14.1. HEAVY-ATOM LOCATION AND PHASE DETERMINATION

14.1.9. Anomalous scattering without isomorphous replacement

The treatment outlined above of phase determination by anomalous scattering assumed that data were available for a parent crystal devoid of anomalous scatters and an anomalously scattering isomorphous heavy-atom derivative. It is not uncommon that the native protein itself contains atoms which scatter anomalously or has been engineered to contain such scatterers. In such cases, measurements will usually be made at multiple wavelengths in order to exploit MAD phasing (Hendrickson, 1991). If, however, measurements are available only at a single wavelength, they can be utilized to obtain some phase information (*e.g.* Matthews, 1970).

14.1.10. Location of heavy-atom sites

During the development of protein crystallography, it was understood that heavy-atom sites might be located from difference Patterson functions, but there was substantial debate as to the type of function that was preferable (Perutz, 1956).

Blow (1958), and also Rossmann (1960), advocated a Patterson function with amplitudes $(F_{PH} - F_P)^2$. It relies on the admittedly crude assumption that the desired scattering amplitude of the heavy atoms, $|F_H|$, can be approximated by

$$|F_H| \simeq |F_{PH} - F_P|. \qquad (14.1.10.1)$$

The approximation does have one very helpful characteristic, namely, that it tends to be most accurate when $|F_{PH} - F_P|$ is large, *i.e.* when F_H is parallel or antiparallel to F_P (*cf.* Fig. 14.1.4.1). Thus, the numerically largest coefficients in the Patterson function tend to represent $|F_H|^2$ correctly. Given a well behaved isomorphous heavy-atom derivative, and accurately measured data, experience has shown that a map with coefficients $(F_{PH} - F_P)^2$ can give an excellent representation of the desired heavy-atom–heavy-atom vector peaks.

14.1.11. Use of anomalous-scattering data in heavy-atom location

A relation exactly analogous to equation (14.1.10.1) can be used to approximate the anomalous heavy-atom scattering amplitude, namely,

$$|F_H''| \simeq \tfrac{1}{2}|F_{PH+} - F_{PH-}| \qquad (14.1.11.1)$$

(see Fig. 14.1.7.1*b*). As noted above, if all the heavy atoms are the same, $F_H = kF_H''$. Thus, a Patterson function with coefficients $(F_{PH+} - F_{PH-})^2$ should also show the desired heavy-atom–heavy-atom vector peaks (Blow, 1957; Rossmann, 1961).

For each individual reflection, however, and as is also the case for phase determination, the information that is provided by the isomorphous-replacement difference $(|F_{PH}| - |F_P|)$ is exactly complementary to that provided by the anomalous-scattering measurement $(|F_{PH+}| - |F_{PH-}|)$. By combining both sets of experimental measurements, it is possible to obtain a much better estimate of the heavy-atom scattering, $|F_H|$, for every reflection (Kartha & Parthasarathy, 1965*a,b*; Matthews, 1966*a*; Singh & Ramaseshan, 1966). One formulation (Matthews, 1966*a*) can be written as

$$F_H^2 = F_P^2 + F_{PH}^2 - 2F_P F_{PH}\{1 - [wk(F_{PH+} - F_{PH-})/2F_P^2]^2\}^{1/2}, \qquad (14.1.11.2)$$

where $F_{PH} = (F_{PH+} + F_{PH-})/2$ and w is a weighting factor (from 0 to 1) that is an estimate of the relative reliability of the measurements of $(F_{PH+} - F_{PH-})$ compared with $(F_{PH} - F_P)$.

14.1.12. Use of difference Fourier syntheses

The discussion above has focused on the use of difference Patterson functions to locate heavy-atom sites. Once one or more putative sites have been located, they can be used to calculate approximate protein phases, which, in turn, can be used to calculate difference Fourier series with coefficients in the form

$$m(F_{PH} - F_H)\exp(-i\varphi_B), \qquad (14.1.12.1)$$

where m is the figure of merit and φ_B is the 'best', albeit approximate, phase of the protein structure factor. Putting aside errors due to inaccuracies in φ_B, such maps do not give the true heavy-atom vector, $\mathbf{F}_H$. Rather, they give, essentially, the projection of F_H along $\mathbf{F}_P$ (*cf.* Fig. 14.1.4.1). Nevertheless, subject to certain limitations, such difference maps are extraordinarily powerful in locating secondary sites in a given heavy-atom derivative, or in using approximate phases from one derivative to search for heavy-atom sites in other putative derivatives. It is in this context, however, that certain limitations of the single-isomorphous-replacement (SIR) method have to be kept in mind. These are noted in the next section.

14.1.13. Single isomorphous replacement

Although phase determination from a single heavy-atom derivative in the absence of anomalous-scattering data is, in principle, ambiguous, it was realized early on that useful phase information can still be obtained (Blow & Rossmann, 1961). As shown in Fig. 14.1.2.1(*a*), the two possible phases for the protein are φ_1 or φ_2. In terms of the analysis of Blow & Crick (1959), the 'best' phase to use for the protein is the average of φ_1 and φ_2. This is also equivalent to using *both* φ_1 *and* φ_2. With this in mind, a situation that is of special concern is one in which the heavy-atom distribution used to determine the phases happens to have a centre of symmetry. One common way in which this can occur is when one has a heavy-atom derivative with a single site in space group $P2_1$. A related situation occurs when there are multiple sites in space group $P2_1$, but all have
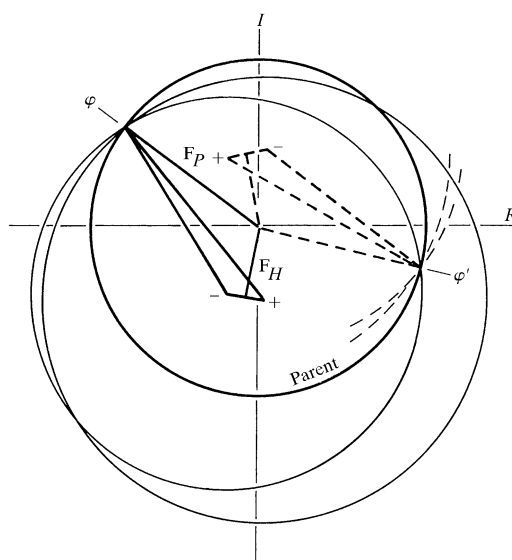

Fig. 14.1.13.1. The consequences of using the incorrect hand for the heavy-atom arrangement in phase determination. The correct heavy-atom arrangement gives the construction drawn in solid lines with φ uniquely determined as the correct phase for $\mathbf{F}_P$. If the enantiomeric arrangement of heavy atoms is used, the dashed construction will result, leading to the incorrect phase φ'. Even though φ is correct and φ' is incorrect, both phase determinations have an identical figure of merit.

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