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Redox Potential of BrCN-Modified Metmyoglobin/Deoxymyoglobin System

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A redox potential of the BrCN-modified metmyoglobin was determined spectrophotometrically to be 0.18 V by using $[Ru(NH_3)_6]^{2+}$ as a redox partner. The self-exchange rate constant for the modified myoglobin system was evaluated to be 1×10^4 dm^3 mol^{-1} s^{-1} based on the Marcus theory, being larger than that for its native system.

A metmyoglobin derivative modified with BrCN[1] is a useful model for myoglobin which lacks a distal histidylimidazole. In this derivative, the distal histidyl residue is cyanated and the sixth coordination site of the central iron atom is vacant. We have recently reported that modification of the heme's distal histidine by BrCN resulted in acceleration of the reduction rate of sperm whale metmyoglobin (metMb) by ascorbate and that the geometry change in the iron site from hexa- to pentacoordination upon reduction of the native metMb(H_2O) to deoxyMb is an important factor.[2] To elucidate the factors controlling the electron-transfer rate of the BrCN-modified metMb, we have attempted to measure the redox potential of this derivative. Redox potentials of hemoproteins have been usually measured electrochemically by use of mediators.[3] This method, however, cannot be applied to the BrCN-modified metMb of unstable nature, because it takes long time to attain the equilibrated potential. We report here the redox potential of this system determined spectrophotometrically in combination with the redox couple of $[Ru(NH_3)_6]^{3+/2+}$ system, and this result allows an estimation of the self-exchange rate constant of the BrCN-metMb/deoxyMb system based on the Marcus theory.

The BrCN-modified metMb was prepared in situ by the method previously described.[2] The titration of the native metMb with BrCN showed a 1:1

stoichiometry. In this work two-fold excess of BrCN was used, under which conditions the only one reaction was observed.[4] $[Ru(NH_3)_6]^{2+}$ was prepared by reduction of $[Ru(NH_3)_6]^{3+}$ with Zn(Hg) in the presence of an equimolar amount of Na_2H_2edta under argon atmosphere. Redox titration was carried out at 25 °C, pH=6.8 (a 0.1 mol dm^{-3} sodium phosphate buffer), an ionic strength (I) of 0.2 mol dm^{-3}, [metMb]=1.00 x 10^{-4} mol dm^{-3}, [Ru(II)]=(0-1.00) x 10^{-4} mol dm^{-3} and [Ru(III)]=(2.6-5.3) x 10^{-3} mol dm^{-3}. The reaction was very fast and the titration was completed within half an hour, while the BrCN-modified myoglobin was sufficiently stable.[5] Figure 1 shows the spectral change during the redox titration of the BrCN-modified metMb with $[Ru(NH_3)_6]^{2+}$.

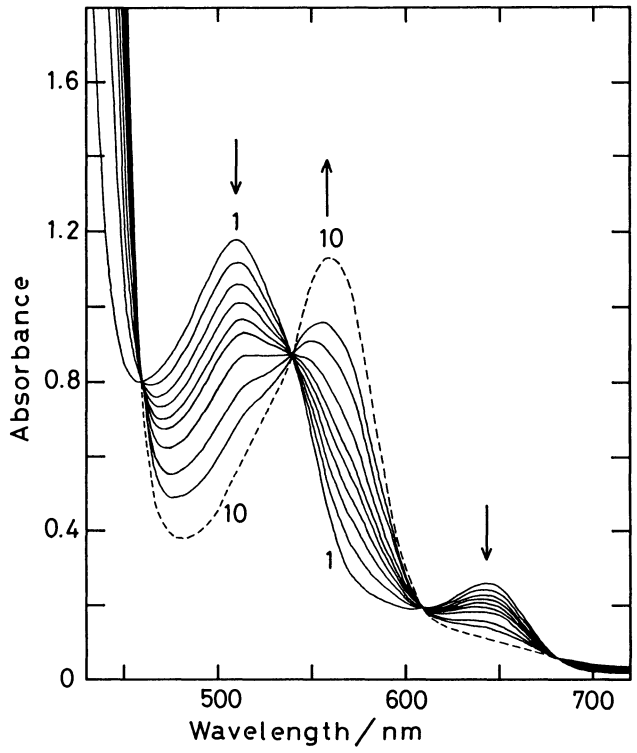

Fig. 1. Redox titration of BrCN-modified metMb (1.0 x 10^{-4} mol dm^{-3}) in the presence of $[Ru(NH_3)_6]^{3+}$ (5.3 x 10^{-3} mol dm^{-3}) with various amounts of $[Ru(NH_3)_6]^{2+}$; 0 (1), 0.14 (2), 0.27 (3), 0.41 (4), 0.54 (5), 0.67 (6), 0.94 (7), 1.32 (8), and 1.95 x 10^{-4} mol dm^{-3} (9). The broken line is the spectrum of deoxyMb.

