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 $W_{cr}(\mathbf{S})$ 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

$$W_{cr}(\mathbf{S}) = \int\limits_{\text{crystal}} \rho(\mathbf{r}) \exp(2\pi i \mathbf{r} \cdot \mathbf{S}) \, \mathrm{d}v_{\text{real}}. \qquad (2.1.7.1)$$

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 $W_{cr}(\mathbf{S})$ is the Fourier transform of $\rho(\mathbf{r})$. It can be shown that $\rho(\mathbf{r})$ is obtained by an inverse Fourier transformation:

$$\rho(\mathbf{r}) = \int\limits_{\mathbf{S}} W_{cr}(\mathbf{S}) \exp(-2\pi i \mathbf{r} \cdot \mathbf{S}) \, \mathrm{d}v_{\text{reciprocal}}. \qquad (2.1.7.2)$$

In contrast to $\rho(\mathbf{r})$, $W_{cr}(\mathbf{S})$ 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), $W_{cr}(\mathbf{S})$ 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, $W_{cr}(\mathbf{S})$ in equation (2.1.7.2) can be replaced by $F(\mathbf{h})/V$, and equation (2.1.7.2) itself by

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

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

$$\rho(xyz) = (1/V) \sum_{h} \sum_{k} \sum_{l} F(hkl) \exp[-2\pi i(hx + ky + lz)]. \qquad (2.1.7.4)$$

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:

$$F(hkl) = V \int\limits_{x=0}^{1} \int\limits_{y=0}^{1} \int\limits_{z=0}^{1} \rho(xyz) \exp[2\pi i(hx + ky + lz)] \, \mathrm{d}x \, \mathrm{d}y \, \mathrm{d}z. \qquad (2.1.7.5)$$

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

$$\rho(xyz) = (1/V) \sum_{h} \sum_{k} \sum_{l} |F(hkl)| \exp[-2\pi i(hx + ky + lz) + i\alpha(hkl)]. \qquad (2.1.7.6)$$

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:

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

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

$$\rho(xyz) = (1/V) \sum_{h} \sum_{k} \sum_{l} |F(hkl)| \cos[2\pi(hx + ky + lz) - \alpha(hkl)] \qquad (2.1.7.8)$$

or

$$\rho(xyz) = F(000)/V + (2/V) {\sum_{h}}' {\sum_{k}}' {\sum_{l}}' |F(hkl)|$$
$$\times \cos[2\pi(hx + ky + lz) - \alpha(hkl)]. \qquad (2.1.7.9)$$

$\sum'$ 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
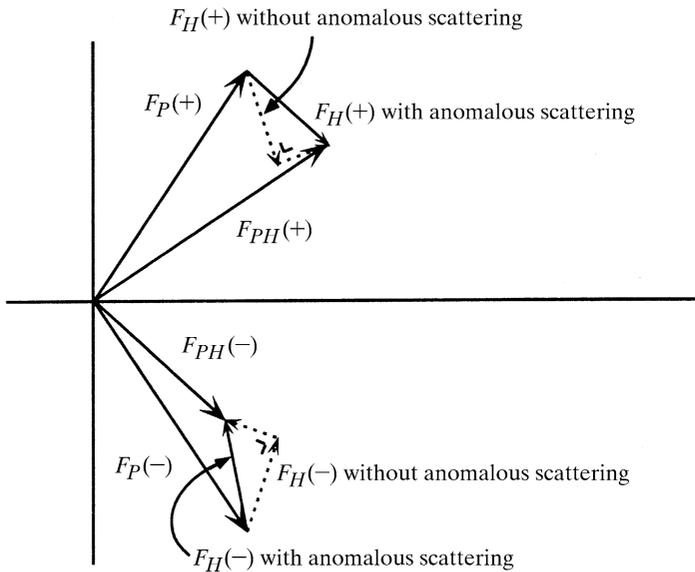
Figure 2.1.7.1

