2.3. PATTERSON AND MOLECULAR-REPLACEMENT TECHNIQUES

Peaks are superimposed; this leads to a small apparent peak-to-noise ratio. The effect can be eliminated by removal of the origins through a modification of the Patterson coefficients. Irrespective of origin removal, a significant peak is one which is more than three r.m.s. deviations from the mean background.

As in all continuous functions sampled at discrete points, a convenient grid size must be chosen. Small intervals result in an excessive computing burden, while large intervals might miss peaks. Furthermore, equal increments of angles do not represent equal changes in rotation, which can result in distorted peaks (Lattman, 1972). In general, a crude idea of a useful sampling interval can be obtained by considering the angle necessary to move one reciprocal-lattice point onto its neighbour (separated by $a^\ast$) at the extremity of the resolution limit, $R$. This interval is given by

$$\Delta \theta = a^\ast/2(1/R) = \frac{\pi}{2}Ra^\ast.$$  

Simple sharpening of the rotation function can be useful. This can be achieved by restricting the computations to a shell in reciprocal space or by using normalized structure factors. Useful limits are frequently 10 to 6 A for an average protein or 6 to 5 A for a virus structure determination.

When exploring the rotation function in polar coordinates, there is no significance to the latitude $\varphi$ (Fig. 2.3.6.4) when $\psi = 0$. For small values of $\psi$, the rotation function will be quite insensitive to $\varphi$, which therefore needs to be explored only at coarse intervals (say 45°). As $\psi$ approaches the equator at 90°, optimal increments of $\varphi$ and $\varphi$ will be about equal. A similar situation exists with Eulerian angles. When $\theta_3 = 0$, the rotation function will be determined by $\theta_1 + \theta_2$, corresponding to $\psi = 0$ and varying $\kappa$ in polar coordinates. There will be no dependence on $(\theta_1 - \theta_2)$. Thus Eulerian searches can often be performed more economically in terms of the variables $\eta = \theta_1 + \theta_2$ and $\Delta = \theta_1 - \theta_2$, where

$$|\mathbf{p}| = \begin{pmatrix} \cos \eta \cos^2 \left(\frac{\theta_2}{2}\right) & \sin \eta \cos^2 \left(\frac{\theta_2}{2}\right) & \sin \theta_2 \sin(\eta - \Delta) \\ + \cos \Delta \sin^2 \left(\frac{\theta_2}{2}\right) & + \sin \Delta \sin^2 \left(\frac{\theta_2}{2}\right) & \\ - \sin \eta \cos^2 \left(\frac{\theta_2}{2}\right) & - \cos \eta \cos^2 \left(\frac{\theta_2}{2}\right) & \sin \theta_2 \cos(\eta - \Delta) \\ + \sin \Delta \sin^2 \left(\frac{\theta_2}{2}\right) & - \cos \Delta \sin^2 \left(\frac{\theta_2}{2}\right) & \\ \sin \theta_2 \sin(\eta + \Delta) & - \sin \theta_2 \cos(\eta + \Delta) & \cos \theta_2 \end{pmatrix},$$

which reduces to the simple rotation matrix

$$|\mathbf{p}| = \begin{pmatrix} \cos \eta & \sin \eta & 0 \\ - \sin \eta & \cos \eta & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

when $\theta_2 = 0$.

The computational effort to explore carefully a complete asymmetric unit of the rotation-function Eulerian group can be considerable. However, unless improper rotations are under investigation (as, for example, cross-rotation functions between different crystal forms of the same molecule), it is not generally necessary to perform such a global search. The number of molecules per crystallographic asymmetric unit, or the number of subunits per molecule, are often good indicators as to the possible types of noncrystallographic symmetry element. For instance, in the early investigation of insulin, the rotation function was used to explore only the $\kappa = 180^\circ$ plane in polar coordinates as there were only two molecules per crystallographic asymmetric unit (Dodson et al., 1966). Rotation functions of viruses, containing 532 icosahedral symmetry elements, are usually limited to exploration of the $\kappa = 180, 120, 72$ and 144° planes [e.g. Raymont et al. (1978) and Arnold et al. (1984)].

In general, the interpretation of the rotation function is straightforward. However, noise often builds up relative to the signal in high-symmetry space groups or if the data are limited or poor. One aid to a systematic interpretation is the locked rotation function (Rossmann et al., 1972) for use when a molecule has more than one noncrystallographic symmetry axis. It is then possible to determine the rotation-function values for each molecular axis for a chosen molecular orientation (Fig. 2.3.6.6).

Another problem in the interpretation of rotation functions is the appearance of apparent noncrystallographic symmetry that relates the self-Patterson of one molecule to the self-Patterson of a crystallographically related molecule. For example, take the case of $\alpha$-chymotrypsin (Blow et al., 1964). The space group is $P_2_1$ with a molecular dimer in each of the two crystallographic asymmetric units. The noncrystallographic dimer axis was found to be perpendicular to the crystallographic $2_1$ axis. The product of the crystallographic twofold in the Patterson with the orthogonal twofold in the self-Patterson vectors around the origin creates a third twofold, orthogonal to both of the other twofolds. In real space this represents a twofold screw direction relating the two dimers in the cell. In other cases, the product of the crystallographic and noncrystallographic symmetry results in symmetry which only has meaning in terms of all the vectors in the vicinity of the Patterson origin, but not in real space. Rotation-function peaks arising from such products are called Klug peaks (Johnson et al., 1975). Such peaks normally refer to the total symmetry of all the vectors around the Patterson origin and may, therefore, be much larger than the peaks due to noncrystallographic symmetry within one molecule alone. Hence the Klug peaks, if not correctly recognized, can lead to erroneous conclusions (Akerall et al., 1972). Litvin (1975) has shown how Klug peaks can be predicted. These usually occur only for special orientations of a particle with a given symmetry relative to the crystallographic symmetry axes. Prediction of Klug peaks requires the simultaneous consideration of the noncrystallographic point group, the crystallographic point group and their relative orientations.

2.3.6.5. The fast rotation function

Unfortunately, the rotation-function computations can be extremely time-consuming by conventional methods. Sasada (1964) developed a technique for rapidly finding the maximum of