et al. (1969) during xanthine oxidase-catalysed oxidation of substrates by oxygen at pH 10. They employed a special method to freeze the reaction solution within a few milliseconds and to cool it to temperatures (below -80°C) at which the radical is stable. Work with less direct methods had earlier indicated that O_2^- might be formed by xanthine oxidase (e.g. Fridovich & Handler, 1958; Totter, Dugros & Riviero, 1960). From the results of Komai, Massey & Palmer, (1969) it seems clear that it is the flavin of the reduced enzyme molecule that reacts with oxygen in producing this radical. Indeed, this group has recently shown that reduced free flavins also give O_2^- (Ballou, Palmer & Massey, (1969). O_2^- has also been detected in the oxidation of reduced ferredoxin from *Clostridium*, but not of that from spinach (Nilsson, Pick & Bray, 1969; Orme-Johnson & Beinert, 1969). Finally there is now electron-paramagnetic-resonance evidence, both direct and indirect, for the participation of O_2^- in the oxidase action of peroxidase (Nilsson *et al.* 1969; see also Ohnishi, Yamazaki, Iyanagi, Nakamura & Yamazaki, 1969). The radical apparently arises here from reaction of a substrate radical with an oxygen molecule. It may be reasonable to generalize that biochemical generation of O_2^- depends on low redox potentials rather than on specific enzyme structures. O_2^- itself seems to be unreactive and its fate is probably generally disproportionation to H_2O_2 and O_2 (cf. Nilsson *et al.* 1969).

OH is the active hydroxylating species in Fenton's reagent (Norman & Lindsay-Smith, 1965). However, it appears that any direct evidence on possible OH involvement in normal biological systems will have to wait for new methods to become available.

- Ballou, D., Palmer, G. & Massey, V. (1969). *Biochem. biophys. Res. Commun.* **36**, 898.
 Bravati, J. A., Symons, M. C. R., Tinling, D. J. A., Wardale, H. W. & Williams, D. O. (1967). *Trans. Faraday Soc.* **63**, 2112.
 Czapski, G. & Dorfman, L. M. (1964). *J. phys. Chem., Ithaca*, **68**, 1169.
 Fridovich, I. & Handler, P. (1958). *J. biol. Chem.* **233**, 1581.
 George, P. (1965). In *Oxidases and Related Redox Systems*, vol. 1, p. 3. Ed. by King, T. E., Mason, H. S. & Morrison, M. New York: John Wiley and Sons Inc.
 Guroff, G., Daly, J. W., Jerina, D. M., Renson, J., Witkop, B. & Udenfriend, S. (1967). *Science, N.Y.* **157**, 1524.
 Knowles, P. F., Gibson, J. F., Pick, F. M. & Bray, R. C. (1969). *Biochem. J.* **111**, 53.
 Komai, H., Massey, V. & Palmer, G. (1969). *J. biol. Chem.* **244**, 1692.
 Mason, H. S. (1965). *A. Rev. Biochem.* **34**, 595.
 Nilsson, R., Pick, F. M. & Bray, R. C. (1969). *Biochim. biophys. Acta*, **192**, 145.
 Norman, R. O. C. & Lindsay-Smith, J. R. (1965). In *Oxidases and Related Redox Systems*, vol. 1, p. 131. Ed. by King, T. E., Mason, H. S. & Morrison, M. New York: John Wiley and Sons Inc.

- Norman, R. O. C. & Radda, G. K. (1962). *Chem. Commun.* p. 138.
 Ohnishi, T., Yamazaki, H., Iyanagi, T., Nakamura, T. & Yamazaki, I. (1969). *Biochim. biophys. Acta*, **172**, 357.
 Orme-Johnson, W. H. & Beinert, H. (1969). *Biochem. biophys. Res. Commun.* **36**, 905.
 Rabani, J. & Matheson, M. S. (1964). *J. Am. chem. Soc.* **86**, 3175.
 Samuel, D. (1968). In *Biochimie des Sauerstoffs*, p. 6. Ed. by Hess, B. & Staudiner, H. Berlin: Springer-Verlag.
 Schested, K., Rasmussen, O. L. & Fricke, H. (1968). *J. phys. Chem., Ithaca*, **72**, 626.
 Staudinger, H. & Ullrich, V. (1964). *Z. Naturf.* **19B**, 877.
 Totter, J. R., Dugros, E. C. & Riviero, C. (1960). *J. biol. Chem.* **235**, 1839.
 Wyard, S. J., Smith, R. C. & Adrian, F. J. (1968). *J. chem. Phys.* **49**, 2780.
 Yamazaki, I. & Piette, L. H. (1963). *Biochim. biophys. Acta*, **77**, 47.

Initial Steps in Oxygen Interaction with Protein-Bound Metals

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In biological systems oxygen usually reacts with metal complexes. Either a transportable (storable) form of oxygen or a reactive intermediate is produced. I shall discuss the nature of the bound oxygen in such complexes, drawing heavily upon model reactions. In the first place two very different cases must be distinguished: one-metal centres and two-metal centres.

