ADVANCES IN EXPERIMENTAL METHODS FOR PRIMARY PHASING IN X-RAY PROTEIN CRYSTALLOGRAPHY

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The estimation of structure factor phases is a crucial step in the elucidation of the three dimensional structure of biological macromolecules by X-ray diffraction methods. Quasi-planar waves diffracted by a single crystal are described by complex numbers; both amplitudes and phases of these numbers are required to compute by Fourier transform the electron density of the macromolecules. Amplitudes only can be directly derived from intensities diffracted by the native crystal; phases have to be estimated by indirect ways.

The principle of primary phasing by experimental methods (i.e. from intensity measurements only) is anchored on the use of partial structure made of a few atoms which diffract X-rays in a particular fashion, due to their large Z numbers and/or measurable anomalous scattering. This partial structure is simple enough to be solved fairly easily, and its contribution to each diffracted beam can then be use as a reference for the estimation of unknown phases.

In the present communication, the emphasis is on applications of anomalous scattering and on refinement of parameters of the partial structure.

The experimental procedure used to prepare xenon complexes of macromolecular crystals was recently investigated at LURE (1). This methods allows now a convenient way for testing this kind of exotic heavy atom and its ability to produce good heavy atom derivatives. Under moderate pressure, xenon can bind to a number of proteins with weak but specific interactions leading to highly isomorphous derivatives (1,2). Xenon has been used successfully for MIR (multiple isomorphous replacement) phasing of several new structures, and its application to SIRAS (single isomorphous replacement plus anomalous scattering) is currently under study.

Following our experience with MAD (multiwavelength anomalous diffraction), we are investigating a novel methods called MASC (multiwavelength anomalous solvent contrast). In this methods, anomalous scatters are randomly dispersed in the crystal mother liquor. A simple theory has been developed (3) on the basis of previous work (4) on X-ray contrast variation. As in the MAD methods, non isomorphism and many systematic errors are eradicated by the use of a single sample for the collection of data sets at the various wavelengths. From MASC data, it is in principle possible to determine the molecular envelope and low resolution phases. The prospect is that multiwavelengths methods will become integral part of the synthesis, within a Bayesian theory of structure determination (5), between direct methods and conventional macromolecular phasing.

In all experimental primary phasing methods, refinement of parameters of the partial structure is essential to get accurate phase statistics. A maximum likelihood methods (6) which gives unbiased estimates of these parameters has been implemented in a program of general applicability which has been successfully tested both on synthetic data and on MAD data (7,8).

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