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MS-02.02.02 PROTEIN DIFFRACTION AROUND THE SULFUR
K-ABSORPTION EDGE USING 5 Å X-RAYS FROM A STORAGE RING.

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Structure analysis of a protein by crystallographic methods is often started by the location of some strongly scattering atoms (Green et al., Proc. R. Soc. London, 1954, 225, 287-307) which are then used as a reference for phasing the diffraction data. They are named 'heavy atoms', and are mostly metal atoms, but an ideal target would be sulfur which is often naturally present in the protein.

The K-absorption edge for sulfur is found at $\lambda_K=5.018$ Å so a major problem is the high absorption of most materials to these X-rays. Using evacuated beam tubes, a diffractometer inside the vacuum chamber, a special sample-holder and back-scattering geometry some first measurements have been made at the instrument A1 (Stuhmann et al., Handbook of Synchrotron Radiation 4, 1990, 555-580) at HASYLAB, and they show the approach to be feasible.

To reach reasonable d-spacings for structure analysis between ca. 7 and 3 Å, the long wavelength imposes large scattering angles, in which case reflection geometry becomes the obvious choice, leading to back-scattering as the limit of 2.5 Å is reached. This is a very favourable geometry for strongly absorbing crystals.

Tetragonal crystals of hen egg-white lysozyme were used, and typically about 30 reflections could be observed twice before the crystal died from radiation damage. In most of the first experiments the d-spacings were in the range from 4.2 Å to 7.2 Å. Typically the agreement with normal wavelength data was between 15 and 20%, while the statistical error was from 1 to 2%. In most cases it was possible to observe all expected reflections within a scan-range.

Some of the extra error is undoubtedly associated with large absorption effects, but the main source is at present most likely the shadows from the window of the detector. This is a multiwire proportional counter where the thin mylar front window (towards the vacuum) is held with a steel grid. The wire thickness of this grid is 0.3 mm, and the spacing between wires of 1.3 mm is comparable to the Bragg spot size.

Measurements were made in a two wavelength mode. These were chosen to get near maximum change in f' and constant value for f'' . Scans were done twice and this gave a measure of the reduction in intensity, about 4% per hour. Ratios between the observed structure factors above and below the absorption edge were then compared to the calculated value. To identify the influence of the anomalous scattering the calculation was done for all possible combinations of the two wavelengths in the range around the absorption edge, and the best agreement was found to occur for the set of wavelengths actually used in the experiment.

Radiation damage is now the most important obstacle, but cooling should possibly handle this. Moreover, the local detector response function can be improved by a change in the material used for the front window.

MS-02.02.03 PARAMETER REFINEMENT IN THE MAD
METHOD BY A MAXIMUM-LIKELIHOOD PROCEDURE. By
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The method of Multiwavelength Anomalous Dispersion (MAD) [Kahn, Fourme, Bosshard, Chiadmi, Risler, Dideberg & Wery (1985). *FEBS Lett.* **179**, 133-137; Hendrickson (1985). *Trans. Am. Cryst. Assoc.* **21**, 11-21] has proved capable of solving small to medium-sized protein structures from which a sufficiently strong anomalous signal can be measured [see the reviews by: Fourme & Hendrickson (1990). In *Synchrotron Radiation and Biophysics*, edited by S.S. HASNAIN, pp. 156-175. Chichester: Ellis Horwood Ltd; Smith (1991). *Curr. Opin. Struct. Biol.* **1**, 1002-1011; and Hendrickson (1991). *Science* **254**, 51-58]. The possibility of producing selenomethionyl proteins (Hendrickson, Horton & LeMaster (1990). *EMBO J.* **9**, 1665-1672] has indeed created hopes that the MAD methodology may offer a universal solution to the phase problem for macromolecules [Moffat (1988). *Nature (London)*, **336**, 422-423].

