02-Methods for Structure Determination and Analysis, Computing and Graphics

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 λ_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 (Stuhrmann 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.

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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.* A**46**, 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 spoilt 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 absorbtion 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/absorbtion 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.* D**49**, 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