Single-metal centres. The obvious biological examples of this case are haemoglobin and certain oxidases. Before turning to them I wish to describe model systems that we have examined. The models are vitamin B_{12} and cobalt(II) porphyrin. In both cases Bayston, King, Looney & Winfield (1969) and ourselves observe that reaction with oxygen leads to the $\text{O}_2^{\cdot-}$ superoxide anion. This can be demonstrated by electron-paramagnetic-resonance measurements. The results show that the single unpaired electron is about 20% on the cobalt. Turning to the biological materials we cannot expect to see the $\text{O}_2^{\cdot-}$ by electron-paramagnetic resonance in the presence of iron(III). We must rely instead on absorption and Mössbauer spectra to give us the electron distribution. The absorption bands of the O_2 complex of myoglobin and haemoglobin are very like those of the CO, NO and RNC complexes of low-spin Fe(II) haem except for a new band at 1000 nm. This could well mean that the ground state is Fe(II)O_2 but that $\text{Fe(III)O}_2^{\cdot-}$ lies very close to it. The Mössbauer spectrum shows a very high quadrupole splitting, and such a spectrum could arise if these two states are mixed in the ground state. [The formation of $\text{M(III)O}_2^{\cdot-}$ from M(II)O_2 is energetically much

easier for cobalt than for iron, as the odd Co(II) electron is more unstable.] The only model mononuclear oxygen complexes for which structures are available have two vacant co-ordination sites for O₂ and are not strictly comparable.

Both in the cases of the haemoproteins and of model porphyrin and vitamin B₁₂ complexes oxidation by molecular oxygen is relatively slow. It can be 'catalysed' by the addition of one-electron reducing agents, implying that oxidation by oxygen needs two electrons, one from the metal, to be fast. Once a peroxide stage of a mononuclear complex is reached the breaking of the O-O bond is easy. Thus we suggest that in oxygen carriers and oxygen-storage systems it is the absence of a source of a second electron that prevents oxidation reactions. In such enzymes as terminal oxidases, cytochrome P-450 etc. we consider that there will be found a distal redox group of the protein, thiol or tyrosine for example, that can act as the source of the second electron, though it is obviously also possible for a substrate to act in this capacity.

The binding of oxygen to the metal in haemoproteins alters the binding to its other ligands, especially as a spin-state change is involved, so that a change in protein conformation is easily achieved, causing allosteric effects.

Two-metal centres. Crystal-structure studies of several model cobalt complexes have been reported. There are two main types of binuclear cobalt complexes: one with a twisted sequence Co-O-O-Co and one with a sequence Co-O(OH)-Co. In the first case two complexes with different charges have been studied. In one of them the cobalt is close to Co(III) and the oxygen is close to O₂^{•-}, whereas in the other the electronic structure is Co(III) and the oxygen is O₂²⁻. In all cases the oxygen is oxygen-bonded and is relatively inactive as an oxidizing agent. In the first case absorption spectroscopy shows a charge-transfer band at 700nm whereas in the second it is at 430nm. With reference to iron-containing proteins, Okamura, Klotz, Johnson, Winter & Williams (1969) have studied in detail the absorption and Mössbauer spectroscopy of Fe-O₂-Fe in haemerythrin. First, they have proved that there is an oxo-bridged Fe(III)-O-Fe(III) complex in the oxidized form of the proteins, so that there are two iron atoms, closely spaced, in the protein. On oxygenation of the Fe(II) protein there is no electron-paramagnetic-resonance spectrum; there is, however, a change in the Mössbauer and absorption spectra, which they interpret as being due to:

b

The uptake of oxygen by haemocyanin probably leads to a similar unit, Cu-O₂-Cu, and the difficulties of giving an electronic distribution to this complex are very similar to those with the iron-containing protein. In many respects the absorption spectrum is most like that expected for Cu(II)-O₂²⁻-Cu(II), but a Cu(I) or a Cu(III) complex could not be excluded. New physical methods, such as X-ray-induced electron-emission spectroscopy, are required to decide which structure is correct.

The overall picture of the first steps in oxygen reaction is that the interaction of oxygen with metal ions can lead to one of several electronic states, O₂, O₂^{•-}, O₂²⁻ (or even 2O²⁻ in some mononuclear models with two vacant co-ordination sites), all of which lie close in energy when the metal atom, or pair of metal atoms, has two or more valence states that are not grossly different in energy. There is no evidence that these complexes are exceedingly reactive in themselves, for internal attack on protein side chains does not occur. Oxygenation or oxidation, rather than the carrying of oxygen, would seem to depend on nearby reactive side chains of the protein (terminal oxidase) or possibly of a second substrate (oxidase) or of both (mixed-function oxidase).

Bayston, J. H., King, N. K., Looney, F. D. & Winfield, M. E. (1969). *J. Am. chem. Soc.* **91**, 2775.

Okamura, M. Y., Klotz, I. M., Johnson, C. E., Winter, M. R. C. & Williams, R. J. P. (1969). *Biochemistry*, *Easton*, **8**, 1951.

Co-operation of Electron-Accepting Sites in Oxygen Reduction by Oxidases

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Oxidases generally require metals for their catalytic function. A natural hypothesis for the role of the metal would be a mechanism in which a high-valence form oxidizes the substrate by accepting an electron, and the metal ion in the reduced enzyme is then reoxidized by molecular oxygen. Such a scheme can be expressed by the following equations in which E and M represent enzyme protein and the metal respectively and AH₂ the oxidizable substrate:

Substrate oxidation (eqn. 1) is here depicted as a one-electron transfer, which is certainly true for cytochrome c oxidase, and also for the only other oxidases reducing oxygen to water, namely the blue copper-containing oxidases (Malkin & Malmström, 1970), e.g. ascorbate oxidase and laccase (*p*-diphenol oxidase) (see Broman, Malmström, Aasa & Vänngård, 1963). Such a reaction is easy to visualize, and the major mechanistic problem is concerned with the