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Effect of the Modification of the Heme Distal Side and of the Heme Propionates on the Reduction by Ascorbate of Metmyoglobin

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The modification of the heme distal histidylimidazole by cyanogen bromide or of the heme propionates to their methyl esters accelerated the reduction rate by ascorbate of metmyoglobin compared with its native form, suggesting that the structural change of the iron site from hexa- to penta-coordination is an important factor.

Reduction kinetics of metmyoglobins have been studied extensively using mostly inorganic reagents and a few organic reductants.[1-3] However, the reaction mechanism has been still uncertain. In the reduction of metmyoglobin (metMb) to deoxymyoglobin (deoxyMb), the structural change of the iron site must be contained: from the hexa-coordinated metMb to the penta-coordinated deoxyMb. The X-ray crystallographic study of sperm whale metMb has shown that the distal histidylimidazole makes a hydrogen bond to the coordinated water and that the heme propionates make hydrogen bonds to Arg45 and His97, thereby stabilizing the structure of the heme pocket.[4] So we have examined the kinetics of the reductions by ascorbate (HA^- and A^{2-}) of the metMb, where the distal histidylimidazole is modified by cyanogen bromide (BrCN) or the heme propionates are replaced by their methyl esters. Cyanogen bromide is a useful reagent for the modification of the distal histidylimidazole, which is recently confirmed by NMR studies by Morishima et al.[5]

The acid-dissociation constant of the coordinated water of metMb reconstituted with protoheminDME[6] was determined spectrophotometrically (pK_a=7.90±0.15 at 25 °C and an ionic strength (I) of 0.30 mol dm^{-3} (NaCl) in a 0.2 mol dm^{-3} Tris/HCl buffer; Tris=tris(hydroxymethyl)aminomethane). The reduction kinetics were carried

out under the pseudo first-order conditions ($[metMb]_0=(0.1-1.0)\times10^{-4}$ mol dm^{-3} and $[NaHA]_0=(0.1-1.5)\times10^{-2}$ mol dm^{-3}) at 25 °C, I=0.30 mol dm^{-3} (NaCl), and pH 7.19-7.87 (a 0.20 mol dm^{-3} Tris/HCl buffer) in an Ar atmosphere. The reaction was followed at 423 nm or 556 nm and the plots of $-\ln(A_\infty-A_t)$ vs. t were linear for at least 80% completion, where A_∞ and A_t represent the absorbance at infinity and time t, respectively. The observed first-order rate constants were linearly dependent on the initial concentrations of ascorbate ($[NaHA]_0$). From the same analysis cited in the previous work,[2] the second-order rate constants were obtained: $k_1=(3.0\pm0.5)\times10^{-1}$ dm^3 mol^{-1} s^{-1} and $k_2=(1.4\pm0.1)\times10^4$ dm^3 mol^{-1} s^{-1} for the reductions by HA^- and A^{2-} of metMb(H_2O) reconstituted with protoheminDME, respectively. These rate constants are 25- to 200-fold greater than those for the native metMb, $k_1=(1.2\pm0.2)\times10^{-2}$ dm^3 mol^{-1} s^{-1} and $k_2=(6.9\pm0.8)\times10$ dm^3 mol^{-1} s^{-1},[2] and for the reconstituted metMb with protohemin, $k_1=(1.2\pm0.2)\times10^{-2}$ dm^3 mol^{-1} s^{-1} and $k_2=(5.6\pm1.0)\times10$ dm^3 mol^{-1} s^{-1}.

When the native metMb was treated with one- to two-fold excess of BrCN, the absorbance at 504 nm increased, reached to the maximum in about 15 min (with isosbestic points at 573 nm, 652 nm, and 695 nm), and then gradually decreased (Fig. 1A). Therefore, the solution of ascorbate was injected at t=15 min after BrCN had been added and the solution had been purged with an Ar gas. The reduction by ascorbate was completed within 5 min under the present experimental conditions (Fig. 1B);[9] the BrCN-modified metMb was sufficiently stable for the reduction by ascorbate to go to completion. The second-order rate constants were much greater than those for the native metMb: $k_1=3.4\pm0.2$ dm^3 mol^{-1} s^{-1} and $k_2=(2.1\pm0.1)\times10^4$ dm^3 mol^{-1} s^{-1}. It is suggested by the NMR studies that the distal histidylimidazole is modified with BrCN and that the BrCN-modified metMb has no coordinated water on the heme distal side.[5] We also confirmed it by the fact that the spectra of the BrCN-modified metMb did not change over the pH range from 6 to 8. Therefore, it is considered that the penta-coordinated BrCN-modified metMb is easy to change by ascorbate to the penta-coordinated deoxymyoglobin.

