

2.4. Isomorphous replacement and anomalous scattering

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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 B₁₂ (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,

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

and

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

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

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

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