The equilibrium constant (K) for the reaction,

$$\text{metMb} + [Ru(NH_3)_6]^{2+} \rightleftharpoons \text{deoxyMb} + [Ru(NH_3)_6]^{3+} \qquad (1),$$

was determined to be 160±40 from the slope of a plot of [deoxyMb]/[metMb] against [Ru(II)]/[Ru(III)] (Fig. 2). When the value of 0.05 V[6] was used for the redox potential of the $[Ru(NH_3)_6]^{3+/2+}$ couple, we obtained 0.18±0.01 V for the BrCN-

modified metMb/deoxyMb system. Absorption coefficients determined in this work were as follows: $\varepsilon_{510}=1.18 \times 10^4$ dm^3 mol^{-1} cm^{-1} and $\varepsilon_{560}=4.3 \times 10^3$ dm^3 mol^{-1} cm^{-1} for metMb, and $\varepsilon_{510}=5.6 \times 10^3$ dm^3 mol^{-1} cm^{-1} and $\varepsilon_{560}=1.13 \times 10^4$ dm^3 mol^{-1} cm^{-1} for deoxyMb.

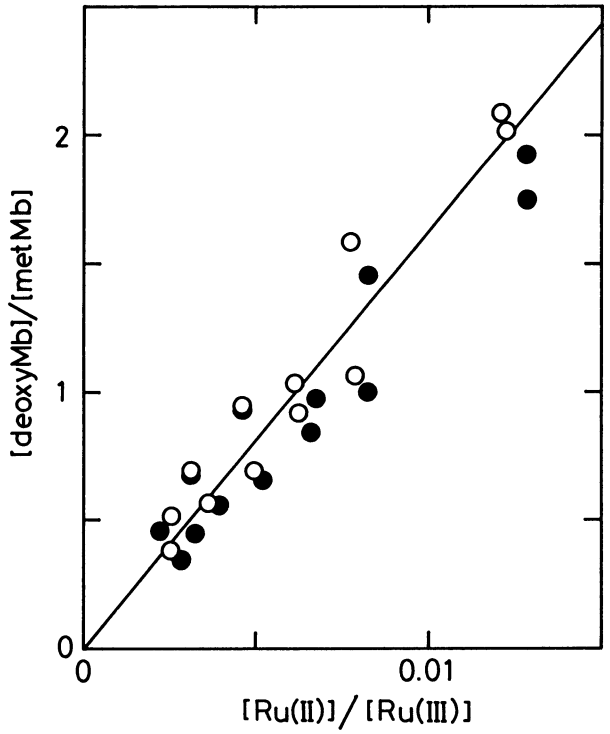

Fig. 2. Plot of [deoxyMb]/[metMb] against [Ru(II)]/[Ru(III)]: $[Mb]_T=1.0 \times 10^{-4}$ mol dm^{-3}, [Ru(III)]=(2.6-5.3) x 10^{-3} mol dm^{-3}, and [Ru(II)]=(0.6-6.8) x 10^{-5} mol dm^{-3} at pH=6.8, I=0.2 mol dm^{-3}, and 25 °C; Wavelengths used, 510 nm(o) and 560 nm(●).

The value of 0.18 V for the modified metMb system is comparable with that for the monomeric hemoglobin or myoglobin which lacks a distal histidylimidazole and a coordinated water molecule (0.13 V for Aplysia limacina Mb and Chironomous thummi Hb[7] and 0.15 V for Glycera dibranchiata Hb[8]). The redox potential of the native myoglobin (sperm whale and horse heart) is 0.06 V.[7] Therefore, it is apparent that the conversion of the pentacoordinated metMb to deoxyMb in the modified Mb is thermodynamically favorable process than that for the native metMb(H_2O).

The rate constant for reduction of the BrCN-modified metMb with $[Ru(NH_3)_6]^{2+}$ was determined under the following conditions: $[metMb]_0=[Ru(II)]_0=(0.5-1.0) \times 10^{-4}$ mol dm^{-3}, pH=6.8 (a 0.1 mol dm^{-3} sodium phosphate buffer), and I=0.2 mol dm^{-3} at 25 °C. The second-order rate constant was found to be $(1.8\pm0.2) \times 10^5$ dm^3 mol^{-1} s^{-1}. The self-exchange rate constant for the BrCN-modified metMb/deoxyMb system was evaluated to be 1×10^4 dm^3 mol^{-1} s^{-1} based on the Marcus equation.[9] This value

is much larger than that for the native metMb(H_2O)/deoxyMb system (1 dm^3 mol^{-1} s^{-1}).[11]

These results show that the pentacoordinated geometry in the iron site of the BrCN-modified metMb favors the reduction kinetically and thermodynamically as compared with its native form which requires Fe^{III}-OH_2 bond breaking.

References

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3) J. F. Taylor, Methods in Enzymology, 76, 577 (1981).

4) When the concentration of BrCN was less than that of metMb, two reactions were observed—faster for the BrCN-modified metMb and slower for the native metMb.

5) Absorption spectrum of the BrCN-modified metMb did not change when $[Ru(NH_3)_6]^{3+}$ was added up to 5.3 x 10^{-3} mol dm^{-3}. However, the absorption intensity was depressed slightly in the presence of 1 x 10^{-2} mol dm^{-3} of $[Ru(NH_3)_6]^{3+}$.

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