3 Space Group Determination and Crystal Characterisation

The Neu5Ac lyase crystals were characterised by small-angle screenless precession photography using a precession angle $\mu$ of 2°, 20 hours of exposure time and a crystal to film distance of 100 mm on an Enraf-Nonius precession camera. The precession method allows the recording of a plane of the reciprocal lattice on a photographic film in an undistorted manner for the zero layer in this case. On a screenless small-angle precession photograph, the circular film area of the zero-level and the annular regions of upper levels which pass through the sphere of reflection are apparent and the center of the zero-level circle corresponds to the straight-through position of the X-ray beam (Buerger, 1964).

The crystals were oriented with the $c$-axis normal to the incident X-ray beam and in order to analyse the symmetry down the $c$-axis it was necessary to mount the long axis of the crystal perpendicular to the capillary axis. For analysis of the repeating distance along the $c$-axis, a precession photograph was also taken with the 110-axis mounted normal to the incident X-ray beam, i.e. with the $c$-axis along the capillary. From the repeating distances on the precession photographs, the unit cell parameters were determined as $a = b = 122.8$ Å, $c = 198.9$ Å, $\alpha = \beta = 90^\circ$ and $\gamma = 120^\circ$, suggesting a hexagonal or trigonal crystal system. The crystals have volume to mass ratio, $V_m$, of 2.8 Å$^3$ Da$^{-1}$ based on the assumption that the crystals contain one tetramer in the asymmetric unit [estimated using the formula given by
Matthews (1968) based on the unit cell contents and the protein molecular weight]. This corresponds to a solvent content of 0.56, which is within the range of normally observed for protein crystals (Matthews, 1968).

A $\mu = 2^\circ$ photograph of Neu5Ac lyase is shown in Figure 4. The precession photograph in Figure 4 shows six-fold symmetry on the zero-level and three-fold symmetry on the upper levels indicating a trigonal crystal system rather than a hexagonal one. Other photographs established that only reflection with $l = 3n$ were present on the line $[0,0,l]$. The upper-level in Figure 4 in fact shows $3m$ symmetry with the mirror planes every $60^\circ$ about $c^*$, along $a^*$ and $b^*$. This indicates the presence of two-fold axes perpendicular to and related by the three-fold axis. The two-fold axes are perpendicular to the mirror planes. Since $a^*$ is perpendicular to $b$ and $b^*$ is perpendicular to $a$, the two-fold axes perpendicular to the mirror planes lie along the real axes $a$ and $b$. This is the case in the space groups $P3_121$ and $P3_221$. The determination of the space group and the repeating distances along the axes was also confirmed by taking $1^\circ$ oscillation photographs on the Mar Research imaging plate (data not shown).
Figure 4: A screenless precession photograph of a Neu5Ac lyase crystal μ = 2° down the c-axis. The zero-level at the film center around the direct beam shows six-fold symmetry whereas the upper levels show only three-fold symmetry.