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Effect of the Chemical Modification by Viologen on the Reduction of Metmyoglobin

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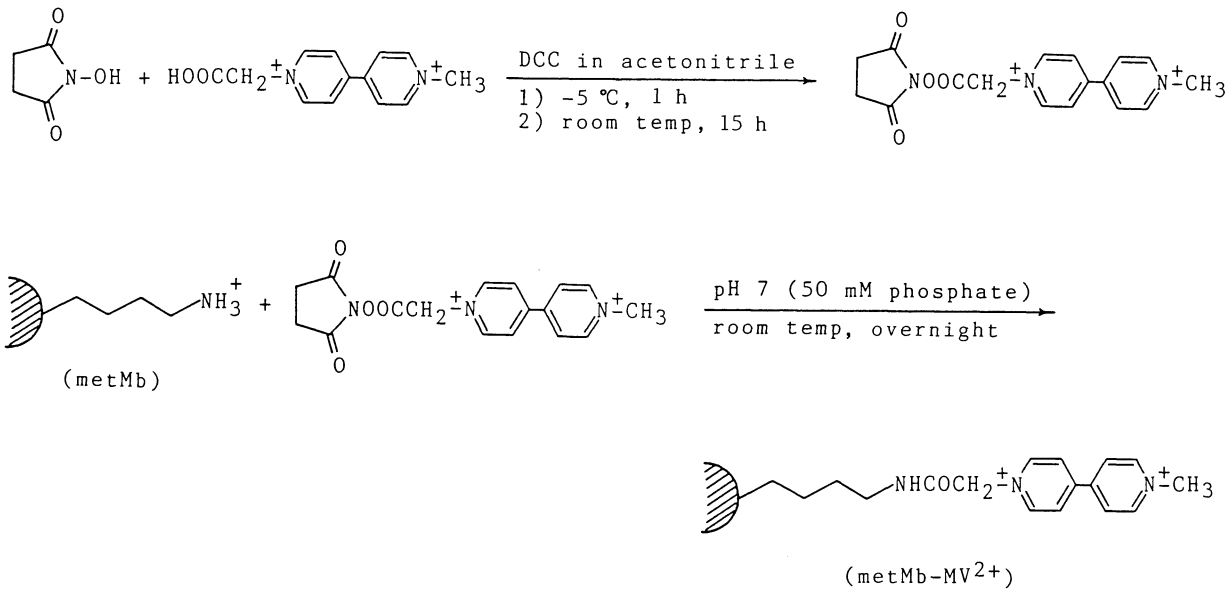
Metmyoglobin covalently linked with viologen was prepared and reduced by dithionite ions faster than the native metmyoglobin, suggesting that the reduction by dithionite of the attached viologen was followed by a rapid intramolecular electron transfer from the viologen radical cation to the heme iron center.

Chemical modification of hemoproteins is a very useful technique for elucidating electron-transfer pathways in biological redox systems. Some approaches have been reported on the modification of cytochrome c, myoglobin, and blue copper proteins with redox active reagents such as ruthenium complexes[1] and a cobalt(III) cage complex.[2] Viologens are diquaternary salts of 4,4'-bipyridine which have been widely used as redox indicators in biological redox systems and as mediators in catalytic photolysis of water.[3] In this letter we report on the first example of the modified metmyoglobin covalently linked with viologen which undergoes a facile redox reaction with external electron donors.

Horse heart muscle metmyoglobin (metMb; Fe(III) form of Mb) possesses 19 lysine residues among which 14 residues are located on the surface of the protein. It is, therefore, possible to modify the lysine residue(s) with the viologen containing carboxylic acid by using 1,3-dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide. Modification of metmyoglobin (Sigma) with viologen was carried out by the method as is shown in Scheme 1.[4,5] Metmyoglobin (0.2 g in 1 cm^3 of 50 mM[6] phosphate buffer) was treated with 10-fold excess of succinimide ester of 1-methyl-1'-carboxymethyl-4,4'-bipyridinium perchlorate in 1 cm^3 of 50 mM phosphate buffer (pH 7.0) at room temperature overnight. After the solution was dialyzed against a 10 mM phosphate buffer (pH 6.3), unreacted viologen was removed by using a Sephadex G-75 column chromatography. After native metMb was eluted with a 30 mM phosphate buffer at pH 6.3 on a Whatman CM-52 cellulose



Scheme 1.

