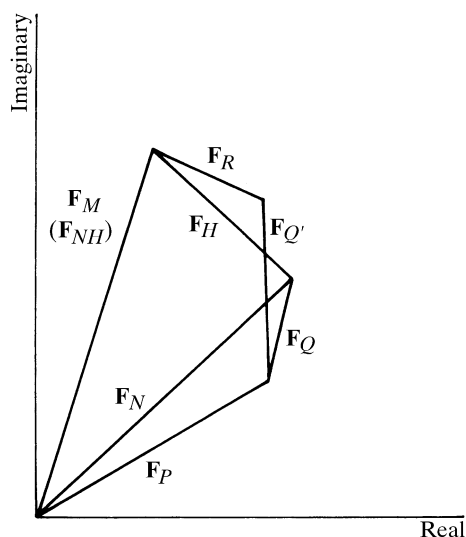2.4. ISOMORPHOUS REPLACEMENT AND ANOMALOUS SCATTERING

Fig. 2.4.2.1. Vector relationship between $\mathbf{F}_N$ and $\mathbf{F}_M$ ($\equiv \mathbf{F}_{NH}$).

replacement' will be used to cover both cases. Also, in as much as $\mathbf{F}_M$ is the vector sum of $\mathbf{F}_N$ and $\mathbf{F}_H$, $\mathbf{F}_M$ and $\mathbf{F}_{NH}$ will be used synonymously. Thus

$$\mathbf{F}_M \equiv \mathbf{F}_{NH} = \mathbf{F}_N + \mathbf{F}_H. \qquad (2.4.2.4)$$

2.4.2.2. *Single isomorphous replacement method*

The number of replaceable (or 'added') atoms is usually small and they generally have high atomic numbers. Their positions are often determined by a Patterson synthesis of one type or another (see Chapter 2.3). It will therefore be assumed in the following discussion that $\mathbf{F}_H$ is known. Then it can be readily seen by referring to Fig. 2.4.2.2 that

$$\alpha_N = \alpha_H - \cos^{-1}\frac{F_{NH}^2 - F_N^2 - F_H^2}{2F_N F_H} = \alpha_H \pm \varphi; \qquad (2.4.2.5)$$

when φ is derived from its cosine function, it could obviously be positive or negative. Hence, there are two possible solutions for α_N. These two solutions are distributed symmetrically about $\mathbf{F}_H$. One of these would correspond to the correct value of α_N. Therefore, in general, the phase angle cannot be unambiguously determined using a pair of isomorphous crystals.

The twofold ambiguity in phase angle vanishes when the structures are centrosymmetric. $\mathbf{F}_{NH}, \mathbf{F}_N$ and $\mathbf{F}_H$ are all real in
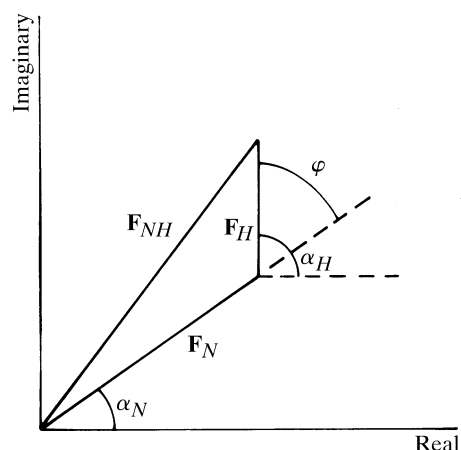
Fig. 2.4.2.2. Relationship between α_N, α_H and φ.

centric data and the corresponding phase angles are 0 or 180°. From (2.4.2.4)

$$F_{NH} \pm F_N = F_H. \qquad (2.4.2.6)$$

The sign of F_H is already known and the signs of F_{NH} and F_N can be readily determined from (2.4.2.6) (Robertson & Woodward, 1937).

When the data are acentric, the best one can do is to use both the possible phase angles simultaneously in a Fourier synthesis (Bokhoven *et al.*, 1951). This double-phased synthesis, which is equivalent to the isomorphous synthesis of Ramachandran & Raman (1959), contains the structure and its inverse when the replaceable atoms have a centrosymmetric distribution (Ramachandran & Srinivasan, 1970). When the distribution is noncentrosymmetric, however, the synthesis contains peaks corresponding to the structure and general background. Fourier syntheses computed using the single isomorphous replacement method of Blow & Rossmann (1961) and Kartha (1961) have the same properties. In this method, the phase angle is taken to be the average of the two possible solutions of α_N, which is always α_H or $\alpha_H + 180°$. Also, the Fourier coefficients are multiplied by $\cos\varphi$, following arguments based on the Blow & Crick (1959) formulation of phase evaluation (see Section 2.4.4.4). Although Blow & Rossmann (1961) have shown that this method could yield interpretable protein Fourier maps, it is rarely used as such in protein crystallography as the Fourier maps computed using it usually have unacceptable background levels (Blundell & Johnson, 1976).

2.4.2.3. *Multiple isomorphous replacement method*

The ambiguity in α_N in a noncentrosymmetric crystal can be resolved only if at least two crystals isomorphous to it are available (Bokhoven *et al.*, 1951). We then have two equations of the type (2.4.2.5), namely,

$$\alpha_N = \alpha_{H1} \pm \varphi_1 \quad \text{and} \quad \alpha_N = \alpha_{H2} \pm \varphi_2, \qquad (2.4.2.7)$$

where subscripts 1 and 2 refer to isomorphous crystals 1 and 2, respectively. This is demonstrated graphically in Fig. 2.4.2.3 with the aid of the Harker (1956) construction. A circle is drawn with F_N as radius and the origin of the vector diagram as the centre. Two more circles are drawn with F_{NH1} and F_{NH2} as radii and the ends of vectors $-\mathbf{F}_{H1}$ and $-\mathbf{F}_{H2}$, respectively as centres. Each of these circles intersects the F_N circle at two points corresponding to the two possible solutions. One of the points of intersection is common and this point defines the correct value of α_N. With the assumption of perfect isomorphism and if errors are neglected, the phase circles corresponding to all the crystals would intersect at a common point if a number of isomorphous crystals were used for phase determination.

2.4.3. Anomalous-scattering method

2.4.3.1. *Dispersion correction*

Atomic scattering factors are normally calculated on the assumption that the binding energy of the electrons in an atom is negligible compared to the energy of the incident X-rays and the distribution of electrons is spherically symmetric. The transition frequencies within the atom are then negligibly small compared to the frequency of the radiation used and the scattering power of each electron in the atom is close to that of a free electron. When this assumption is valid, the atomic scattering factor is a real positive number and its value decreases as the scattering angle increases because of the finite size of the atom. When the binding energy of the electrons is appreciable, the atomic scattering factor at any given angle is given by

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references