However the current methods used for extracting phase information from MAD measurements [Karle (1980). *Int. J. Quant. Chem. Symp.* **7**, 357-367; Hendrickson (1985). *Trans. Am. Cryst. Assoc.* **21**, 11-21] make assumptions about data quality and completeness which are difficult to meet in practice, particularly with respect to wavelength definition and stability. The phenomenon of anisotropy of anomalous scattering [Fanchon & Hendrickson (1990). *Acta Cryst.* **A46**, 809-820] can also render inaccurate the algebraic relations on which phase determination is based. The much emphasized perfect structural isomorphism between the normally-scattering parts of the crystal at the various wavelengths may therefore be spoiled by substantial uncertainties in the quantitative characterisation of the behaviour of the anomalous scatterers at those wavelengths - in other words, by non-isomorphism in a new guise affecting the anomalous scatterers. The main consequence of the experimental difficulties in maintaining a precisely-defined value and spread of the wavelength and in taking into account the fine structure of the absorption edges is that the scattering factors f' and f'' are not known well enough to be treated as constants and must be refined. Furthermore, the practical difficulties in obtaining complete data sets with significant anomalous effects at all the desired wavelengths result in many reflexions not having the full complement of measurements required to determine their phases uniquely by the algebraic method so as to make the scattering factor refinement straightforward.

This situation is identical to that found in the Multiple Isomorphous Replacement (MIR) method with respect to the refinement of occupancies from acentric reflexions only, with low signal-to-noise ratios and uncertain (usually bimodal) phase information. This problem has a long history of difficulties and a conceptually satisfactory solution allowing bias-free refinement of all parameters (including those measuring the lack of isomorphism) has only recently been obtained by a recourse to the method of maximum-likelihood estimation [Bricogne (1991). In *Isomorphous Replacement and Anomalous Scattering*, edited by W. Wolf, P.R. Evans & A.G.W. Leslie, pp. 60-68. Warrington: SERC Daresbury Laboratory].

We have undertaken the systematic implementation of this approach in a computer program designed and written from scratch. The hierarchy used to define the successive levels of parametrisation in the substitution models and the associated observed data was designed to accommodate both MIR and MAD data, or any mixture of them. It uses flexible binning according to resolution and solid angle so as to operate on unmerged data and refine local scaling/absorption parameters, and also according to time of recording to allow wavelength tracking. The program has been tested systematically, from the lowest to highest levels of functional dependence, on synthetic data generated internally by the program itself, an approach which has made possible the *post mortem* investigation of the pathologies associated with the previous parameter refinement methods. Our long-term goal is to interface the MIR and MAD methods with the phasing procedure based on entropy maximisation and likelihood ranking [Bricogne (1993). *Acta Cryst.* **D49**, 37-60]. Our rationale is that the MAD method is intrinsically unable to phase reflexions for which the transform of the constellation of anomalous scatterers is weak, so that some other procedure will in general be needed in order

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to propagate phase information from those reflexions where it is strongly indicated to those where it is essentially absent. Maximum-entropy extrapolation will perform this task in an optimal fashion.

MS-02.02.04 DETERMINATION OF THE STRUCTURE OF A LARGE PROTEIN BY MAD PHASING: GLUTAMINE PRPP AMIDOTRANSFERASE. by J.L.Smith*, E.J.Zaluzec, and J.-P.Wery, Dept. of Biological Sciences, Purdue Univ., West Lafayette, IN 47907 USA, and Y.Satow, Faculty of Pharmaceutical Science, University of Tokyo, Bunkyo-ku, Tokyo, Japan

