**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

\[
G(h) = V^{-1} \sum_{k} F(h) F(h-k) \cdots (1)
\]

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

\[
F(h) = f(h) \exp\left(2\pi i h.r_n\right) + \sum_{j=1}^{L} f(h) \exp(2\pi i h.r_j) \cdots (2)
\]

\[
G(h) = g(h) \exp\left(2\pi i h.r_n\right) + \sum_{j=1}^{L} g(h) \exp(2\pi i h.r_j) \cdots (3)
\]

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

\[
F(h) = \theta(h) V^{-1} \sum_{k} F(h) F(h-k) + (\theta(h)^{-1} - 1) x
\]

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

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

\[
(\theta(h)^{-1} - 1) g(h) \exp(2\pi i h.r_n) \cdots (4a)
\]

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. Cascaranono(+), C. Giacovazzi(†), A.G.G. Molteni(+), M.C. Burla(†) & G. Polidori(+), (+Istituto di Ricerca per lo Sviluppo delle Cristallografie - CNR e/o Dipartimento Geomineralogico - Campus Universitário Via E. Orabona, 4 - 70125 Bari - Italy; †Istituto di Scienze della Terra, Università di Bari, 70100 Bari, 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 HYDROXYLAMINE OXIDOREDUCTASE. Igarashi N, Moriyama H, Fujimura T, Fukuomi Y and Tanaka N, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, 226 Japan.

The autotrophic bacterium Nitrrosomonas 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 monoxygenase 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 hexahedrally 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 heavy 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 heavy symmetry operation disappeared in the difference Patterson map. The observation of the crystallographic process shows 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 unwinned data set, the common 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 HgAs2 and 1.4mM K2PtCl4, respectively. The heavy-atom binding sites were confirmed by both isomorphous and Bijvoet difference Patterson map. The structure determination by MIAS method is now in progress.

**PS04.02.49** THE CRYSTAL STRUCTURE OF Asn694His MUTANT OF L-1 ISOZYME OF SOYBEAN L IPOXENASE. K.Leewinski, J. Steczko, T.Holman*, E.Sigal, B. Sec*, W.Minor*, E. 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 (His698, His694 and His690), the C-terminal carbonyl group of Asp699, a water molecule and the OR of Asp696. 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 remains 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 His690, the carboxyl end of His699 and a water molecule. Changes in geometry of the mutated enzyme and their impact on activity will be discussed.