column, the modified myoglobin was eluted with a 20 mM phosphate buffer at pH 7.0; yield, 1.5%. The absorption spectra are shown in Fig. 1. Absorbance ratios are as follows: A_{409}/A_{270} = 3.30 for the modified metmyoglobin (metMb-MV^{2+}) and A_{409}/A_{280} = 5.60 for native metMb. It is found that the modified metmyoglobin contains equimolar amounts of metmyoglobin and viologen on the basis of the calculation of the concentration of hemin and viologen from the absorption maxima at 409 nm and 270 nm, respectively.[7] When metMb-MV^{2+} was treated with sodium dithionite in excess under Ar atmosphere, deoxymyoglobin (deoxyMb; Fe(II) form of Mb) with the viologen radical cation was observed (Fig. 1). The difference absorption spectrum between the fully reduced modified myoglobin (deoxyMb-$MV^{+\cdot}$) and native deoxyMb shows the characteristics of viologen radical cations (Fig. 1B),[3] indicating also the presence of equimolar amounts of viologen and myoglobin.

The reduction of metMb-MV^{2+} by dithionite was followed by a Photal RA-401 stopped-flow spectrophotometer at pH 7.0 (10 mM phosphate buffer), I = 0.02 M, and 25 ℃ (Fig. 2). The decay of metmyoglobin and the formation of deoxymyoglobin for the modified system were of single exponential and were faster than those for the native myoglobin. The rate was linearly dependent on the concentration of $SO_2^{-\cdot}$ which was calculated from the known value of the dissociation constant of $S_2O_4^{2-}$ (K_D = 1.4 x 10^{-9} M):[8]

$$S_2O_4^{2-} \rightleftharpoons 2SO_2^{-\cdot} \qquad K_D \qquad (1)$$

$$-d[Fe(III)]/dt = d[Fe(II)]/dt = kK_D^{1/2}[S_2O_4^{2-}]_0^{1/2}[Fe(III)] \qquad (2)$$

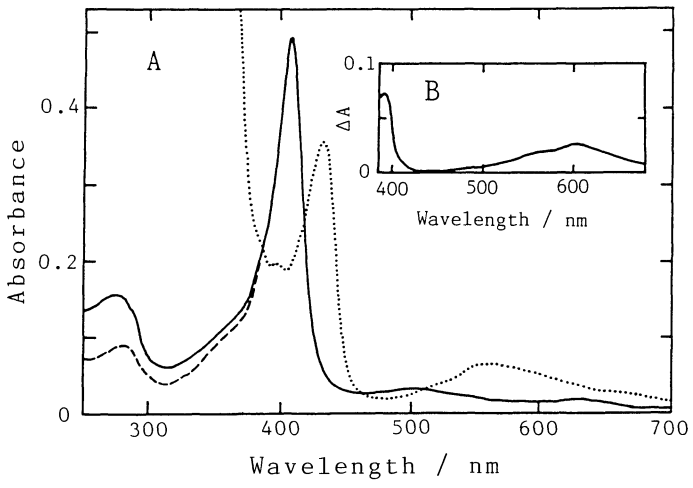


Fig. 1A. Absorption spectra of metMb-MV^{2+} (——), native metMb (---), and deoxyMb-$MV^{+\cdot}$ (·····) at pH 7.0 (20 mM phosphate). The concentration of myoglobins is 2.6×10^{-6} M.

Fig. 1B. The difference absorption spectrum between deoxyMb-$MV^{+\cdot}$ and native deoxyMb.

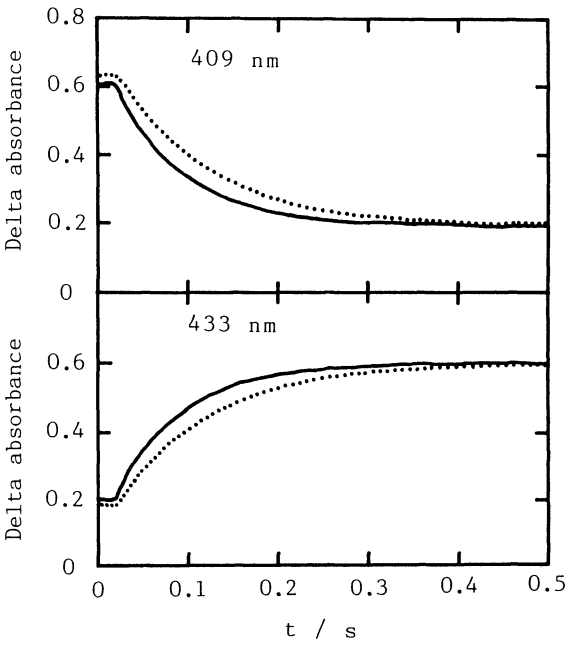


Fig. 2. The absorbance changes during the reaction of metMb-MV^{2+} (——) and native metMb (·····) with dithionite ions at pH 7.0 (10 mM phosphate buffer), $[metMb]_0$ = 3.85×10^{-6} M, $[S_2O_4^{2-}]_0$ = 2.50×10^{-3} M, I = 0.02 M, and 25 °C.

