IMPACT 12 ON BIOCHEMISTRY

X-ray crystallography of biological macromolecules

X-ray crystallography is the deployment of X-ray diffraction techniques for the determination of the location of all the atoms in molecules as complicated as biopolymers. The success of modern biochemistry in explaining such processes as DNA replication, protein biosynthesis, and enzyme catalysis is a direct result of developments in preparatory, instrumental, and computational procedures that have led to the determination of large numbers of structures of biological macromolecules by X-ray crystallography. Most work is now done not on fibres but on crystals, in which the large molecules lie in orderly ranks.

A technique that works well for charged proteins consists of adding large amounts of a salt, such as $(NH_4)_2SO_4$, to a buffer solution containing the biopolymer. The increase in the ionic strength of the solution decreases the solubility of the protein to such an extent that the protein precipitates, sometimes as crystals that are amenable to analysis by X-ray diffraction. A common strategy for inducing crystallization involves the gradual removal of solvent from a biopolymer solution by *vapour diffusion*. In one implementation of the method, a single drop of biopolymer solution hangs above an aqueous solution (the reservoir), as shown in Fig. 1. If the reservoir solution is more concentrated in a non-volatile solute (for example, a salt) than is the biopolymer solution, then solvent will evaporate slowly from the drop. At the same time, the concentration of biopolymer in the drop increases gradually until crystals begin to form.

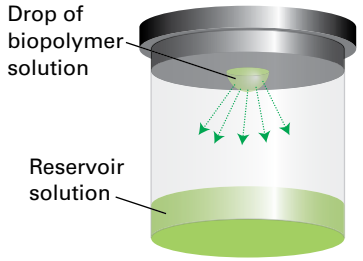

Fig. 1 In a common implementation of the vapour diffusion method of biopolymer crystallization, a single drop of biopolymer solution hangs above a reservoir solution that is very concentrated in a non-volatile solute. Solvent evaporates from the more dilute drop until the vapour pressure of the solvent in the closed container reaches a constant equilibrium value. In the course of evaporation (denoted by the downward arrows), the biopolymer solution becomes more concentrated and, at some point, crystals may form.

Special techniques are used to crystallize hydrophobic proteins, such as those spanning the bilayer of a cell membrane. In such cases, surfactant molecules, which like phospholipids contain polar head groups and hydrophobic tails, are used to encase the protein molecules and make them soluble in aqueous buffer solutions. Vapour diffusion may then be used to induce crystallization.

After suitable crystals are obtained, X-ray diffraction data are collected and analysed, as described in the text. The three-dimensional structures of a very large number of biological polymers have been determined in this way. However, the techniques discussed so far give only static pictures and are not useful in studies of dynamics and reactivity. This limitation stems from the fact that the Bragg method requires stable crystals that do not change structure during the lengthy data acquisition times required. However, special time-resolved X-ray diffraction techniques have become available in recent years and it is now possible to make exquisitely detailed measurements of atomic motions during chemical and biochemical reactions.

Time-resolved X-ray diffraction techniques make use of synchrotron sources, which can emit intense polychromatic pulses of X-ray radiation with pulse widths varying from 100 ps to 200 ps ($1\ ps = 10^{-12}\ s$). Instead of the Bragg method, the Laue method is used because many reflections can be collected simultaneously, rotation of the sample is not required, and data acquisition times are short. However, good diffraction data cannot be obtained from a single X-ray pulse and reflections from several pulses must be averaged together. In practice, this averaging dictates the time resolution of the experiment, which is commonly tens of microseconds or less.

The progress of a reaction may be studied either by real-time analysis of the evolving system or by trapping intermediates by chemical or physical means. Regardless of the strategy, all the molecules in the crystal must be made to react at the same time, so special reaction initiation schemes are required. One way to initiate a reaction is to allow a solution containing one of the reactants to diffuse into a crystal containing the other reactant. This method is simple, but limited to relatively long reaction times, as diffusion of solutions into crystals large enough for crystallographic measurements is of the order of seconds to minutes.