

CRYSTAL GROWTH OF ENZYMES IN LOW GRAVITY
L-5

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Recent developments in protein engineering have expanded the possibilities of studies of enzymes and other proteins. Now such studies are not limited to the elucidation of the relationship between the structure and function of the protein. They also aim at the production of proteins with new and practical functions, based on results obtained during investigation of structure and function. For continuing research in this field, investigation of the tertiary structure of proteins is important. X-ray diffraction of single crystals of protein is usually used for this purpose. The main difficulty is the preparation of the crystals. The theme of the present research is to prepare such crystals at very low gravity, with the main purpose being to obtain large single crystals of proteins suitable for x-ray diffraction studies.

Single crystals of protein are prepared in a low-gravity field in space by use of the conditions found suitable on Earth for the growth of single crystals of protein and the crystals grown in space must be compared with those grown on Earth. Furthermore, x-ray crystallographic analysis of single crystals of protein grown at low gravity should be done at high resolution. The influence of low-gravity fields on the protein structure can be studied by comparison with the already published structures of crystals grown at one gravity.

Large single crystals of proteins of 5 mm in each dimension that would be difficult to grow on Earth might be prepared and used in physical measurements such as neutron diffraction, as well as in x-ray diffraction analysis.

The results of these experiments should make a practical contribution to the invention of new protein foodstuffs and to the development of medical supplies. Also, studies will help to clarify protein structures, especially the surface structure of the protein molecule, which cannot yet be completely elucidated.

The airtight cell for enzyme crystallization shown in Figure 1 was designed and produced for use in the Spacelab experiment and is adapted for operation at very low gravity. It is composed of the middle part on which metal fittings are fixed for photography, a knob, and two pairs of cylinders, and pistons located on both ends of the cell.

For the preparation of single crystals of protein, several principles have been proposed, and investigators have been conducting trials of the principles by using a variety of procedures. In the present experiment, we decided to use the batch method in which a supersaturated solution is left undisturbed, taking into account that single crystals must be grown at 20 °C during the 7-day flight of the shuttle. Outline and merits of the present method are as follows: The crystallization solution contains a salt such as sodium chloride or ammonium sulfate, and it produces a supersaturated solution when mixed with the protein solution (Figure 2). The concentration and pH of the buffer used, the concentration of the protein solution, and the concentration of salt of the crystallization solution are selected to give a mixture suitable for crystal formation. The two solutions are mixed and then allowed to stand. This method is suitable for crystallization within a short time because the conditions necessary for crystallization are fulfilled soon after the solutions are mixed. Irrespective of the large volume of the solution, the procedures are simple and time-saving.

We selected three functional proteins and three kinds of enzymes as samples for this experiment. The functions of these proteins are as follows:

1. Lysozyme from hen egg white. Lysozyme is an enzyme that catalyzes the hydrolysis of β -(1-4) glycoside linkages between N-acetylmuramic acid and N-acetylglucosamine (Figure 3). These are basic components of bacterial cell walls. This enzyme also hydrolyzes chitin, a biopolymer of N-acetylglucosamine.
2. Myoglobin from horse skeletal muscle. Myoglobin is a functional protein found in skeletal muscle cells (Figure 3). It is particularly abundant in diving mammals such as whales, seals, and walruses. It serves not only to store oxygen but also to enhance the rate of diffusion of oxygen through the cell.
3. ω -amino acid: pyruvate aminotransferase from *Pseudomonas* sp. F-126. ω -amino acid transferase is an enzyme that is involved in the reversible transfer of amino group from ω -amino acids to amino acceptors with the aid of pyridoxal 5'-phosphate as a coenzyme (Figure 3c). For this enzyme, pyruvate is the only amino acceptor, and β -alanine is used as the best amino donor among the various ω -amino acids.
4. Glucoamylase from *Rhizopus delemar*. Glucoamylase is an exo-splitting enzyme that consecutively removes the glucose unit from the non-reducing of starch and related malto-oligosaccharides. This enzyme is found in various microorganisms, and it occurs almost exclusively in fungi, but far less in bacteria and yeasts. Fungal glucoamylase is very useful for industrial production of glucose or oligo-saccharides.

