resonance Raman spectra of native and reconstituted Mbs in a 0.1 M phosphate buffer solution (pH 7.0) suggested that the proto-III and proto-XIII Mbs in oxidized forms showed a typical high-spin state, suggesting their aquomet forms (a water molecule is coordinated to the heme iron as an axial ligand) at a neutral pH region like native Mb.

The proto-III and proto–XIII Mbs showed well-defined reversible redox waves at an In2O3 electrode with the highly hydrophilic surface in a 0.1 M bis-Tris buffer solution (pH 6.5), as in the case of native Mb [4]. The $E^{0'}$ values, estimated as the midpoint of anodic and cathodic peak potentials of the quasi-reversible redox waves, were –145 mV for proto-III Mb, –160 mV for proto-XIII Mb and –140 mV vs. Ag□AgCl□Sat. KCl for native Mb. Since the increase in the exposure of the heme moiety to the solvent is known to cause the negative shift of $E^{0'}$, and according to the suggested equation ($E^{0'}$ shifts by –15 (% exposure) mV [5]), ca. 1 % increase in exposure of the heme for proto-III Mb is suggested compared with proto-XIII Mb may be a possible reason for the change in redox potential.

The formal heterogeneous electron transfer rate constants, $k^{0'}$, were estimated by using a digital simulation technique for the observed voltammograms to be ca. 5.7 (± 0.5) x 10^{-4} cm s^{-1} for proto-III Mb, 7.0 (± 0.5) x 10^{-4} cm s^{-1} for proto-XIII Mb and 6.5 (± 0.5) x 10^{-4} cm s^{-1} for native Mb.

In 0.1 M phosphate buffer solution at pH 7.0 from the titration curve showed the oxygen binding constants (K_{O2}) for the equation (deoxy Mb + O2 = oxy Mb) were 1.2 (± 0.3) x 10^6 M^{-1} for proto-III Mb, 1.0 (± 0.3) x 10^6 M^{-1} for proto-XIII Mb and 7.9 (± 0.3) x 10^5 M^{-1} for native Mb. The observed K_{O2} value for native Mb in the present study was in good agreement with the reported value (7.5 x 10^5 M^{-1}) (estimated by the different method).

At pH 7.0 and 35 ˚C, the observed first-order rate constants (k_{auto}) for the auto-oxidation of heme iron were calculated to be 8.1 (± 0.5) x 10^{-5} s^{-1} for proto-III Mb, 6.5 (± 0.5) x 10^{-5} s^{-1} for proto-XIII Mb and 7.9 (± 0.5) x 10^{-4} s^{-1} for native Mb.

The present results strongly indicate that the difference in symmetry for the heme structure gives no significant change in Mb function.

References

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