01.5–9 CRYSTAL STRUCTURE REFINEMENT OF APO- AND PARTIALLY LIGANDED GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) AT 4 A RESOLUTION.

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GAPDH is a tetramer of four chemically identical subunits which requires the cofactor nicotinamide adenine dinucleotide (NAD) for activity. The structure of the holo-enzyme from Bacillus stearothermophilus has recently been refined at 2.4A resolution. This has facilitated the structure determination of the apo-enzyme and the enzyme with one molecule of NAD bound to the tetramer. These structures have been refined at 4A resolution using the constrained-restrained parameter structure factor least squares refinement program CORELS. Each structure was divided into rigid groups corresponding to secondary structural elements such as alpha helices or strands of β-sheet for the structure refinement. The crystallographic residuals for data of 3A resolution are 30.2% and 30.4% for the apo-enzyme and the 1 NAD enzyme respectively. Difference electron density maps calculated at 3A resolution clearly demonstrate where the rigid-body description of the structures is inadequate.

The apo-enzyme displays 222 molecular symmetry, although small but significant deviations from this symmetry are present. These can be ascribed to the presence of residual amounts of NAD in the crystalline enzyme which binds preferentially to one subunit in the tetramer. The structure of an apo-enzyme subunit is related to that of the holo-enzyme by an approximate rigid body rotation of the coenzyme binding domain by 5^0 relative to the catalytic domain. The effect of the rotation is to shield the active site from solvent in the holo-enzyme.

The structure of the 1 NAD per tetramer species is highly asymmetric. The subunit containing the bound NAD adopts a conformation very similar to that of a holo-enzyme subunit while the other three subunits are very similar to the apo-enzyme subunit conformation.

01.5–10 REFINEMENT OF A MOLECULAR MODEL FOR LAMPREY HEMOGLOBIN. By R. B. Honzatko, Dept. of Biochem., Iowa State University, Ames, IA 50011, W. A. Hendrickson, Code 6030, Naval Research Lab., Washington, DC 20375 and W. E. Love, Dept. of Biophys., Johns Hopkins Univ., Baltimore, MD 21218.

A molecular model for the protein and ambient solvent of the complex of cyanide with methemoglobin V from the sea lamprey Petromyzon marinus yields a R-factor of 14.2% against X-ray diffraction data to 2.0 Å resolution. The root-mean-square discrepancies from ideal bond length and angle are respectively 0.014 Å and 1.5 degrees. Atoms which belong to planar groups, deviate by 0.012 Å from planes determined by least squares. The average standard deviation for chiral volumes, peptide torsion angle and torsion angles of side chains are 0.150 $Å^3$, 2.0 degrees and 19.4 degrees, respectively. The root-mean-square variation in the thermal parameters of atoms of the polypeptide backbone is 1.21 $Å^2$; the variation in thermal factors for side chain atoms is 2.13 $Å^2$. The model includes multiple conformations for ten side chains of the 148 amino acids of the protein and 231 of the approximately 550 water molecules contained within a crystallographic asymmetric unit.

Lamprey hemogloblin has the familiar fold of other globins. The superposition of the lamprey hemoglobin structure on the refined models of sperm whale myoglobin, α chain of horse hemoglobin, β chain of horse hemoglobin, and leghemoglobin reveals the G-H corner of lamprey hemoglobin as structurally unique among the globins. We explore the possiblity of a tetramer for the polymeric form of lamprey hemoglobin by superposition of the refined model for the monomer on the R and T states of mammalian hemoglobin. We evaluate the relevance of the postulated tetramer to biochemical properties of cooperativity in ligand association.

01.5–11 PROGRAM CONSTRUCTION FOR REFINEMENT OF THE ATOMIC STRUCTURE OF MACROMOLECULES BASED ON THE FAST DIFFERENTIATION ALGORITHM. By V.Yu.Lunin and A.G.Urzhumtsev, Research Computer Centre, USSR Academy of Sciences, Pushchino, Moscow region, USSR.

The large amount of computation required for refinement of the macromolecule structure is due to a great number of the parameters affecting the minimized criterion $f(\vec{q})$. The most time-consuming is the computation of gradient ∇f of this criterion. If the vector $\nabla^2 f \cdot \vec{e}$ is to be calculated, one uses commonly one or another approximation of the matrix $\nabla^2 f$. However, as is shown by Kim et al.(Dokl.Acad.Nauk SSSR, in the press), the program computation of all the components of the gradient and the vector $\nabla^2 f \cdot \vec{e}$ for a function $f(\vec{q})$ takes about the same time as the computation of one value of $f(\vec{q})$ does. This program can be constructed by expanding the computation of $f(\vec{q})$ in the superposition of elementary changes of the variables

$$\vec{q} \to \vec{y}^1 \to \vec{y}^2 \to \ldots \to \vec{y}^N \to R(\vec{y}^N)=f(\vec{q})$$

and in series of sequential transforms into the gradients over the corresponding variables

$$\nabla_{y^N} R \to \nabla_{y^{N-1}} R \to \ldots \to \nabla_{y^1} R \to \nabla_q R = \nabla_q f$$

Thus, the problem of the speed of refinement programs comes to that of fast computation of $f(\vec{q})$. The model of the object can be described in the different ways: by the individual atomic coordinates $\vec{r}_i$ of the atoms, by the dihedral angles φ_i and ψ_i, by the parameters defining the position of several large parts of the molecule or by another generalized parameters $\vec{q}$. The most complicated is the calculation of the part of the minimized criterion describing the fit of the calculated structure factors to the X-ray experiment. This calculation may be carried out the most quickly as the chain of superpositions:

$$\vec{q} \to \{\vec{r}_j\} \to \varrho \to \{F(\vec{s})\} \to R(\{F(\vec{s})\}).$$

Here, only the first and the last transforms depend on the choice of $\vec{q}$ and R, the central part being made uniformly for all programs. Analogously, in the gradient computation only the transforms $R \to \nabla_F R$ and $\nabla_r R \to \nabla_q R$ are specific for this program. As a criterion of good agreement between the model and the X-ray experimental data we propose the criterion

$$R=\sum_{\vec{s}} (F_c^2-F_o^2)^2-(A\cos\varphi_c+B\sin\varphi_c+C\cos 2\varphi_c+D\sin 2\varphi_c)$$

where A,B,C,D are the coefficients (Hendrickson, Lattman, Acta Cryst. (1970) B26, 136) of the probability distribution of the phase $\varphi_c(\vec{s})$. The minimization of the criterion may be interpreted as the determination of structure parameters from the maximum of the likelihood function. Such a criterion would by prefered to the ordinary ones, because
a) the refinement implies use of the phase information, i.e. the information on other X-ray experiments with heavy-atom derivatives
b) this information is reliable, in particular, the reflexions with both determined and undetermined phases can be included in the refinement (if phases are undetermined, A=B=C=D=0).