International Tables for Crystallography (2006). Vol. B, Section 2.4.2.1, pp. 264–265.

2.4. Isomorphous replacement and anomalous scattering

BY M. VIJAYAN AND S. RAMASESHAN

2.4.1. Introduction

Isomorphous replacement is among the earliest methods to be employed for crystal structure determination (Cork, 1927). The power of this method was amply demonstrated in the classical X-ray work of J. M. Robertson on phthalocyanine in the 1930s using centric data (Robertson, 1936; Robertson & Woodward, 1937). The structure determination of strychnine sulfate pentahydrate by Bijvoet and others provides an early example of the application of this method to acentric reflections (Bokhoven *et al.*, 1951). The usefulness of isomorphous replacement in the analysis of complex protein structures was demonstrated by Perutz and colleagues (Green *et al.*, 1954). This was closely followed by developments in the methodology for the application of isomorphous replacement to protein work (Harker, 1956; Blow & Crick, 1959) and rapidly led to the first ever structure solution of two related protein crystals, namely, those of myoglobin and haemoglobin (Kendrew *et al.*, 1960; Cullis *et al.*, 1961*b*). Since then isomorphous replacement has been the method of choice in macromolecular crystallography and most of the subsequent developments in and applications of this method have been concerned with biological macromolecules, mainly proteins (Blundell & Johnson, 1976; McPherson, 1982).

The application of anomalous-scattering effects has often developed in parallel with that of isomorphous replacement. Indeed, the two methods are complementary to a substantial extent and they are often treated together, as in this article. Although the most important effect of anomalous scattering, namely, the violation of Friedel's law, was experimentally observed as early as 1930 (Coster *et al.*, 1930), two decades elapsed before this effect was made use of for the first time by Bijvoet and his associates for the determination of the absolute configuration of asymmetric molecules as well as for phase evaluation (Bijvoet, 1949, 1954; Bijvoet *et al.*, 1951). Since then there has been a phenomenal spurt in the application of anomalous-scattering effects (Srinivasan, 1972; Ramaseshan & Abrahams, 1975; Vijayan, 1987). A quantitative formulation for the determination of phase angles using intensity differences between Friedel equivalents was derived by Ramachandran & Raman (1956), while Okaya & Pepinsky (1956) successfully developed a Patterson approach involving anomalous effects. The anomalous-scattering method of phase determination has since been used in the structure analysis of several structures, including those of a complex derivative of vitamin B_{12} (Dale *et al.*, 1963) and a small protein (Hendrickson & Teeter, 1981). In the meantime, the effect of changes in the real component of the dispersion correction as a function of the wavelength of the radiation used, first demonstrated by Mark & Szillard (1925), also received considerable attention. This effect, which is formally equivalent to that of isomorphous replacement, was demonstrated to be useful in structure determination (Ramaseshan *et al.*, 1957; Ramaseshan, 1963). Protein crystallographers have been quick to exploit anomalous-scattering effects (Rossmann, 1961; Kartha & Parthasarathy, 1965; North, 1965; Matthews, 1966; Hendrickson, 1979) and, as in the case of the isomorphous replacement method, the most useful applications of anomalous scattering during the last two decades have been perhaps in the field of macromolecular crystallography (Kartha, 1975; Watenpaugh *et al.*, 1975; Vijayan, 1981). In addition to anomalous scattering of X-rays, that of neutrons was also found to have interesting applications (Koetzle & Hamilton, 1975; Sikka & Rajagopal, 1975). More recently there has been a further revival in the development of anomalous-scattering methods with the advent of synchrotron radiation, particularly in view of the possibility of choosing any desired wavelength from a synchrotron-radiation source (Helliwell, 1984).

It is clear from the foregoing that the isomorphous replacement and the anomalous-scattering methods have a long and distinguished history. It is therefore impossible to do full justice to them in a comparatively short presentation like the present one. Several procedures for the application of these methods have been developed at different times. Many, although of considerable historical importance, are not extensively used at present for a variety of reasons. No attempt has been made to discuss them in detail here; the emphasis is primarily on the state of the art as it exists now. The available literature on isomorphous replacement and anomalous scattering is extensive. The reference list given at the end of this part is representative rather than exhaustive.

During the past few years, rapid developments have taken place in the isomorphous replacement and anomalous-scattering methods, particularly in the latter, as applied to macromolecular crystallography. These developments will be described in detail in *International Tables for Crystallography*, Volume F (2001). Therefore, they have not been dealt with in this chapter. Significant developments in applications of direct methods to macromolecular crystallography have also occurred in recent years. A summary of these developments as well as the traditional direct methods on which the recent progress is based are presented in Chapter 2.2.

