New developments in iron-sulphur proteins

from R. Cammack

A NOVEL kind of iron-sulphur cluster has been found in a ferredoxin from the nitrogen-fixing bacterium *Azotobacter vinelandii*. The cluster is unusual in that it contains a ring of three iron atoms linked by labile sulphide atoms. The iron-sulphur proteins are a class of proteins that contain clusters of iron atoms bound to cysteine and sulphide sulphur. They are an important group of biological electron transfer agents. For example, more than a dozen different types of iron-sulphur clusters have been detected in mitochondria where they are concerned with energy metabolism. Two years ago, the possibility that iron-sulphur clusters can have other functions was raised when F. J. Ruzicka and H. Beinert (*J. Biol. Chem.* **253**, 2514; 1978) found that aconitase is an iron-sulphur protein. This enzyme of the Krebs tricarboxylic acid cycle of mitochondria has no known electron transfer function. It now seems likely that the iron-sulphur cluster in this enzyme is unusual in another respect, in that it may be a new type of three-iron cluster, similar to those recently proposed in certain bacterial proteins.

In the early 1970's it became established, by a combination of X-ray crystallography, spectroscopy and synthesis of chemical analogues, that all the iron-sulphur proteins contained either 1-iron, (Rd), 2-iron [2Fe-2S] or 4-iron [4Fe-4S] clusters (see figure). The 1-iron centres are found only in the rubredoxins of certain bacteria but the [2Fe-2S] and [4Fe-4S] clusters, in which iron is attached to sulphide sulphur and cysteine sulphur from the protein, has been found in many electron-transferring systems and enzymes. The iron-sulphur proteins are often described by the low-temperature electron-paramagnetic-resonance (EPR) spectra, since this is a good way of detecting them in biological systems. The [2Fe-2S] clusters and some of the [4Fe-4S] clusters give EPR signals in the reduced state at $g=1.94$. Other [4Fe-4S] clusters

give EPR signals around $g=2.01$. However recent results suggest that a novel type of cluster may also give a $g=2.01$ signal.

The centre of attention is a ferredoxin from *Azotobacter vinelandii*. This protein contains two iron-sulphur clusters with widely different redox potentials, +320mV and -424mV. Both give EPR signals at $g=2.01$. The first cluster seems to be a conventional [4Fe-4S] type. The second one, however, seems to be different. Chemical analysis (which is, admittedly, notoriously difficult with these proteins) indicates that it contains fewer than four iron atoms. Preliminary X-ray crystallography of the ferredoxin at 4 Å resolution by C. D. Stout of the University of Pittsburgh showed that this cluster was smaller than the other and last year it was proposed (Stout *Nature* **279**, 83; 1979) that it might be a two-iron cluster. A clearer picture of the cluster has now emerged from an electron density map at 2.5 Å resolution (Stout, Ghosh, Patabni & Robbins *J. Biol. Chem.* **255**; 1979; 1980).

A model (see figure) has now been fitted to the X-ray data and can be considered as a two-iron cluster which has opened at one side to introduce a third iron atom (numbered 3) and sulphide atom. Unlike all other iron atoms in iron-sulphur clusters, atom 3 does not have tetrahedral symmetry but appears to be almost square planar. Also, the ligands from the protein could not all be cysteine sulphurs as there are insufficient residues of this amino acid available.

The results of the X-ray analysis are still preliminary. Refinement of the full structure of the protein is not expected before the end of this year. However support for the existence of a three-iron cluster is provided by a totally different technique, ^{57}Fe Mössbauer spectroscopy. A paper from groups in Wisconsin and Minnesota (Emptage, Kent, Huynh, Rawlings, Orme-Johnson & Münck *J. Biol. Chem.* **255**, 17973; 1980) looks at the iron atoms in *A. vinelandii* ferredoxin. After subtracting the spectrum of the [4Fe-4S] cluster, the second cluster appears to contain two types of iron atom, in the ratio 2:1. As in other iron-sulphur clusters, the

iron atoms are antiferromagnetically coupled together. The observation that the cluster gives the EPR signal at $g=2.01$ in the oxidized form, but is non-magnetic in the reduced state, can readily be explained by a three-iron cluster. (This was a difficulty with the previously proposed two-iron cluster). The valence states of the iron atoms would correspond to 3Fe^{3+} in the oxidized state, and 2Fe^{3+} and Fe^{2+} in the reduced state, giving net charges for the cluster of +3 and +2, respectively.

If the existence of three-iron clusters is confirmed, there are already a number of proteins known that may also contain them. All of these given the EPR signal at $g=2.01$. Mössbauer spectra of ferredoxin II from *Desulfovibrio gigas* indicate that it contains a [3Fe-3S] cluster on each polypeptide chain. Since the protein is a tetramer it appears to contain four such clusters (Interestingly ferredoxin I from the same organism contains [4Fe-4S] clusters in a trimeric unit of exactly the same polypeptide). Other, more complex proteins, may also contain the cluster. Emptage *et al.* report that they have observed similar Mössbauer spectra from the enzyme glutamate synthase of *A. vinelandii* and from aconitase of beef heart mitochondria.

The presence of this iron-sulphur cluster in aconitase is intriguing. The cluster does not appear to participate in the enzyme reaction directly, but the enzyme is inactive unless the cluster is first reduced. It has therefore been suggested that the cluster somehow regulates the activity of the enzyme. If this idea becomes accepted, there are other examples known. Glutamine amidoribosyl transferase is an enzyme concerned with nucleic acid synthesis. The enzyme, both from human tissue and from *Bacillus subtilis* has been found to contain an iron-sulphur cluster (possibly of the [4Fe-4S] type). Again this has no obvious role in catalysis, but the enzyme is only active when the cluster is reduced.

It seems, as more types of iron-sulphur proteins continue to be discovered, that we have much to learn about the versatility of iron-sulphur clusters. □

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Atomic arrangements in the one-iron (rubredoxin, Rd) two-iron and four-iron clusters, and the proposed three-iron cluster.