Biochemistry

Dressing the SOD

from Roger H. Pain

ONE of the most intriguing points at which biology and inorganic chemistry converge — almost justifying the term 'inorganic biochemistry' — is the superoxide radical O_2^-. This negatively charged radical is a common intermediate of the reduction of oxygen and is generated by a wide range of enzymatic oxidation reactions in living, respiring cells. It is potentially damaging and indeed appears to be involved in the destruction of cells by macrophages[1] and by natural killer cells[2]. Superoxide spontaneously undergoes a dismutation reaction

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

producing hydrogen peroxide. The reaction is fast, with a rate constant of $2 \times 10^5 M^{-1}s^{-1}$ so that O_2^- is normally present in only minute concentrations.

It is presumably an indication of the toxicity of O_2^- that an enzyme catalysing the dismutation is present in relatively high concentrations in respiring cells. This activity was only discovered in 1969 although the protein had been known for many years, masquerading under various names, including haemocuprein, hepatocuprein, erythrocuprein and cerebrocuprein. The metalloenzyme superoxide dismutase, now rejoicing in the acronym SOD, catalyses the dismutation of O_2^- with a rate constant of $2 \times 10^9 M^{-1}.s^{-1}$. Taking into account the levels of enzyme and substrate in human liver, for example, this means that the decay of O_2^- in that tissue is speeded up by a factor of 10^9(ref. 3). Just how SOD captures and catalyses the dismutation of such a small species so efficiently is beginning to become clear by looking at studies of the molecular structure of the enzyme in the light of the considerable biochemical literature on the reaction. Two important new studies of the enzyme, both from David and Jane Richardson and their colleagues at Duke University, the University of California, San Francisco and the Scripps Clinic, appear in this issue of *Nature* (p.284 and 287).

The copper, zinc superoxide dismutase comprises two identical subunits, each with an eight stranded β-barrel in the familiar Greek key conformation and three large loops of aperiodic structure. The Richardsons and their colleagues have refined the earlier 2Å structure and included the ordered water molecules in the vicinity of the active site[4]. They find that two of the loops form the walls of a deep channel on the outside surface of the barrel, with the active site copper ion Cu (II) slightly exposed on the floor of the channel and close to a completely buried zinc ion, Zn(II). The amino acid residues making up this channel are highly conserved in different species, suggesting that the channel is important. The active site has been identified as a small pit over the copper ion by using interactive computer graphics to fit the substrate O_2^- into position. The copper ion is held in place by four histidine residues while the neighbouring zinc is similarly held by three histidines, one of which acts as a bridge between the copper and the zinc. A feature of this region is the interlocking network of hydrogen bonds which stabilises the location of the ligands and hence the metal ions.

The formal reaction scheme for the superoxide dismutation

$$E\text{-}Cu(II) + O_2^- + H^+ \gtrless E\text{-}Cu(I) + O_2$$
$$E\text{-}Cu(I) + O_2^- + H^+ \gtrless E\text{-}Cu(II) + H_2O_2$$

looks unexceptional. The mechanism which the authors propose, however, is both elegant and interesting. Tetrahedral distortion of the Cu(II) ligands, itself a consequence of the protein conformation, in combination with the presence of the neighbouring zinc ion, accounts for the unusually high oxidation reduction potential of the copper. The strain in the zinc-ligand conformation helps to draw the enzyme-substrate complex into the first intermediate state on the catalytic pathway. In addition, the zinc also ensures that the bridging histidine 61 picks up a hydrogen atom during the catalytic process, later to be donated to a second O_2^- to form hydrogen peroxide.

The precise location and stabilization of the active site, metal-binding residues, allowing only histidine 61 to rock gently away from the copper when the latter is in its Cu(I) state, fits one's preconceptions of an enzyme designed to catalyse the reactions of a small, rigid substrate. Other factors are required, however, to explain the particularly high rate enhancement over an already rapid reaction. The rate constant for SOD is within an order of magnitude of the theoretical limit, itself governed by the rate at which substrate can diffuse to the enzyme. This is despite the fact that the copper containing pit to which substrate must bind forms only a fraction of a percent of the total surface area exposed to the solvent. The probability of a productive interaction resulting from random collisions between SOD and O_2^- is therefore low.

In their second paper[5] the Richardsons and their colleagues have calculated the electrostatic potential in and around the active site channel. Although an acidic protein, the long, deep channel is positively charged with residues critically placed to guide an incoming negatively charged ion towards the copper ion in the active site. It seems reasonable to suppose that the rate enhancement arises from relatively long range electrostatic forces which talk the incoming substrate down on to the active site, so reducing the number of non-productive collisions.

SOD thus provides a particularly good example of an asymmetric distribution of charged groups on the surface of a protein which plays a role in rate enhancement. Other examples of this phenomenon are the dipolar cytochrome *c* (ref. 6) and ribonuclease T1 (ref. 7). It will be interesting to see whether a similar system operates in the iron and manganese SOD family whose structures have recently been confirmed to be completely different from that of the copper and zinc class[8,9].

The two new papers appearing in *Nature* are notable for the successful use of computer graphics in investigating an enzyme mechanism. The communication of structural and electrostatic field information has been tackled effectively by 'dressing' the skeleton model with electrostatic field potential and field vectors in colour, with the flair associated with the Duke University laboratory. Indeed, the illustrations on p.288 might suggest a suitable epitaph for the artistic contribution of Jane Richardson:

"She then shall dress a sweeter SOD
Than Fancy's feet have ever trod"
Carter, 1748.

Roger H. Pain is Professor of Physical Biochemistry at the University of Newcastle upon Tyne, Newcastle NE1 7RU.

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THE following is an illustration of what private enterprise may effect for the benefit of science. When the Swedish ship *Monark* was leaving Sweden last year for Australia the second officer on board applied to the Zoological Museum at Upsala for the loan of a trawl and some vessels for preserving natural history objects. The results have been a collection of some 120 species of fish, 50 of insects and some birds.

The *Times* Calcutta Correspondent, in speaking of the possibility of opening up Thibet to Indian trade by way of Darjeeling, states that the Prime Minister of the Lama at Shigatze, said to be a most intelligent man, sent recently to Darjeeling for a supply of English books, photographic and other scientific apparatus.

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