2.4.2. Isomorphous replacement method

2.4.2.1. *Isomorphous replacement and isomorphous addition*

Two crystals are said to be isomorphous if (*a*) both have the same space group and unit-cell dimensions and (*b*) the types and the positions of atoms in both are the same except for a replacement of one or more atoms in one structure with different types of atoms in the other (isomorphous replacement) or the presence of one or more additional atoms in one of them (isomorphous addition). Consider two crystal structures with identical space groups and unit-cell dimensions, one containing N atoms and the other M atoms. The N atoms in the first structure contain subsets P and Q whereas the M atoms in the second structure contain subsets P, Q' and R. The subset P is common to both structures in terms of atomic positions and atom types. The atomic positions are identical in subsets Q and Q', but at any given atomic position the atom type is different in Q and Q'. The subset R exists only in the second structure. If $\mathbf{F}_N$ and $\mathbf{F}_M$ denote the structure factors of the two structures for a given reflection,

$$\mathbf{F}_N = \mathbf{F}_P + \mathbf{F}_Q \qquad (2.4.2.1)$$

and

$$\mathbf{F}_M = \mathbf{F}_P + \mathbf{F}_{Q'} + \mathbf{F}_R, \qquad (2.4.2.2)$$

where the quantities on the right-hand side represent contributions from different subsets. From (2.4.2.1) and (2.4.2.2) we have

$$\mathbf{F}_M - \mathbf{F}_N = \mathbf{F}_H = \mathbf{F}_{Q'} - \mathbf{F}_Q + \mathbf{F}_R. \qquad (2.4.2.3)$$

The above equations are illustrated in the Argand diagram shown in Fig. 2.4.2.1. $\mathbf{F}_Q$ and $\mathbf{F}_{Q'}$ would be collinear if all the atoms in Q were of the same type and those in Q' of another single type, as in the replacement of chlorine atoms in a structure by bromine atoms.

We have a case of 'isomorphous replacement' if $\mathbf{F}_R = 0$ ($\mathbf{F}_H = \mathbf{F}_{Q'} - \mathbf{F}_Q$) and a case of 'isomorphous addition' if $\mathbf{F}_Q = \mathbf{F}_{Q'} = 0$ ($\mathbf{F}_H = \mathbf{F}_R$). Once $\mathbf{F}_H$ is known, in addition to the magnitudes of $\mathbf{F}_N$ and $\mathbf{F}_M$, which can be obtained experimentally, the two cases can be treated in an equivalent manner in reciprocal space. In deference to common practice, the term 'isomorphous

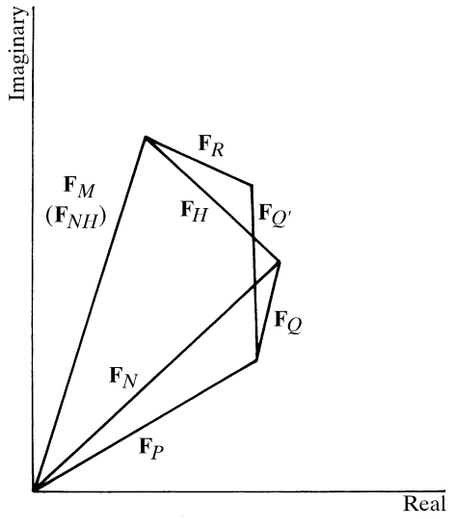

Fig. 2.4.2.1. Vector relationship between $\mathbf{F}_N$ and $\mathbf{F}_M$ $(\equiv \mathbf{F}_{NH})$.

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 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 φ, 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.

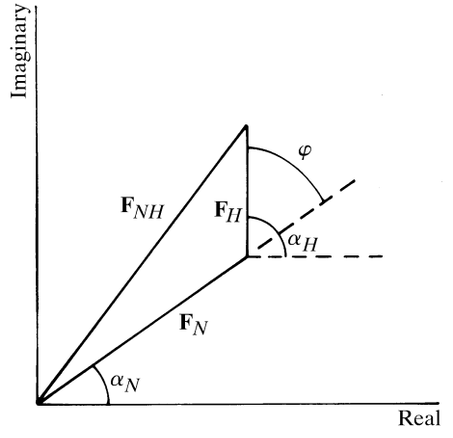

Fig. 2.4.2.2. Relationship between α_N, α_H and φ.

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

references