02.2–1 A FAST METHOD OF COMPARING THREE DIMENSIONAL STRUCTURE OF PROTEINS.

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Comparative studies on protein structures form an integral part of protein crystallography. They provide important information regarding the patterns of protein folding, evolutionary relationships and similarities of active site geometries. The methods currently available for the quantitative comparison of chain folds require considerable computing time. Hence, a new method has been developed by representing protein structures as a set of secondary structural elements. This method is a three dimensional extension of Levitt and Chotias' (Nature, 261, 552 (1976)) diagrammatic representation of helices and sheets. Equivalence between secondary structural elements of two proteins are determined based on their relative orientation using a procedure developed by Needleman and Wunch (J. Mol. Biol., 48, 443 (1970)). The method provides information regarding statistical significance of similarities, insertions and deletions and preferred packing arrangements. The latter information is not easily obtained by earlier methods. The present method can be used to compare any newly determined structure with all the previously known structures without demanding excessive computing time. However, in contrast to the earlier methods, the present one can be used only for proteins with some secondary structure. The usefulness of the method will be illustrated with examples from globin folds, cytochrome and dehydrogenases.

02.2–2 THE THREE-DIMENSIONAL STRUCTURE OF A 'SALT-LOVING' FERREDOXIN FROM HALOBACTERIUM OF THE DEAD SEA.

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Extreme 'halophilic' bacteria are unique in the realm of living organisms since their cellular interiors are approximately 4 M in KCl (Werber et al. in "Energetics & Structure of Halophilic Microorganisms" (1978) Caplan & Ginzburg, Eds., pp. 427-445; Rao & Argos, Biochemistry (1981) 20, 6536-6543). The survival of these microorganisms depended upon the ability of their proteins to have adapted to their extreme environment; moreover, most of these proteins require high salt for their stability, in contrast to most 'nonhalophilic' proteins which are inactive under such conditions.

A 2Fe-ferredoxin has been isolated and purified from Halobacterium of the Dead Sea. Large red-colored hexagonal plate crystals were grown of it in 4 M phosphate buffer (Sussman et al., J. Mol. Biol. (1979) 134, 375-377). A partially-refined model of a 'nonhalophilic' 2Fe-ferredoxin from a blue green algae, S. platensis, was recently published (Fukuyama et al., Nature (1980) 286, 522-524). Although there is some amino acid sequence similarity between these two ferredoxins, the 'halophilic' ferredoxin is about 30% larger and has a significantly higher net negative charge at neutral pH than its 'nonhalophilic' counterpart. Therefore the S. platensis structure can at most serve as a possible model for part of the halophilic structure.

A 5 Å resolution electron density map was computed based on a single isomorphous heavy atom derivative (SIR) and native X-ray diffraction data from crystals of the 'halophilic' 2Fe-ferredoxin. It clearly showed the molecular envelope, but was not of high enough quality to trace the polypeptide chain. Attempts to obtain better phases through additional heavy atom derivatives were not successful.

Therefore some novel crystallographic computational techniques were developed and implemented for improving an SIR electron density map. These consist of 1) the use of anomalous diffraction data from the Fe-S cluster in the native protein to resolve the phase ambiguity inherent in the SIR methods (Hendrickson & Teeter, Nature (1981) 290, 107-113, Smith & Hendrickson, in "Computational Crystallography", (1982) Sayre, ed., pp 209-222), 2) real space three-dimensional search techniques using the S. platensis structure as a model (Steigemann (1983), personal communication), 3) automatic enveloping, density modification, and phase extension & combination (Bhat & Blow, Acta Cryst. (1982) A38, 21-29) and 4) least-squares refinement of a partial structure (Sussman & Podjarny, Acta Cryst. (1983) B39, 495-505); Bhat & Blow, Acta Cryst (1983) A39, 1666-170). These techniques have been employed in conjunction with real-time computer graphics model fitting (Jones, J. Appl. Cryst. (1978) 11, 268).

Use of these methods in an iterative manner resulted in a greatly improved electron density map at 2.2 Å resolution, from which we have been able to trace about 90% of the polypeptide chain and to see most of the side chain groups.

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02.2–3 THE CRYSTAL STRUCTURE OF T STATE MET-HAEMOGLOBIN,

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The aim of our work is to define the structural perturbations produced by ligation of T state haemoglobin. This has been achieved by studying the molecule in a crystal lattice which inhibits the switch to the R conformation.

The structure of a partially oxygenated crystal has been described (Brzozowski et al., Nature, 307, 74-76) and the study has now been extended to the T state met-haemoglobin crystal. Diffraction data have been collected to 2.1A spacing on these crystals (in which all four haems are oxidised). With the coordinates of partially oxygenated haemoglobin as the starting model, the R factor was .46. This has been reduced to .29 by automatic refinement, using Agarwal's FFT routines in conjunction with the simultaneous application of geometrical restraints (Konnert-Hendrickson). Rebuilding of this structure is now in progress. Preliminary results indicate that all four haems are ligated. Comparison with the structure of deoxyhaemoglobin reveals that the haem geometry has responded to the change in Fe's co-ordination, and that these effects have been transmitted to the surrounding globin and extend to the subunit interfaces. The magnitude of these movements (∿1Å) is greater than that observed in the partially oxygenated structure. We believe that these structural comparisons will shed new light on the mechanism of haem-haem interaction in haemoglobin.