The pK_a of the coordinated water of the metMb reconstituted with protoheminDME decreases to 7.90 from 9.16 of the native one. Since the electron density on Fe(III) is considered to decrease with a decrease in pK_a of metMb(H_2O), it is likely that an electron of ascorbate is more easily transferred to Fe(III). The decrease in pK_a may be ascribable to weakening the hydrogen bond of the distal

imidazole to the coordinated water or changing the coordination nature of the proximal imidazole, arisen from the modification of the heme propionates to their methyl esters.

In conclusion it is considered that the important factor of the reduction rate of metMb is the structural change of the iron site from the hexa-coordinated metMb(H_2O) to the penta-coordinated deoxyMb. This may explain the slowness of the self-exchange rate of the native metMb/deoxyMb compared with its model system.[10]

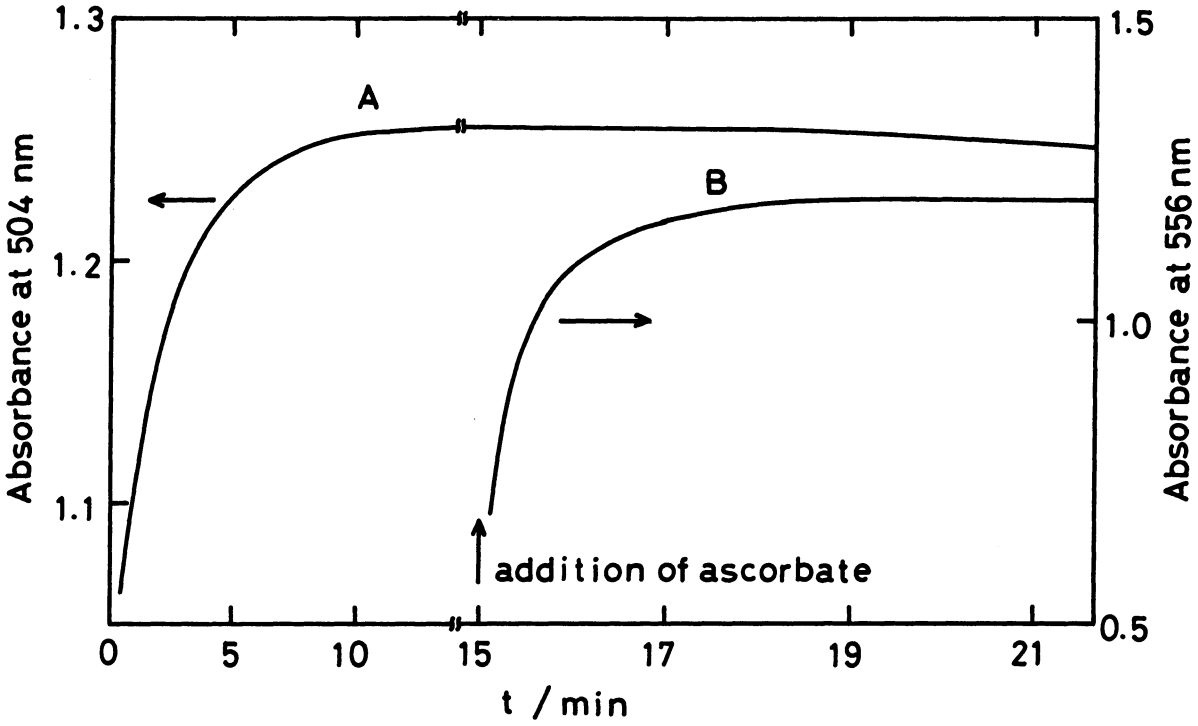

Fig. 1. The absorbance changes during the reaction of metMb with BrCN and the reduction by ascorbate of the BrCN-modified metMb. A: the reaction of metMb with a two-fold excess of BrCN. B: the reduction by ascorbate of the BrCN-modified metMb at 25 °C, I=0.30 mol dm^{-3} (NaCl), pH=7.87 (a 0.20 mol dm^{-3} Tris/HCl buffer), $[metMb]_0$=1.0x10^{-4} mol dm^{-3}, and $[NaHA]_0$=1.0x10^{-3} mol dm^{-3}.

References

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6) ChloroprotoporphyrinIX dimethyl ester iron(III) (protoheminDME) was prepared by incorporation of iron into the porphyrin dimethyl ester and purified by the method in the literature.[7] Recombination of protoheminDME with apomyoglobin was carried out by the method in the literature[8] with a slight modification. The reaction mixture of protoheminDME with apomyoglobin was stood in a refrigerator for 24 h and was subjected to a dialysis and to a CM-52 cellulose column chromatography.

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9) When air was introduced into the product solution (BrCN-modified deoxyMb, λ_{max}= 559 nm), the α- and β-bands (λ_{max}=575 nm and λ_{max}=540 nm), characteristic of MbO_2, were detected and this was stable for a few hours. It is suggested that the redox catalysis must be contained: the autoxidation of BrCN-modified MbO_2 to metMb is followed by the reduction by ascorbate to deoxyMb, which is then converted to MbO_2.

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