5. Ovotransferrin from hen egg white. Transferrins are a family of iron-binding glycoproteins found in serum, secretory fluids, milk, and egg white. They are dimeric proteins and each monomer can reversibly bind to two atoms of iron. The high affinity for iron of ovotransferrin can retard microbial growth by making the iron relatively unavailable.

6. Insulin from humans. Insulin is a polypeptide hormone secreted by the B cells of the islets of Langerhans. It affects the entire intermediary metabolism, especially of the liver adipose tissue and muscle. Insulin is the only hormone that decreases the blood glucose concentration, and is concerned with the regulation of the rate of carbohydrate metabolism.

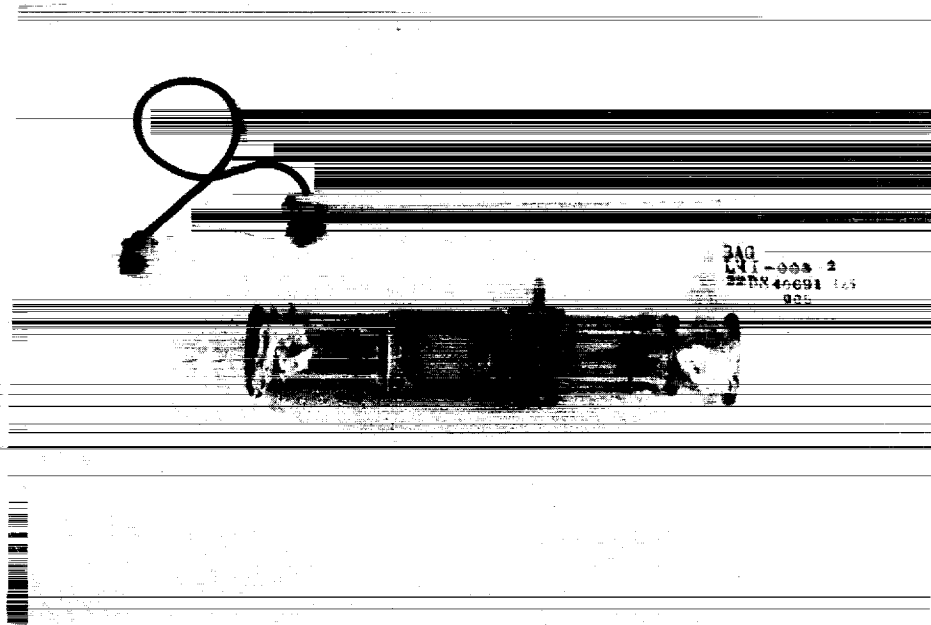


Figure 1. Enzyme crystallization cell.

