PS02.03.20 EFFECT OF ATOMIC HETEROGENEITY ON SAYRE'S EQUATIONS Sabita Das, Victoria College, 78B Acharya Prafulla Chandra Road, Calcutta-700009, India and G.B. Mitra, Indian Association for the Cultivation of Science, Calcutta-700032, India.

An extension of Sayre's equation is developed for a structure formed by a single heavy atom and L number of equal and well resolved light atoms in the unit cell. Sayre's equation may be written as

$$G(h) = V^{-1} \sum_K F(h) \ F(h-k) \qquad \cdots (1)$$

for a squared structure of electron density p^2 (r). The structure factors corresponding to normal and squared atoms can be written as, respectively,

$$F(h) = f_H(h)\exp\left(2\pi \ i \ h.r_H\right) + \sum_{j=1}^{L} f(h)\exp(2\pi \ i \ h.r_j \quad \cdots (2)$$

$$G(h) = g_H(h)\exp\left(2\pi \ i \ h.r_H\right) + \sum_{j=1}^{L} g(h)\exp(2\pi \ i \ h.r_j \quad \cdots (3)$$

where the H stands for heavy atom and f(h) , g(h) being the atomic scattering factors of normal and squared atoms respectively. From equations (1) and (3), we get

$$F(h) = \theta(h)V^{-1} \sum_K F(h) \ F(h-k) + (\theta(h)^{-1} - 1) \ x$$

$$g_H(h)\exp(2\pi \ i \ h.r_H) \ [\text{where}, \ \theta(h) = f(h)/g(h)] \quad \cdots (4)$$

$$|F(h)| \ e^{i\phi(h)} = \theta(h)V^{-1} \sum_K |F(h)| \ |F(h-k|e^{i \ [\phi(h)+\phi(h-k)]}$$

$$+ (\theta \ (h)^{-1} - 1)g_H(h)\exp(2\pi \ i \ h.r_H) \quad \cdots (4a)$$

Equations (4) give modified Sayre's equation for the direct determination of phases of light atoms, having the position of the heavy atom been pre-determined by Patterson or any other method.

PS02.05.18 ACCURACY OF STRUCTURE FACTOR AMPLITUDES FROM A POWDER DIFFRACTION PATTERN: THE ROLE OF THE PRIOR INFORMATION. A. Guagliardi(+), A. Altomare(+), G. Cascarano(+), C. Giacovazzo(+), A.G.G. Moliterni(+), M.C. Burla(*) & G. Polidori(*), (+)Istituto di Ricerca per lo Sviluppo di Metodologie Cristallografiche - CNR c/o Dipartimento Geomineralogico - Campus Universitario Via E. Orabona, 4 - 70125 Bari - Italy,(*) Dipartimento di Scienze della Terra, Universita', 06100 Perugia, Italy

The two-stage method is often used for analysis and crystal structure solution from powder diffraction data. In the first stage the integrated intensity of each Bragg reflection is obtained by a pattern decomposition process; the second stage uses these intensities to solve the structure by Direct or Patterson Methods.

The accuracy of structure factor amplitudes supplied by the first step is often not sufficient for the success of such an approach.

Besides the quality of the experimental pattern and the efficiency of the extraction procedures, the possibility of exploiting some prior information in the Le Bail algorithm [Le Bail, A., Duroy, H. & Fourquet, J.L. (1988). Math. Res. Bull. **23**,447-452] greatly contributes to improve the accuracy of structure factor moduli. Different kinds of prior information will be discussed and used and the optimized algorithms for their management will be described in detail.

PS04.02.48 X-RAY CRYSTAL STUDIES OF HYDROXYL-AMINE OXYDOREDUCTASE. Igarashi N, Moriyama H, Fujiwara T, Fukumori Y and Tanaka N, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, 226 Japan

The autotropic bacterium Nitrosomonas europaea obtains energy for growth by the oxidation of ammonia to nitrite. Hydroxylamine oxidoreductase (HAO) catalyzes the oxidation of hydroxylamine to nitrite. The enzyme contains eight hemes per subunit (63kDa) which participate in catalytic function and electron transport. The electrons released in the reaction are partitioned to ammonium monooxygenase and to the respiratory chain. Crystallization of HAO was performed by the vapor diffusion method using ammonium sulfate as precipitant. The space group was P63 with cell dimensions a = b = 96.2 Å c = 265.6 Å and with two subunits per asymmetric unit. It was found that the crystal specimen was hemihedrally twinned, which was firstly detected by the facts that the distribution of intensities reflected on a varied degree of twinning for each crystal and showed a pseudo 6/mmm Laue symmetry and lack of heavy-atom cross vectors between positions related by the pseudo heigher symmetry operation in MIR. To deconvolute the intensities into the separated ones, the Britton's principle (Acta Cryst., A28, 296-297, 1972) was applied. The resultant indicated that the pseudo higher symmetry operation disappeared in the difference Patterson map. The observation of the crystallizing process shown that HAO crystal was composed of the two twin domains, which was caused by the crystal growth from two very fine crystals orientated exactly in the opposite direction from each other. To collect the untwinned data set, the uniform part at the edge of a crystal was aimed to input the X-ray using the fine focused SR beam (BL6A at PF). Two heavy atom derivatives were prepared by soaking in 1mM $HgAc_2$ and 1.4mM K_2PtCl_4, respectively. The heavy-atom binding sites were confirmed by both isomorphous and Bijvoet difference Patterson map. The structure determination by MIRAS method is now in progress.

PS04.02.49 THE CRYSTAL STRUCTURE OF Asn694His MUTANT OF L-1 ISOZYME OF SOYBEAN LIPOXYGENASE. K.Lewinski*, J.Steczko+, T.Holman♠, E.Sigal♣, B.Stec♦, B.Axelrod+, W.Minor*. *University of Virginia, +Purdue University, ♠University of California-Riverside, ♦Boston College, ♣Mercator Genetics

Lipoxygenases are non-heme, non-sulfur iron dioxygenases catalyzing the hydroperoxidation of polyunsaturated fatty acids as linoleic, linolenic and arachidonic acid, the precursor of a number of physiologically potent effectors critical in human physiology. In soybeans there are three classes of lipoxygenase L-1, L-2 and L-3, all containing one iron atom per molecule and exhibiting >70% similarity in sequence.

Low temperature crystallographic studies at 1.4 Å resolution showed that in the L-1 isozyme the iron atom is coordinated octahedrally to three histidines (His499, His504 and His690), the C-terminal carboxylate group of Ile839, a water molecule and the $O\delta_1$ of Asn694. As the distance of Asn694 to iron was significantly larger than for histidine ligands (3.0 Å vs. 2.3 Å) the role of this residue in iron binding and mechanism of activity remained uncertain.

In some lipoxygenase sequences the position equivalent to Asn694 is occupied by a histidine residue. Recent studies of the L-3 isozyme in which asparagine was substituted by histidine showed no significant changes in activity. This result is contrary to our studies of Asn694His mutant of L-1 isozyme which shows only very limited activity.

The crystal structure of the Asn694His mutant is to high degree isomorphous to structure of wt-enzyme. Diffraction data were collected at 100K with the use of a standard RAXIS-II set-up. The position of His694 was found on the omit map in close vicinity of the iron atom. Refinement of the structure was performed in the resolution range 10-2 Å using the XPLOR program.

The iron atom is octahedrally coordinated by four histidine residues, including His694, the carboxyl end of Ile839 and a water molecule. Changes in geometry of the mutated enzyme and their impact on activity will be discussed.