The second-order rate constant of the $SO_2^{-\cdot}$ reduction of metMb-MV^{2+} was determined to be $(7.2\pm0.3) \times 10^6$ M^{-1} s^{-1}, being larger than that for native metMb under the same experimental conditions ($(5.4\pm0.3) \times 10^6$ M^{-1} s^{-1}). The reaction scheme may be represented as follows:

$$\text{metMb-}MV^{2+} + SO_2^{-\cdot} \longrightarrow \text{deoxyMb-}MV^{2+} + SO_2 \qquad k_0 \quad (3)$$

$$\text{metMb-}MV^{2+} + SO_2^{-\cdot} \longrightarrow \text{metMb-}MV^{+\cdot} + SO_2 \qquad k_1 \quad (4)$$

$$\text{metMb-}MV^{+\cdot} \longrightarrow \text{deoxyMb-}MV^{2+} \qquad \text{fast} \quad (5)$$

$$\text{deoxyMb-}MV^{2+} + SO_2^{-\cdot} \longrightarrow \text{deoxyMb-}MV^{+\cdot} + SO_2 \qquad k_2 \quad (6)$$

Here, $k = k_0 + k_1$. The intramolecular electron transfer (Reaction 5) might be faster than the reductions by $SO_2^{-\cdot}$ ion.[9] If the rate of Reaction 3 is comparable with that of the reaction of native metMb ($k_0 \simeq 5.4 \times 10^6$ M^{-1} s^{-1}), the value of k_1 can be estimated to be 1.8×10^6 M^{-1} s^{-1}. This is a reasonable value for the reduction of viologen by $SO_2^{-\cdot}$ ions.[10]

In conclusion we have prepared the metmyoglobin covalently linked with methylviologen and found the increase of the reduction rate by dithionite

of viologen-linked metmyoglobin. The modified metmyoglobin with viologen prepared in this work must contain several isomers where viologen is bound to the different site of lysine residue. We are trying to separate these isomers and to investigate the electron-transfer reactions.

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2) D. W. Conrad and R. A. Scott, J. Am. Chem. Soc., 111, 3461 (1989).
3) K. Tsukahara, Trends Inorg. Chem., 2, 17 (1991).
4) 1-Methyl-1'-carboxymethyl-4,4'-bipyridinium perchlorate was synthesized by the reaction of bromoacetic acid ethyl ester with 1-methyl-4,4'-bipyridinium iodide in methanol, followed by an acid hydrolysis and by an SP-Sephadex C-25 (Na^+ form) column chromatography (a $NaClO_4$ solution as an eluent). Satisfactory results are obtained for an elementary analysis and a ^{1}H-NMR spectrum (unpublished results). Cyclic voltammetry at a glassy carbon electrode in a 10 mM phosphate buffer (pH 7.0): E_1^0 = -0.42 V and E_2^0 = -0.78 V vs. NHE.
5) Succinimide ester of 1-methyl-1'-carboxymethyl-4,4'-bipyridinium perchlorate: ^{1}H-NMR (270 MHz, CD_3CN) δ2.83 (4H, s, CH_2CH_2), 4.43 (3H, s, CH_3), 5.98 (2H, s, CH_2), 8.45 (2H, d, J 6.0 Hz, bpy), 8.54 (2H, d, J 7.3 Hz, bpy), 8.92 (2H, d, J 6.8 Hz, bpy), and 9.04 (2H, d, J 6.8 Hz, bpy).
6) 1 M = 1 mol dm^{-3}.
7) Molar absorption coefficients for metMb and viologen at pH 7.0: ε_{270} = 3.10 x 10^4 M^{-1} cm^{-1}, ε_{280} = 3.36 x 10^4 M^{-1} cm^{-1}, and ε_{409} = 1.88 x 10^5 M^{-1} cm^{-1} for metMb and ε_{270} = 1.95 x 10^4 M^{-1} cm^{-1} for 1-methyl-1'-carboxymethyl-4,4'-bipyridinium perchlorate.
8) D. O. Lambeth and G. Palmer, J. Biol. Chem., 248, 6095 (1973).
9) The rate of reaction monitored at 600 nm, which is an absorption maximum of the viologen radical cation, was also first-order and the same as those monitored at 409 and 433 nm.
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