An Argand diagram for the structure factors of the two members of a Friedel pair. (+) represents hkl and (−) represents $\overline{hkl}$. F_P is the contribution to the structure factor by the non-anomalously scattering protein atoms and F_H is that for the anomalously scattering atoms. F_H consists of a real part with an imaginary part perpendicular to it. The real parts are mirror images with respect to the horizontal axis. The imaginary parts are rotated counterclockwise with respect to the real parts (Section 2.1.4.4). The result is that the total structure factors, $F_{PH}(+)$ and $F_{PH}(-)$, have different amplitudes and phase angles. Reproduced with permission from Drenth (1999). Copyright (1999) Springer-Verlag.

rotation of the reciprocal lattice. If this rotation is the result of a symmetry operation around an axis, the crystal structure looks exactly the same as before the rotation, and the same must be true for the weighted reciprocal lattice. However, screw axes in the crystal lattice reduce to normal (non-screw) rotation axes in the weighted reciprocal lattice, as has been shown by Waser (1955). We follow his arguments, but must first introduce matrix notation for convenience.

If $\mathbf{r}$ is a position vector and $\mathbf{h}$ a vector in reciprocal space, the scalar product

$$\mathbf{h} \cdot \mathbf{r} = (h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*) \cdot (\mathbf{a}x + \mathbf{b}y + \mathbf{c}z) = hx + ky + lz,$$

or in matrix notation,

$$(hkl)\begin{pmatrix} x \\ y \\ z \end{pmatrix} = \mathbf{h}^T \mathbf{r},$$

where $(hkl) = \mathbf{h}^T$ is a row vector and

$$\begin{pmatrix} x \\ y \\ z \end{pmatrix} = \mathbf{r}$$

is a column vector. $\mathbf{h}^T$ is the transpose of column vector $\mathbf{h}$ (rows and columns are interchanged). In this notation, the structure factor is given by

$$F(\mathbf{h}) = \int_{\text{cell}} \rho(\mathbf{r}) \exp(2\pi i \mathbf{h}^T \cdot \mathbf{r}) \, dv_{\text{real}}. \qquad (2.1.8.1)$$

The symmetry operation of a screw axis is a combination of a rotation and a translation. The rotation can be represented by the matrix $\mathbf{R}$ and the translation by the vector $\mathbf{t}$. Because of the screw-axis symmetry, $\rho(\mathbf{R} \cdot \mathbf{r} + \mathbf{t}) = \rho(\mathbf{r})$.

$F(\mathbf{h})$ can also be expressed as

$$F(\mathbf{h}) = \int_{\text{cell}} \rho(\mathbf{R} \cdot \mathbf{r} + \mathbf{t}) \exp[2\pi i \mathbf{h}^T \cdot (\mathbf{R} \cdot \mathbf{r} + \mathbf{t})] \, dv_{\text{real}}$$

$$= \exp(2\pi i \mathbf{h}^T \cdot \mathbf{t}) \int_{\text{cell}} \rho(\mathbf{r}) \exp(2\pi i \mathbf{h}^T \cdot \mathbf{R} \cdot \mathbf{r}) \, dv_{\text{real}}. \qquad (2.1.8.2)$$

Because $\mathbf{h}^T \cdot \mathbf{R} = (\mathbf{R}^T \cdot \mathbf{h})^T$, where $\mathbf{R}^T$ is the transpose of the matrix $\mathbf{R}$, equation (2.1.8.2) can be written as

$$F(\mathbf{h}) = \exp(2\pi i \mathbf{h}^T \cdot \mathbf{t}) \int_{\text{cell}} \rho(\mathbf{r}) \exp[2\pi i (\mathbf{R}^T \cdot \mathbf{h})^T \cdot \mathbf{r}] \, dv_{\text{real}}.$$

$$(2.1.8.3)$$

By definition, the integral in equation (2.1.8.3) is $F(\mathbf{R}^T \cdot \mathbf{h})$, and, therefore

$$F(\mathbf{h}) = \exp(2\pi i \mathbf{h}^T \cdot \mathbf{t}) F(\mathbf{R}^T \cdot \mathbf{h}).$$

