

BIOCHEMICAL LETTERS

A controversy on the mechanism of autoxidation of oxymyoglobin and oxyhaemoglobin: oxidation, dissociation, or displacement?

The stability properties of the iron(II)-dioxygen bond in Mb and Hb are of particular importance, since the oxygenated form is known to be oxidized easily to the ferric met-form, which cannot be oxygenated and is therefore physiologically inactive. Since the early work of Brooks (1931, 1935) on HbO₂ and that of George & Stratmann (1952, 1954) on MbO₂, it has been observed that the autoxidation rate increases with decreasing partial pressure of O₂ and increases with increasing H⁺ concentration. Several proposals have therefore been made concerning the mechanism of this autoxidation reaction. These can be classified into the following three types that provide an interesting basis for future study.

(a) *Oxidation.* Along with the early work, Brown & Meibine (1969) and Wallace *et al.* (1982), among others, all agree that the first step in autoxidation of MbO₂ and HbO₂ is the dissociation of the oxygen ligand followed by the oxidation of the deoxy species by free O₂ to produce metMb or metHb and the superoxide anion. In the case of myoglobin, therefore, one may write the autoxidation reaction as



In this scheme, however, the differences in the deoxy species which allow them to react with oxygen in one instance to become oxygenated (eqn. 1) and in another instance to become oxidized (eqn. 2) are completely unknown (Snyder, 1963). Wallace *et al.* (1974, 1982) presented the idea that anion binding to the deoxy species mediates one-electron transfer from iron(II) to free O₂ through the porphyrin or aromatic amino acid residues of the protein, and that under physiological conditions one of the most potential anions is Cl⁻.

At this point it should be noted that free dioxygen is a poor electron acceptor with a lower redox potential, $E'_0(\text{O}_2/\text{O}_2^{\cdot -}) = -0.27$ to -0.33 V

Abbreviations used: Hb, haemoglobin; Mb, myoglobin.

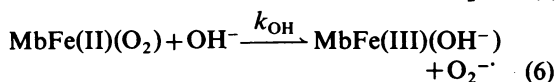
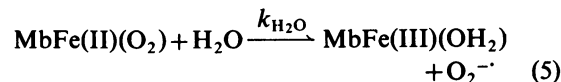
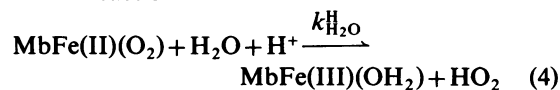
(Sawada *et al.*, 1975), than that, $E'_0 = +0.046$ V, for the MbFe(III)/MbFe(II) system (Taylor & Morgan, 1942). Furthermore, evidence for free dioxygen as the real and the sole oxidant for Mb and Hb at low O₂ levels still remains indirect.

(b) *Dissociation.* Weiss (1964) proposed that oxyHb could be described as a superoxo-ferrahaem complex formed by electron transfer from iron to bound dioxygen. Therefore, it has been suggested that a superoxide anion may be split off directly from the iron during autoxidation of MbO₂ or HbO₂ to its met-form, i.e. that autoxidation is essentially the dissociation of O₂^{·-}:



In fact, generation of O₂^{·-} has been demonstrated during autoxidation of shark HbO₂ (Misra & Fridovich, 1972), bovine HbO₂ (Wever *et al.*, 1973), isolated α - and β -chains of human HbO₂ (Brunori *et al.*, 1975), and bovine MbO₂ (Gotoh & Shikama, 1976). As a direct electron-acceptor from iron(II), the co-ordinated O₂ would be much more acceptable than free O₂. Nevertheless, this scheme seems to be too simple to provide any basis for interpretation of the pH- or dioxygen-dependence of the autoxidation rate. Since the spontaneous dissociation of O₂^{·-} from the FeO₂ centre in hydrophobic haem environments is an energetically unfavourable process (George, 1961), there must be involved some specific mechanism that makes it possible to produce O₂^{·-} from the FeO₂.

(c) *Displacement.* Under air-saturated conditions, the rate of autoxidation of MbO₂ to metMb increases rapidly with increasing H⁺ concentration, a rate minimum appears at about pH 9 and a small increase occurs at higher pH values (Yamazaki *et al.*, 1964; Gotoh & Shikama, 1974; Shikama & Sugawara, 1978). Kinetic and thermodynamic analyses of this pH-dependence have recently revealed that the following three types of displacement processes are involved in the autoxidation reaction:



where for bovine MbO₂ the values of $k_{\text{H}_2\text{O}}^{\text{H}} = 0.25 \times 10^4 \text{ M}^{-2} \cdot \text{h}^{-1}$, $k_{\text{H}_2\text{O}} = 0.47 \times 10^{-4} \text{ M}^{-1} \cdot \text{h}^{-1}$, and $k_{\text{OH}} = 0.18 \times 10^2 \text{ M}^{-1} \cdot \text{h}^{-1}$ were obtained in the neutral pH range at 25°C (Sugawara & Shikama, 1980). The extent of contribution of these elementary processes can vary with changes in concentrations of H⁺ and OH⁻ ions, and can give rise primarily to a strong pH-dependence of the overall autoxidation rate.