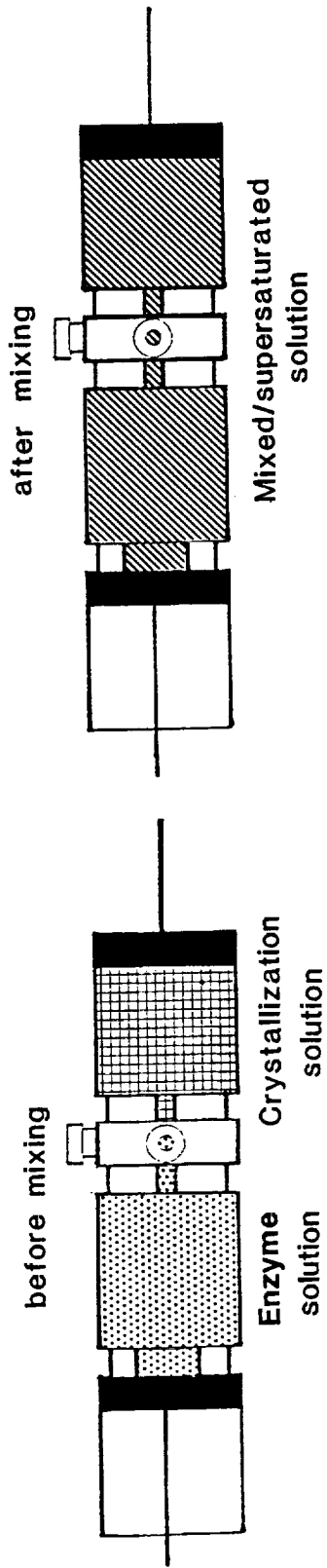
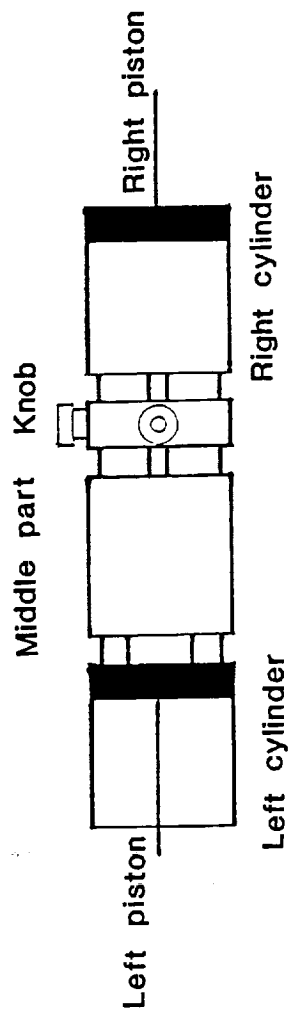
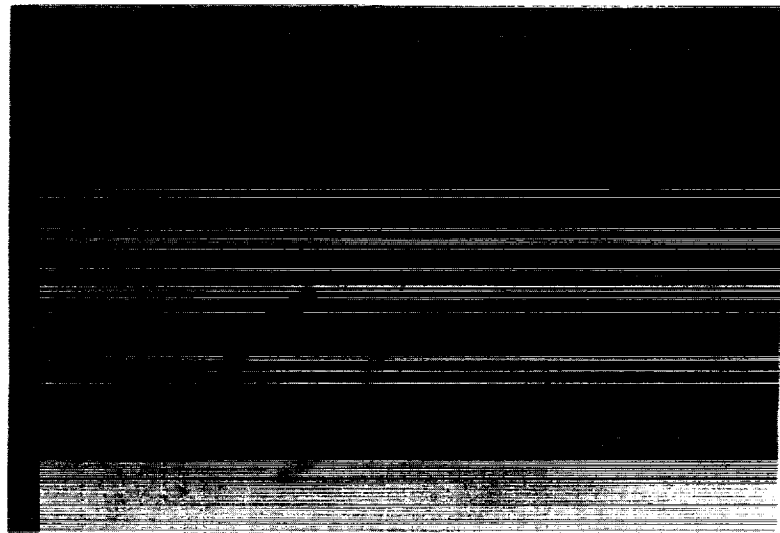
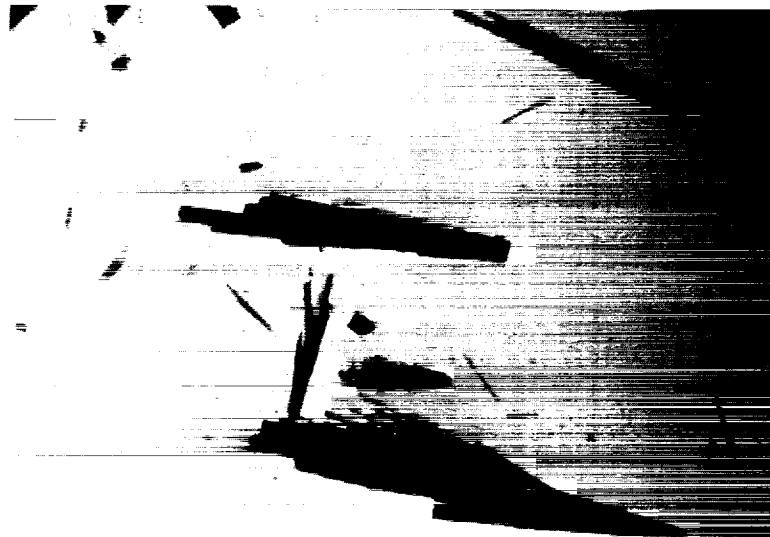


Figure 2. Operations of enzyme crystallization cell.



(a)



(b)



(c)

Figure 3. Single crystals of: (a) hen egg lysozyme, (b) horse skeletal muscle myoglobin, and (c) *Pseudomonas* ω -amino acid: Pyruvate Aminotransferase.