Conclusion: The phase angles of the two structure factors are different for $\mathbf{t} \neq 0$:

$$\alpha(\mathbf{h}) = \alpha(\mathbf{R}^T \cdot \mathbf{h}) + 2\pi \mathbf{h}^T \cdot \mathbf{t}, \qquad (2.1.8.4)$$

but the structure-factor amplitudes and, therefore, the intensities are always equal:

$$I(\mathbf{h}) = I(\mathbf{R}^T \cdot \mathbf{h}) \quad \text{or} \quad I[(\mathbf{R}^T)^{-1} \cdot \mathbf{h}] = I(\mathbf{h}). \qquad (2.1.8.5)$$

The matrices $(\mathbf{R}^T)^{-1}$ in reciprocal space and $\mathbf{R}$ in direct space denote rotation over the same angle. Therefore, both an n-fold screw axis and an n-fold rotation axis in the crystal correspond to an n-fold axis in the weighted reciprocal lattice.

However, screw axes distinguish themselves from non-screw axes by extinction of some reflections along the line in reciprocal space corresponding to the screw-axis direction. This will be shown for a twofold screw axis along the monoclinic b axis.

The electron density at $\mathbf{r}$, $\rho(\mathbf{r})$, is then equal to the electron density at $\mathbf{R} \cdot \mathbf{r} + \mathbf{t}$, where $\mathbf{R} \cdot \mathbf{r}$ is a rotation that leaves the value of the y coordinate unchanged. $\mathbf{t}$ is equal to $\mathbf{b}/2$.

$$F(\mathbf{h}) = \int_{\text{half the cell}} \rho(\mathbf{r})\{\exp(2\pi i \mathbf{h}^T \cdot \mathbf{r}) + \exp[2\pi i \mathbf{h}^T (\mathbf{R} \cdot \mathbf{r} + \mathbf{t})]\} \, dv_{\text{real}}.$$

$$(2.1.8.6)$$

For the $(0k0)$ reflections, ($\mathbf{h}$ along $\mathbf{b}^*$) is $\mathbf{h} = k\mathbf{b}^*$, giving

$$\mathbf{h}^T \cdot \mathbf{r} = \mathbf{h}^T \cdot \mathbf{R} \cdot \mathbf{r} = 0 + ky + 0 \text{ and } \mathbf{h}^T \cdot \mathbf{t} = k/2.$$

This simplifies equation (2.1.8.6) to

$$F(0k0) = [1 + \exp(\pi i k)] \int_{\text{half the cell}} \rho(\mathbf{r}) \exp(2\pi i ky) \, dv_{\text{real}}.$$

$$(2.1.8.7)$$

If k is odd, $F(0k0) = 0$, because $1 + \exp(\pi i k) = 0$.

This type of systematic absence, due to screw components in the symmetry elements, occurs along lines in reciprocal space. Other types of absence apply to all hkl reflections. They result from the centring of the unit cell (Fig. 2.1.1.4). Suppose the unit cell is centred in the ab plane (C centring). Consequently, the electron density at $\mathbf{r}$ is equal to the electron density at $\mathbf{r} + \mathbf{t}$, with $\mathbf{t} = \mathbf{a}/2 + \mathbf{b}/2$ and $\mathbf{h}^T \cdot \mathbf{t} = h/2 + k/2$. The structure factor can then be written as

$$F(\mathbf{h}) = \{1 + \exp[\pi i(h + k)]\} \int_{\text{half the cell}} \rho(\mathbf{r}) \exp(2\pi i \mathbf{h}^T \cdot \mathbf{r}) \, dv_{\text{real}}.$$

$$(2.1.8.8)$$

The conclusion is that when $(h + k)$ is odd, the structure factors are zero and no diffracted intensity is observed for those reflections.

references