Chapter 6

Protein crystallization
**Introduction**

Crystallography is the most powerful tool for the elucidation of the detailed structure of large biomolecules. The most popular methods for crystallization are slow evaporation, vapour diffusion by the hanging drop, the sitting drop methods and microdialysis. Automated machines for setting up crystallization experiments have been developed by Cox *et al.*, (1987).

Factors such as availability of the protein, purification procedure, yield of the protein, the presence of cofactors and metal ions required for its activity, conformational changes due to binding the substrates, etc. play a role in the success of obtaining protein crystals.

The recent revival of protein crystallography is due to the increased availability of protein as a result of cloning techniques in addition to the development in computer technology and the availability of synchrotron radiation. Through crystallography high resolution structures have been obtained for single polypeptides larger than 100 kDa such as the photosynthetic reaction centre (Deisenhofer, *et al.*, 1985), medium resolution has been obtained for histone octamer and low resolution for molecules such as the nucleosome (Richmond *et al.*, 1984). At present the crystal structures of more than 30,000 proteins are available in the Protein Data Bank (http://www.rcsb.org).

**X-ray** crystallography is an experimental technique that exploits the fact that X-rays are diffracted by crystals. It is not an imaging technique. X-rays have the proper wavelength (in the Angstrom range, \( \sim 10^{-10} \text{ cm} \)) to be scattered by the electron cloud of
density can be reconstructed. Crystals are generally formed from a pure homogenous preparation of a protein. True crystals often feature sharp edges. These appear as needles, blades, spherulites etc. There are also crystals formed from proteins after the removal of small irregular domains by proteolysis, and these cleaved proteins have all the activities of the intact protein. A protein crystal should have the size of 0.5 x 0.5 x 0.3 mm, without cracks or defects, that is ideal to diffract X rays to high resolution. Phase information must be extracted either from the diffraction data or from supplementing diffraction experiments to complete the reconstruction (the phase problem in crystallography). The methods used to determine the phases are the molecular replacement method, the multiple isomorphous replacement method and the multiwavelength anomalous dispersion method. The electron density maps are calculated and the protein model is then progressively built into the experimental electron density, refined against the data and the result is a quite accurate molecular structure.

The knowledge of accurate molecular structures is a prerequisite for rational drug design and for structure based functional studies to aid the development of effective therapeutic agents and drugs. Crystallography can reliably provide the answer to many structure related questions, from global folds to atomic details of bonding. In contrast to NMR, no size limitation exists for the molecule or complex to be studied by X-ray crystallography.
Materials

*Dolichos lablab seeds*, affinity purified lectin and different crystal screen kits

Methodology

Purification of the galactose specific bean lectin has been carried out as described in Chapter I. Suitable crystallization conditions were screened using different methods at 20 °C. The concentration of protein taken was 20 mg/mL. The hanging drop consisted of 2 μL of protein containing 20 times molar excess of galactose and 2 μL of the well solution (precipitant). The drops were equilibrated against 0.5 mL of the precipitant.

Crystallization was set up using different screening kits. In Grid screening 24 different PEG 6K conditions were used, in Hampton crystal Screen 1, 48 conditions were used, in Hampton Screen 2, 48 conditions were used and Wizard Screen kit with 96 conditions.
Results

Small crystals were obtained in conditions in which polyethylene glycol (PEG, 6K) of different conditions was used as precipitant at pH 6.0 (Fig. 41). Optimization of these conditions resulted in obtaining bigger crystals which diffracted to about 3.0 Å. The crystals were mounted in glass capillaries and exposed to X-rays generated by a rotating anode X-ray generator. The diffraction images were recorded with a mar imaging plate. Attempts are being made to improve the size and the quality of the crystals to obtain diffraction data to higher resolution for structure determination and analysis.
Figure 41: Crystals of galactose specific lectin obtained in PEG 6K

A: Crystals obtained in the presence of 20% PEG 6K, pH 6.0.

B: Crystals obtained in the presence of 30% PEG 6K, pH 6.0.
Discussion

The 3 D structures of the legume lectins are dimers or tetramers made of dimmers. Each subunit is formed of 3 p sheets: an antiparallel back sheet that is almost flat, a concave 7 stranded front sheet and a smaller five stranded sheet that plays a role in holding the two large sheets together. Most of the other residues form loops and bends forming the "lectin fold" (Srinivasan et al., 1996). The carbohydrate binding sites of the legume lectins are located mostly in the p strands of the curved seven chain sheet. The Ca$^{2+}$ and Mir$^{2+}$ are in close proximity to the carbohydrate binding site. Crystals have also been obtained from demetallised legume lectins (Bouckaert et al., 2000) which differ from their native lectins by lacking carbohydrate binding ability. Lectins dimerise and oligomerise in several modes (Elgavish and Shaanan, 2001) and small differences in the primary structure may lead to large changes in the quaternary structures and thus its biological properties (Prabu, Suguna & Vijayan, 1999; Manoj & Suguna, 2001).

The galactose specific lectin purified in this study was found to be more stable in 30% PEG 6 K, pH 6.0 when this was used as the precipitant. Further analysis of the 3 dimensional structure of the galactose specific lectin has to be determined to elucidate the structural and functional relationship of this lectin with the glucose/mannose specific lectin.