CRYSTALLIZATION OF BIOLOGICAL MACROMOLECULES IN A REDUCED GRAVITY ENVIRONMENT

by

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Application of the techniques of x-ray diffraction to crystal structures of biological macromolecules has been overwhelmingly successful. The three-dimensional molecular structure of over 70 proteins and the nucleic acid t-RNA^{phe} have been determined (1). These structural studies, together with other biochemical techniques, have provided a molecular basis for understanding the biological activities of enzymes, hormones, antibodies, redox, and transport proteins.

There are, however, limitations associated with the use of x-ray diffraction studies that can be overcome using neutron radiation. The x-ray scattering factor of an atom is proportional to the number of electrons it contains, thus scatter from hydrogen is very weak. As a consequence, the positions of hydrogen atoms in macromolecules cannot be determined experimentally using x-ray radiation and can only be inferred from the locations of non-hydrogen atoms. Experimentally determined hydrogen positions would be valuable considering the importance of hydrogen bonding in maintaining protein and nucleic acid structures, and the putative roles of hydorgen atoms in the catalytic mechanisms of a number of enzymes. Neutrons, on the other hand, in non-magnetic materials are scattered by the nucleus and all atoms including light ones have significant scattering factors. Thus, the positions of hydrogen atoms in biological macromolecules could be determined using neutron diffraction techniques.

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There are other advantages offered by neutron radiation. Since it is non-ionizing, there is no radiation damage and the entire data set may be collected from a single crystal. X-ray induced radiation damage often necessitates the use of several crystals and data from individual crystals must be scaled together to obtain a complete data set. Absorption problems are minimized using neutron radiation, while useful anomalous dispersion effects are large. X-ray scattering factors also display a large dependence on scattering angle and fall off rapidly at high resolution, neutron scattering factors lack this strong angular dependence. More high resolution reflections should therefore be observable using neutrons, theoretically yielding more accurate results.

Despite all these advantages and the existence of excellent facilities for collecting neutron diffraction data, very few proteins have been examined using this technique. The difficulty and indeed the rate limiting step is the growth of single crystals of sufficient size. The neutron flux available at suitable wavelengths is approximately 10^3 times less than that available for x-rays. A high resolution x-ray study of a protein of approximately 50,000 molecular weight would require a crystal about 0.3 mm in all dimensions, while a neutron diffraction study would require a crystal in excess of 3 mm in all dimensions.

Methodologies need to be developed for growing large crystals required for neutron diffraction studies. The factors controlling nucleation and growth of simple systems such as crystals of metals and salts have received much attention, yet very little work has been done to understand these factors in protein systems. It is also difficult to extend the theoretical work obtained in these simple systems to biological macromolecules. The absence of a good theoretical model hinders efforts to obtain improved crystals; nevertheless, the importance of this class of molecules warrants their detailed study.

Current strategies for growing large protein crystals attempt to limit the number of nucleation sites and to very, very slowly approach the point of insufficient solvation. This is based on evidence demonstrating the correlation of crystal size and quality with the rate of growth. Amorphous precipitate or many small crystals are formed if the macromolecule is rapidly forced out of solution, while slower rates of growth result in larger, more perfectly formed crystals. The low gravity environment of space may have special advantages when trying to obtain low growth rates. In the absence of gravity driven convection and sedimentation, diffusion becomes the principle means of material transport. The rate of transport can then be controlled by the concentration of the diffusing substances and by the diffusion pathlength.

When attempting to crystallize a protein or nucleic acid, the crystallographer or biochemist must examine its solubility as a function of a number of parameters. These include the concentration of macromolecule, pH, temperature, ionic strength, choice of buffer, choice and concentration of "precipitating agent." Special cofactors, metal ions, or reducing agents may also be required.

The task then is to search this multiparameter space for solubility minima. The fact that proteins commonly grow in many different crystal forms demonstrates the possibility of many local minima (2,3). A battery of micro techniques have been developed which allow the screening of a large number of conditions using only a small amount of the macromolecule. Experience indicates that if a water soluble globular protein with molecular weight less than 100,000 can be isolated in sufficient quantity and purity, the chance of obtaining crystals are excellent. Once conditions that produce crystals have been determined, the task then is to grow them to sufficient size. Again, either micro or macro techniques can be used, and a wide variety of methods is available. These methods include diffusion techniques that could be readily adapted for flight experiments.

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