14.1. HEAVY-ATOM LOCATION AND PHASE DETERMINATION

Individual probability distributions for the additional heavy-atom derivatives are derived in an analogous manner and may be multiplied together to give an overall probability distribution. The joint probability distribution corresponding to Fig. 14.1.3.1(*a*) is shown in Fig. 14.1.3.1(*b*), and in this case the most probable phase is that which simultaneously fits best the observed data for the two isomorphous derivatives.

The main objection which may be made to the Blow & Crick treatment is that it assumes that there is no error in F_P. In practice, however, this is not a serious limitation.

14.1.5. The best Fourier

A protein crystallographer desires to obtain a Fourier synthesis that can most readily be interpreted in terms of an atomic model of the structure. One synthesis which could be calculated is the 'most probable Fourier', obtained by choosing the value of $F_P(\mathbf{h})$ for each reflection which corresponds to the highest value of $P(\varphi)$. Blow & Crick pointed out that although this Fourier is the most likely to be correct, it has certain disadvantages. In the first place, it might tend to give too much weight to uncertain or unreliable phases, and, in the second place, for cases where $P(\varphi)$ is bimodal, there is a strong chance of making a large error in the phase angle. Blow & Crick suggested that in cases such as this, a compromise is needed, and that the centroid of the phase probability distribution provides just the required compromise. They showed that the corresponding synthesis is the 'best Fourier', which is defined to be *that Fourier transform which is expected to have the minimum mean-square difference from the Fourier transform of the true F's when averaged over the whole unit cell.*

The centroid of the phase probability distribution may be defined as a point on the phase diagram with polar coordinates (mF_P, φ_B), where φ_B is the 'best' phase angle. The quantity m, which acts as a weighting factor for F_P, is called the 'figure of merit' of the phase determination. Its magnitude, between 0 and 1, is a measure of the reliability of the phase determination.

14.1.6. Anomalous scattering

All atoms, particularly those used in preparing heavy-atom isomorphs, give rise to anomalous scattering, especially if the energy of the scattered X-rays is close to an absorption edge. The atomic scattering factor of the atom in question can be expressed as

$$\mathbf{f} = f_0 + \Delta f' + if'' = f' + if'', \qquad (14.1.6.1)$$

where f_0 is the normal scattering factor far from an absorption edge, and $\Delta f'$ and f'' are the correction terms which arise due to dispersion effects. The quantity $\Delta f'$, in phase with f_0, is usually negative, and f'', the imaginary part, is always $\pi/2$ ahead of the phase of the real part $(f_0 + \Delta f')$. It may be noted that by using different wavelengths, the term $\Delta f'$ is equivalent to a change in scattering power of the heavy atom and produces intensity differences similar to a normal isomorphous replacement, except that in this case the isomorphism is *exact* (Ramaseshan, 1964). This is the basis of the multiwavelength-anomalous-dispersion (MAD) method (Hendrickson, 1991) discussed in Chapter 14.2. Here we focus on measurements based on a single wavelength, traditionally referred to as the 'anomalous-scattering method'.

The anomalous scattering of a heavy atom is always considerably less than the normal scattering (for Cu $K\alpha$ radiation, $2f''/f'$ ranges from about 0.24 to 0.36), but there are several factors which tend to offset this reduction in magnitude (*e.g.* see Blow, 1958; North, 1965).
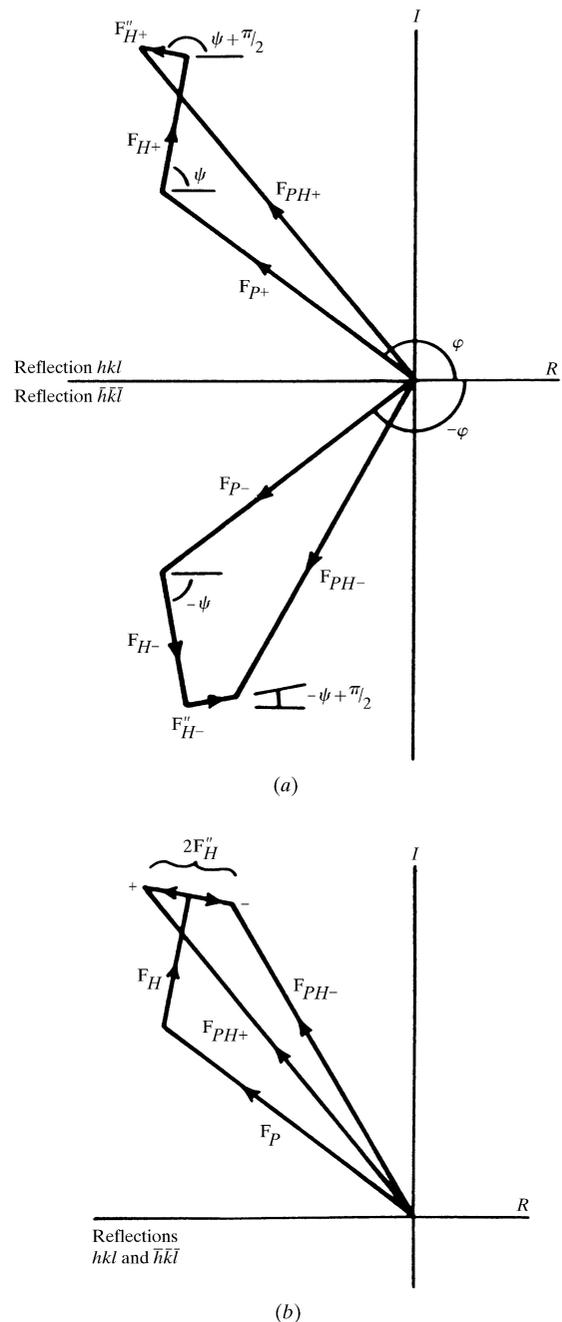

(*a*)

(*b*)

Fig. 14.1.7.1. (*a*) Vector diagrams illustrating anomalous scattering for the reflections *hkl* and $\overline{hkl}$. (*b*) Combined vector diagram for reflections *hkl* and $\overline{hkl}$.

14.1.7. Theory of anomalous scattering

Suppose that two isomorphous crystals are differentiated by N heavy atoms of position $\mathbf{r}_n$ and scattering factor $(\mathbf{f}'_n + i\mathbf{f}''_n)$. Then, for the reflection *hkl*, the calculated structure factor of the N atoms is

$$F_H(\mathbf{h}) + iF''_H(\mathbf{h}) = \sum_{n=1}^{N} f'_n(\mathbf{h}) \exp(2\pi i\mathbf{h} \cdot \mathbf{r}_n)$$

$$+ i\sum_{n=1}^{N} f''_n(\mathbf{h}) \exp(2\pi i\mathbf{h} \cdot \mathbf{r}_n). \qquad (14.1.7.1)$$

If the heavy atoms are all of the same type, *i.e.* they all have the same ratio of $f'_n/f''_n (= k)$, then F_H and F''_H are orthogonal, and $F''_H = F_H/k$.

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