AN X-RAY INVESTIGATION OF THE STRUCTURE OF A BACTERIAL FERREDOXIN

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Ferredoxin is a nonheme iron-containing protein isolated from Clostridium pasteurianum by Mortensen, Valentine and Carnahan (1962). It has also been found associated with other bacteria (Whiteley and Woolfolk, 1962; Buchanan, Lovenberg and Rabinowitz, 1963; Valentine, Mortensen and Carnahan, 1963). While there appear to be differences among some of the ferredoxins occurring in different bacteria, they are characterized by molecular weights $\sim$ 6,000 and the presence of 5-7 Fe atoms and an equivalent number of S atoms per molecule of the protein. This communication reports the preliminary X-ray work on the structure of ferredoxin isolated from Micrococcus aerogenes.

Experimental. The material used in this study was kindly supplied by Dr. H. R. Whiteley of the Department of Microbiology of this University. The crude substance was obtained from supernatants from a cell suspension of the bacteria in distilled H_2O. The ferredoxin was purified by adsorption on a DEAE-cellulose column and elution with 0.5 M Tris-HCl buffer at pH 7.3 and desalted with a sephadex G-25 column (Lovenberg, Buchanan and Rabinowitz, 1963).

Single crystals satisfactory for use in collecting X-ray diffraction data were grown from aqueous solution by salting out with 85% saturated $(NH_4)_2SO_4$ at pH 7.3. The crystals are lath-like needles elongated along c and grow to lengths of a millimeter or more and a thickness $\sim$ 0.1 mm in a few days. The crystals are orthorhombic with unit cell dimensions

$$a = 30.583 \pm 0.003 \text{ Å}$$

$$b = 37.865 \pm 0.007$$

$$c = 39.288 \pm 0.007$$

as determined with CuK_α radiation ($\lambda = 1.5418$ Å).

The only extinctions observed are h00, 0k0 and 00ℓ absent for h, k and ℓ odd respectively. The space group was assumed, therefore, to be $P2_12_12_1$. There are 4 molecules per unit cell on the assumption that the density of the crystal is 1.4 g. cc^{-3} and that about 35% of its mass is solvent.

Three-dimensional counter data to a resolution of 5 Å have been collected from a single crystal of dimensions 0.07 x 0.1 x 0.3 mm. Of the 228 possible reflections, all but 11 were significantly above the background count when a 37 second 2θ scan was used.

Discussion. While no attempt has been made to determine how far out in reciprocal space it is possible to observe reflections, it is worth noting that reflections occur virtually to the edge of a 10-hour, 30° precession photograph from a crystal of volume 3×10^{-6} cc. It appears likely, therefore, that it will be possible to observe reflections with spacings well below 1.5 Å and that atomic resolution can be attained in an electron density map if the structure is not complicated by disorder or similar adverse circumstances.

A three-dimensional Patterson function (vector map) has been calculated using the data to 5 Å resolution. In contrast to the usual appearance of such functions for native proteins, it is characterized by prominent peaks which are probably primarily due to Fe-Fe and Fe-S interactions. Blomstrom et al. (1964) have postulated that the Fe atoms in ferredoxin from Clostridium pasteurianum are arranged in a linear fashion, being linked together by sulfur bridges. If the Fe and S atoms were, in fact, linked in this type of linear array in the ferredoxin from Micrococcus aerogenes, the vector map would, in general, show eight long rods of outstanding vector density radiating from the origin. That these are not found may be taken

as strong evidence that the Fe atoms are not arranged in a single long linear array. The vector map at a resolution of 5 Å, however, is not incompatible with several short linear arrays of the heavy atoms.

At this point, no attempt has been made to locate all the Fe atoms directly from the vector map though this may be possible. A more positive approach is by way of isomorphous heavy atom derivatives (Harker, 1956; Perutz, 1956). A single such derivative along with anomolous dispersion data for both the derivative and the parent compound should provide ample data for the solution of this structure.

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