Coordination Geometry of Haem in Cyanogen Bromide Modified Myoglobin and its Effect on the Formation of Compound I

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By use of nuclear relaxation time measurements of the water proton, it is demonstrated unambiguously that water is not coordinated to the haem iron in BrCN-modified myoglobin; by stopped-flow measurements it is also shown that the rate of formation of compound I in the modified Mb is very similar to that in horseradish peroxidase, having similar coordination geometry.

The distal histidine (His) is known to play an important role in controlling the haem reactivities in haemoproteins.[1-3] Its effects on ligand binding are shown in the kinetic properties of mutant myoglobin (Mb) and haemoglobin (Hb) in which the distal His is replaced by both amino acid residues.[4,5] Chemical modification of the distal His in haemoproteins is, therefore, expected to affect the properties of the haem prosthetic group.

Morishima *et al.*[2,3] have recently chemically modified the distal His of sperm whale metmyoglobin by cyanogen bromide (BrCN) and have indirectly inferred from the hyperfine-shifted haem NMR resonances that the water molecule coordinated to the haem iron at the sixth coordination site is expelled upon BrCN modification, making the haem iron in the modified Mb five-coordinated. Such five-coordinated haem structure is known to be present in horseradish peroxidase (HRP) and lactoperoxidase (LPO).[6,7]

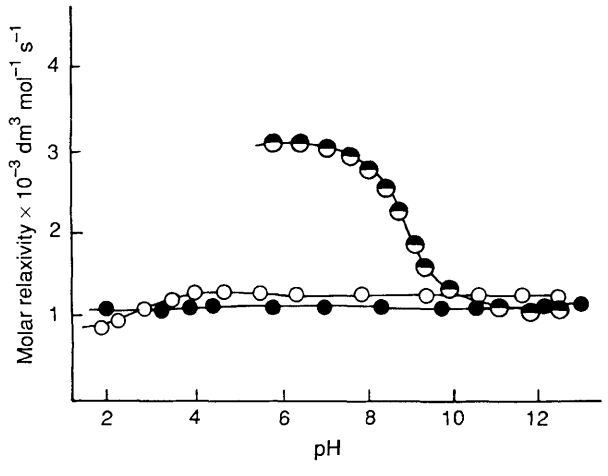

Fig. 1 Variation of molar relaxivity with pH for metMb(◓), HRP(○) and CNBr-modified Mb(●)

By use of nuclear relaxation time measurements of water proton molecule, we demonstrate here directly and unambiguously that the water molecule is indeed not coordinated to the haem iron in the BrCN-modified Mb. We further show that the rate of formation of compound I in the modified Mb is similar to that in HRP but distinctly different from that in the native Mb.

Sperm whale metmyoglobin obtained from Sigma was further purified.[3] The modification of metMb by BrCN was carried out by the method described previously.[2,3] The paramagnetic spin-lattice relaxation time (T_{1p}) was measured on a Bruker 500 MHz NMR machine using inversion recovery method with the pulse sequence (180°–τ–90°).[8,9] The molar relaxivity is expressed as[7,8] in eqn. (1),

$$\text{Molar relaxivity} = \frac{1}{T_{1p}[\text{Enz}]} = \frac{q}{111}(T_{1m} + \tau_m)^{-1} \qquad (1)$$

where q is the number of exchangeable water protons in the first coordination sphere, T_{1m} is the relaxation time of the bound water molecule and τ_m is the residence time of the protons in the bound state. T_{1m} is obtained from eqn. (1) in the limit of fast exchange (which was shown to exist here). The distance between the haem iron and the water proton is

obtained from eqn. (2),[7,9] where τ_c is the correlation time and other terms are as defined earlier.

$$r = 813 \left[T_{1m} q \left\{ \frac{3\tau_c}{1 + \omega_I^2 \tau_c^2} + \frac{7\tau_c}{1 + \omega_S^2 \tau_c^2} \right\} \right]^{1/6} \quad (2)$$

Fig. 1 summarises the pH dependence of the molar relaxivity of the water proton in the BrCN-modified Mb, HRP and native Mb. Using the values of relaxivity at pH = 7.0, the distance between the water proton and the ferric ion (r) was calculated from eqn. (2), which gave r = 2.7, 3.8 and 3.7 Å for metMb, modified Mb and HRP respectively. The value of r = 2.7 Å in metMb compares well with the value of 2.67 Å obtained from X-ray structural analysis. The 3.8 Å distance between the water proton and the haem iron clearly excludes the possibility of the water molecule being coordinated to the haem iron in the modified Mb, as is also true for HRP.

The pH curves further support the above conclusion (Fig. 1). The molar relaxivity of the modifed Mb is observed to be almost constant over the entire pH range and shows close similarity to that of HRP. The behaviour of native metMb is, however, strikingly different, showing drastic changes in the relaxivity in the pH range 7.0–9.5 which is known[11] to be associated with the aquo ↔ hydroxo transition of the coordinated water with pK_a = 8.8.

The foregoing results unambiguously establish that the water molecule is not coordinated to the haem iron in the BrCN-modified Mb, the sixth coordination site of the haem being vacant. In this respect the haem coordination geometry of the modified Mb resembles closely that of HRP (and LPO). The vacant sixth coordination site in the modified Mb and HRP is, therefore, quite favourable for the binding of hydrogen peroxide to the haem iron, which is postulated[12] to be essential for the formation of compound I. The rate of formation of compound I from the parent BrCN-modified Mb was determined by the stopped-flow method monitored at 403 nm by keeping the myoglobin concentration constant at 2×10^{-8} mol dm^{-3} and varying the concentration of hydrogen peroxide between 2×10^{-7} to 12×10^{-7} mol dm^{-3}. The rate was determined to be 5.2×10^7 dm^3 mol^{-1}, which is close to the value[13] of 1.2×10^7 dm^3 mol^{-1} s^{-1} for HRP, but significantly different[14] from that of native metMb (1.0×10^3 dm^3 mol^{-1} s^{-1}). The nature of the coordination geometry around the haem iron is, therefore, important in controlling the rate of formation of compound I in these haemoproteins.

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