14. ANOMALOUS DISPERSION

14.1. Heavy-atom location and phase determination with single-wavelength diffraction data

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14.1.1. Introduction

As is well known, the successful introduction of the method of isomorphous replacement by Green *et al.* (1954) was the turning point in the subsequent development of protein crystallography as we now know it.

The idea that the phases of X-ray reflections from a protein crystal could be obtained by the introduction of heavy atoms into the crystal was not new, having been suggested by J. D. Bernal in 1939 (Bernal, 1939). The isomorphous-replacement method was used as early as 1927 by Cork (1927) in studying the alums. Bokhoven et al. (1951) subsequently extended the method to the study of a noncentrosymmetric projection of strychnine sulfate, using what would now be termed the method of single isomorphous replacement. They also suggested that by using a double isomorphous replacement, a unique phase determination could be obtained, even for noncentrosymmetric reflections. The details of the double (or multiple) isomorphous-replacement method were worked out by Harker (1956), who introduced the very useful concept of phase circles. Another contribution which was of great practical value, and which will provide the basis for much of the subsequent discussion, is the method introduced by Blow & Crick (1959) for the treatment of errors in the isomorphous-replacement method. In addition to the determination of protein phases by the method of substitution with heavy atoms, it is now routine to supplement this information by utilizing the anomalous scattering of the substituted atoms. The underlying principles trace back to articles by Bijvoet (1954), Ramachandran & Raman (1956), and Okaya & Pepinsky (1960). The first application of the anomalousscattering method to protein crystallography was by Blow (1958), who used the anomalous scattering of the iron atoms to determine phase information for a noncentrosymmetric projection of horse oxyhaemoglobin.

In the following discussion, we first review the classical method of phase determination by isomorphous replacement, then discuss the inclusion of single-wavelength anomalous-scattering data, and conclude by discussing the use of such data for heavy-atom location. Part of the review is based on Matthews (1970).

14.1.2. The isomorphous-replacement method

Consider a protein crystal with an isomorphous heavy-atom derivative, *i.e.* a modified crystal in which heavy atoms occupy specific sites throughout the crystal, but which is in all other respects identical to the unsubstituted 'parent' crystal. Let the structure factors of the protein crystal be $\mathbf{F}_P(\mathbf{h})$, of the isomorph be $\mathbf{F}_{PH}(\mathbf{h})$, and of the heavy atoms $\mathbf{F}_H(\mathbf{h})$. (Note: Structure *amplitudes* are indicated by italic type, *e.g.* F_P , and *vectors* by bold-face type, *e.g.* \mathbf{F}_P .) In practice, one can measure the structure amplitudes F_P and F_{PH} , and it is desired to obtain from these observable quantities the value of the phase angle of $\mathbf{F}_P(\mathbf{h})$ so that a Fourier synthesis showing the electron density of the protein structure may be calculated. It will be assumed, for the moment, that the positions and occupancy of the sites of heavy-atom binding have been determined as accurately as possible.

From the heavy-atom parameters, the corresponding structure factor $\mathbf{F}_{H}(\mathbf{h})$ is calculated. To determine φ , the phase of $\mathbf{F}_{P}(\mathbf{h})$, we

construct a set of phase circles, as proposed by Harker (1956). From a chosen origin O (Fig. 14.1.2.1*a*), the vector OA is drawn equal to $-\mathbf{F}_{H}$. Circles of radius F_{P} and F_{PH} are then drawn about O and A, respectively. The intersections of the phase circles at B and B' define two possible phase angles for F_{P} . Note that the angles are



Fig. 14.1.2.1. (a) Harker construction for a single isomorphous replacement. φ_1 and φ_2 are the 'most probable' phases for \mathbf{F}_P . (b) Phase probability distribution for a single isomorphous replacement. This and subsequent probabilities are unnormalized. [All figures in this chapter are reproduced with permission from Matthews (1970). Copyright (1970) International Union of Crystallography.]