Anomalous Dispersion and the Phase Problem in Biocrystallography

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The anomalous dispersion of x-rays is an essential tool for the determination of structure factor phases in macromolecular crystallography. Multiple wavelength methods (MAD and MASC), applied to a cryocooled crystal, put into actual practice the ideal scheme of *ab initio* phase determination where the waves diffracted by the unknown structure should remain invariant. MAD on crystals of purposely bio-engineered proteins is a rather systematic approach to the solution of the phase problem, and might become the method of choice for structural genomics. MASC may provide information on the macromolecular envelope and low resolution phases. We have shown that reliable values of amplitudes of the envelope structure factors can be derived from MASC experiments, but phasing these structure factors is still an open question. Single wavelength experiments will be powerful when isomorphism between a native and a heavy atom derivative can be preserved, which is the case in SIRAS experiments with noble gas (Xe, Kr) derivatives. The development of anomalous dispersion methods from pioneering experiments to the present situation results from a number of experimental advances (in synchrotron radiation sources, instrumentation, and molecular biology) and theoretical and computational advances. The impact of maximum likelihood methods is especially noteworthy. They have provided a rigorous and common frame to all *ab initio* phasing methods. Unbiased phase information produced by maximum likelihood, combined with solvent flattening, produces high quality results and has allowed solution of structures from minimal information (for instance SAD). Current developments aim at parameterizing the anisotropy of anomalous scattering by a label atom (e.g. selenium). These improvements will remove remaining systematic errors in the MAD phase estimates and can ultimately be exploited to give additional phase information.