

2. BASIC CRYSTALLOGRAPHY

ejected from the atom or are elevated to a higher-energy bound state, the photons disappear completely and the X-ray beam is strongly absorbed. This is called *photoelectric absorption*. At an absorption edge, the frequency of the X-ray beam ν is equal to the frequency ν_K , ν_L or ν_M corresponding to the energy of the K , L or M state. According to equation (2.1.4.4), anomalous scattering is maximal at an absorption edge.

2.1.7. Calculation of electron density

In equation (2.1.4.6), the wave $W_{\text{cr}}(\mathbf{S})$ scattered by the crystal is given as the sum of the atomic contributions, as in equation (2.1.4.5) for the scattering by a unit cell. In the derivation of equation (2.1.4.5), it is assumed that the atoms are spherically symmetric (Section 2.1.4.3) and that density changes due to chemical bonding are neglected. A more exact expression for the wave scattered by a crystal, in the absence of anomalous scattering, is

$$W_{\text{cr}}(\mathbf{S}) = \int_{\text{crystal}} \rho(\mathbf{r}) \exp(2\pi i \mathbf{r} \cdot \mathbf{S}) \, dV_{\text{real}}. \quad (2.1.7.1)$$

The integration is over all electrons in the crystal. $\rho(\mathbf{r})$ is the electron-density distribution in each unit cell. The operation on the electron-density distribution in equation (2.1.7.1) is called Fourier transformation, and $W_{\text{cr}}(\mathbf{S})$ is the Fourier transform of $\rho(\mathbf{r})$. It can be shown that $\rho(\mathbf{r})$ is obtained by an inverse Fourier transformation:

$$\rho(\mathbf{r}) = \int_{\mathbf{S}} W_{\text{cr}}(\mathbf{S}) \exp(-2\pi i \mathbf{r} \cdot \mathbf{S}) \, dV_{\text{reciprocal}}. \quad (2.1.7.2)$$

In contrast to $\rho(\mathbf{r})$, $W_{\text{cr}}(\mathbf{S})$ is not a continuous function but, because of the Laue conditions, it is only different from zero at the reciprocal-lattice points \mathbf{h} ($= hkl$). In equation (2.1.4.6), $W_{\text{cr}}(\mathbf{S})$ is the product of the structure factor and three delta functions. The structure factor at the reciprocal-lattice points is $F(\mathbf{h})$, and the product of the three delta functions is $1/V$, the volume of one reciprocal unit cell. Therefore, $W_{\text{cr}}(\mathbf{S})$ in equation (2.1.7.2) can be replaced by $F(\mathbf{h})/V$, and equation (2.1.7.2) itself by

$$\rho(\mathbf{r}) = (1/V) \sum_{\mathbf{h}} F(\mathbf{h}) \exp(-2\pi i \mathbf{r} \cdot \mathbf{h}). \quad (2.1.7.3)$$

If x , y and z are fractional coordinates in the unit cell, $\mathbf{r} \cdot \mathbf{S} = (\mathbf{a} \cdot x + \mathbf{b} \cdot y + \mathbf{c} \cdot z) \cdot \mathbf{S} = \mathbf{a} \cdot \mathbf{S} \cdot x + \mathbf{b} \cdot \mathbf{S} \cdot y + \mathbf{c} \cdot \mathbf{S} \cdot z = hx + ky + lz$, and an alternative expression for the electron density is

$$\rho(xyz) = (1/V) \sum_h \sum_k \sum_l F(hkl) \exp[-2\pi i(hx + ky + lz)]. \quad (2.1.7.4)$$

Instead of expressing $F(\mathbf{S})$ as a summation over the atoms [equation (2.1.4.5)], it can be expressed as an integration over the electron density in the unit cell:

$$F(hkl) = V \int_{x=0}^1 \int_{y=0}^1 \int_{z=0}^1 \rho(xyz) \exp[2\pi i(hx + ky + lz)] \, dx \, dy \, dz. \quad (2.1.7.5)$$

Because $F(hkl)$ is a vector in the Argand diagram with an amplitude $|F(hkl)|$ and a phase angle $\alpha(hkl)$,

$$F(hkl) = |F(hkl)| \exp[i\alpha(hkl)]$$

and

$$\rho(xyz) = (1/V) \sum_h \sum_k \sum_l |F(hkl)| \exp[-2\pi i(hx + ky + lz) + i\alpha(hkl)]. \quad (2.1.7.6)$$

By applying equation (2.1.7.6), the electron-density distribution in the unit cell can be calculated, provided values of $|F(hkl)|$ and $\alpha(hkl)$ are known. From equation (2.1.6.1), it is clear that $|F(hkl)|$ can be derived, on a relative scale, from $I_{\text{int}}(hkl)$ after a correction for the background and absorption, and after application of the Lorentz and polarization factor:

$$|F(hkl)| = \left[\frac{I_{\text{int}}(hkl)}{LPT} \right]^{1/2}. \quad (2.1.7.7)$$

Contrary to the situation with crystals of small compounds, it is not easy to find the phase angles $\alpha(hkl)$ for crystals of macromolecules by direct methods, although these methods are in a state of development (see Part 16). Indirect methods to determine the protein phase angles are:

- (1) isomorphous replacement (see Part 12);
- (2) molecular replacement (see Part 13);
- (3) multiple-wavelength anomalous dispersion (MAD) (see Part 14).

From equation (2.1.7.5), it is clear that the reflections hkl and \overline{hkl} have the same value for their structure-factor amplitudes, $|F(hkl)| = |F(\overline{hkl})|$, and for their intensities, $I(hkl) = I(\overline{hkl})$, but have opposite values for their phase angles, $\alpha(hkl) = -\alpha(\overline{hkl})$, assuming that anomalous dispersion can be neglected. Consequently, equation (2.1.7.6) reduces to

$$\rho(xyz) = (1/V) \sum_h \sum_k \sum_l |F(hkl)| \cos[2\pi(hx + ky + lz) - \alpha(hkl)] \quad (2.1.7.8)$$

or

$$\rho(xyz) = F(000)/V + (2/V) \sum'_h \sum'_k \sum'_l |F(hkl)| \times \cos[2\pi(hx + ky + lz) - \alpha(hkl)]. \quad (2.1.7.9)$$

\sum' denotes that $F(000)$ is excluded from the summation and that only the reflections hkl , and not \overline{hkl} , are considered.

The two reflections, hkl and \overline{hkl} , are called Friedel or Bijvoet pairs.

If anomalous dispersion cannot be neglected, the two members of a Friedel pair have different values for their structure-factor amplitudes, and their phase angles no longer have opposite values. This is caused by the f'' contribution to the anomalous scattering (Fig. 2.1.7.1). Macromolecular crystals show anomalous dispersion if the structure contains, besides the light atoms, one or more heavier atoms. These can be present in the native structure or are introduced in the isomorphous replacement technique or in MAD analysis.

2.1.8. Symmetry in the diffraction pattern

In the previous section, it was noted that $I(hkl) = I(\overline{hkl})$ if anomalous scattering can be neglected. In this case, the effect is that the diffraction pattern has a centre of symmetry. This is also true for the reciprocal lattice if the reciprocal-lattice points (hkl) are weighted with their $I(hkl)$ values. If the crystal structure has symmetry elements, they are also found in the diffraction pattern and in the weighted reciprocal lattice. Macromolecular crystals of biological origin are enantiomorphic and the symmetry operators in the crystal are restricted to rotation axes and screw axes. It is evident that a rotation of the real lattice will cause the same