It becomes thus quite evident that the proton-catalysed process with the rate constant $k_{\text{H}_2\text{O}}^{\text{H}}$ promotes mainly the autoxidation reaction of MbO₂ above the spontaneous process in water with the rate constant $k_{\text{H}_2\text{O}}$. In fact, the catalytic proton enhances the rate by a factor of $5 \times 10^7/\text{mol}$. In this proton catalysis (eqn. 4), the distal histidine, which forms a hydrogen bond to the bound dioxygen (Phillips & Schoenborn, 1981), appears to participate in facilitating the effective movement of a catalytic proton from the solvent to the bound dioxygen via its imidazole ring by a proton-relay mechanism (Sugawara & Shikama, 1980; Suzuki & Shikama, 1983). This proton transfer can lead to a favorable displacement of O₂^{-·} as the hydroperoxyl radical HO₂, which departs and, since its pK_a is 4.8 (Fridovich, 1975), then dissociates into the superoxide anion and a catalytic proton again.

To elucidate further the molecular mechanism of these substitution reactions (eqns. 4–6) leading to metMb formation *in vivo*, Satoh & Shikama (1981) have studied the oxidation of MbO₂ induced by excess anion. The anions examined were SCN⁻, F⁻, OCN⁻, N₃⁻ and CN⁻, whose nucleophilicity differs from that of H₂O and OH⁻. In each case, the observed oxidation rate was linearly dependent upon the concentration of an added anion. A Brønsted plot for the series showed that the rates correlated with the pK_a of the conjugate acid, a measure of the nucleophilicity of the anion. These results clearly indicate that the mechanism of autoxidation is not a simple, dissociative loss of O₂^{-·} from MbO₂. Rather, the oxidation of MbO₂ proceeds by way of a nucleophilic attack of anions at the iron centre; only in the presence of attacking nucleophiles is a full charge transfer from Fe(II) to O₂ produced. They also concluded that, as the most common nucleophiles *in vivo*, both H₂O and OH⁻ can react with MbO₂, and thereby displace the bound dioxygen in the form of O₂^{-·} so that the iron is converted to the ferric form.

Unfortunately it seems that there is no provision in this scheme for the inverse dependence of the autoxidation rate upon oxygen pressure. In this respect, however, it is very interesting to note that H₂O₂ can oxidize deoxyMb more than 100 times more easily than can oxyMb (Yusa, 1984). Since

H₂O₂ may be produced by dismutation of the superoxide anion generated from autoxidation of the oxy-form, it must act as at least one of the potent oxidants of the deoxy-form that increases with decreasing O₂ pressures. This idea also seems to be attractive for a possible interpretation of the oxygen dependence, and remains open to future study.

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- Brooks, J. (1931) *Proc. R. Soc. London Ser. B* **109**, 35–50
 Brooks, J. (1935) *Proc. R. Soc. London Ser. B* **118**, 560–577
 Brown, W. D. & Mebine, L. B. (1969) *J. Biol. Chem.* **244**, 6696–6701
 Brunori, M., Falcioni, G., Fioretti, E., Giardina, B. & Rotilio, G. (1975) *Eur. J. Biochem.* **53**, 99–104
 Fridovich, I. (1975) *Annu. Rev. Biochem.* **44**, 147–159
 George, P. (1961) in *Haematin Enzymes* (Falk, J. E., Lemberg, G. & Morton, R. K., eds.), p. 103, Pergamon, Oxford
 George, P. & Stratmann, C. J. (1952) *Biochem. J.* **51**, 418–425
 George, P. & Stratmann, C. J. (1954) *Biochem. J.* **57**, 568–573
 Gotoh, T. & Shikama, K. (1974) *Arch. Biochem. Biophys.* **163**, 476–481
 Gotoh, T. & Shikama, K. (1976) *J. Biochem. (Tokyo)* **80**, 397–399
 Misra, H. P. & Fridovich, I. (1972) *J. Biol. Chem.* **247**, 6960–6962
 Phillips, S. E. V. & Schoenborn, B. P. (1981) *Nature (London)* **292**, 81–82
 Satoh, Y. & Shikama, K. (1981) *J. Biol. Chem.* **256**, 10272–10275
 Sawada, Y., Iyanagi, T. & Yamazaki, I. (1975) *Biochemistry* **14**, 3761–3764
 Shikama, K. & Sugawara, Y. (1978) *Eur. J. Biochem.* **91**, 407–413
 Snyder, H. E. (1963) *Biochim. Biophys. Acta* **69**, 200–202
 Sugawara, Y. & Shikama, K. (1980) *Eur. J. Biochem.* **110**, 241–246
 Suzuki, T. & Shikama, K. (1983) *Arch. Biochem. Biophys.* **224**, 695–699
 Taylor, J. F. & Morgan, V. E. (1942) *J. Biol. Chem.* **144**, 15–20
 Wallace, W. J., Maxwell, J. C. & Caughey, W. S. (1974) *FEBS Lett.* **43**, 33–36
 Wallace, W. J., Houtchens, R. A., Maxwell, J. C. & Caughey, W. S. (1982) *J. Biol. Chem.* **257**, 4966–4977
 Weiss, J. J. (1964) *Nature (London)* **202**, 83–84
 Wever, R., Oudega, B. & Van Gelder, B. F. (1973) *Biochim. Biophys. Acta* **302**, 475–478
 Yamazaki, I., Yokota, K. & Shikama, K. (1964) *J. Biol. Chem.* **239**, 4151–4153
 Yusa, K. (1984) M.Sc. Thesis, Tohoku University