Glutamine PRPP amidotransferase is a unusual FeS protein that catalyzes the first step of *de novo* purine biosynthesis. The enzyme is a tetramer or 50-kDa subunits; each subunit contains one Fe_4S_4 cluster. MAD phasing was based on K-edge anomalous scattering by Fe. Multiwavelength data to 3.0Å spacings were measured on imaging plates at beamline 14A at the Photon Factory. The asymmetric unit of the crystals contains one tetrameric molecule. Positions of the Fe_4 clusters were initially determined using data to 5.5Å spacings, and individual Fe atoms were located by analysis of partial-structure Fourier maps at 3.0Å resolution. Experimental phases to 3.0Å were derived from the multiple |Fobs| by a probability treatment similar to the Blow-Crick analysis of errors in isomorphous replacement (Pahker, Smith & Hendrickson(1990) Acta Cryst. A46, 537-540). Once the Fe partial structure was known, phase probability expressions were computed for all experimental observations. The resulting phase coefficients were combined for all observations of each unique reflection without regard to the crystal, asymmetric unit or time of the measurement. The final MAD phase set was used as a starting point for phase refinement by local symmetry averaging and solvent flattening. A procedure was developed for automatic mask generation and rapid, automatic phase refinement (J.T.Bolin, S.W.Muchmore & J.L.Smith, Unpublished), which allowed us to experiment with the phase refinement protocols until we found the most interpretable map. The final 3.0Å electron density was of excellent quality and was easily interpreted for the structure.

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PS-02.02.05 ANHARMONIC THERMAL VIBRATION OF RUTILE (TiO_2) DETERMINED FROM NUCLEAR DENSITY DISTRIBUTION OF MAXIMUM ENTROPY METHOD ANALYSIS

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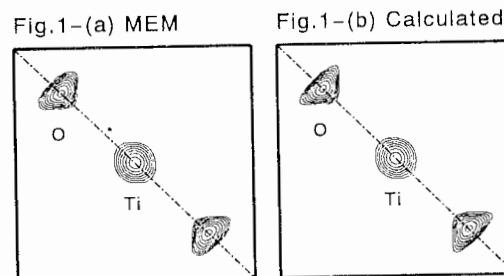
Maximum Entropy Method (MEM) analysis enables us to have an electron density distribution which is consistent with the observed structure factors and least biased with the not-observed structure factors when it is applied to X-ray diffraction data. By applying it to neutron diffraction data, a nuclear density distribution can be obtained, which is considered to describe the thermal

smearing of the nuclei. Such a thermal smearing must be influenced, by all kinds of thermal vibrations regardless harmonic and anharmonic vibrations. It is, therefore, possible to determine thermal parameters of the constituent atoms by the analysis of the nuclear density distributions obtained by the MEM analysis. In this work, the MEM nuclear density distribution of rutile (TiO_2) were analysed by least square refinement and thermal parameters for an effective one particle potential (OPP) were determined. This is a second example of determining the thermal parameters from the nuclear density distribution. First example was done by Takata, Sakata, Larsen, Kubota & Iversen; (1992) *Inaugural Conference, AsCA'92* in the case of Be. In this case, Be atom is located at a special position and it was not necessary to determine the atomic position beforehand. In rutile case, x-coordinate of oxygen has to be determined before the thermal parameters are analysed. In the present study, the atomic position was defined and determined as the position for which the first order moment of nuclear density becomes zero. The obtained x-coordinate was 0.30477, which shows excellent agreement with the previous result of Rietveld refinement by Howard, Sabime & Dickson; (1991) Acta Cryst. B47, 462-468, i.e. 0.30478 (6). The higher moments which are related to thermal behaviors are also calculated, which is very helpful to consider what kind of OPP model to be employed for thermal parameters analysis.

The least square refinement was successfully performed and thermal parameters are obtained as well as the harmonic ones. More specifically to say about anharmonicities of rutile, it was found that Ti atoms had the 4th order anharmonic terms and that O atoms had not only 4th order but also 3rd order anharmonicities, which cause substantial skewness of O atoms in rutile. In Fig.1. both (a) the MEM nuclear densities and (b) the calculated densities from the final thermal parameters determined by the present least square refinement are shown for (002) plane, which are beautifully agreed.

Fig. 1-(a) MEM

Fig. 1-(b) Calculated



PS-02.02.06 Determination of the Anharmonicity Constant of GaAs by Means of the Bijvoet-Relation of the weak (666) Reflection

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Due to the influence of anomalous dispersion the weak (h,h,h) and (-h,-h,-h) reflections of the zinc-blende structure differ from each other. At large scattering vectors this difference, scaled by the Bijvoet-relation