three-dimensional analysis, while to gain information about the protein itself it would be necessary to have extremely accurate intensity data which are difficult to obtain when large absorption corrections have to be made. A more profitable approach to the structure determination would be to prepare and examine lysozyme derivatives containing only one or two heavy atoms per molecule, as has been done for other proteins.

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Acta Cryst. (1959). **12**, 219

The Observation of Growth Steps on Sucrose Crystals

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A method is described for the examination of growth surfaces of sucrose crystals whilst growing from solution and also two procedures whereby the crystals may be removed from solution without destroying growth features. The growth of sucrose crystals by a dislocation mechanism was established by using these methods. Some theoretical predictions of the behaviour of growth steps have been confirmed experimentally. Rates of crystal growth under controlled conditions have been determined by measurement of the movement of steps.

1. Introduction

The purpose of this investigation was to develop a method of measuring the rate of growth of sucrose crystals. In order to decide between a dislocation mechanism or a surface nucleation process in this case, a study of the crystal surface was necessary. For sucrose crystals growing from solution the theoretical considerations of the mechanism of growth from the vapour advanced by Burton, Cabrera & Frank (1951) may not be relevant.

Many observations of growth steps on crystal surfaces have been reported since the prediction of spiral growth by Frank (1949). However, in many cases the examination of the surfaces of crystals has failed to reveal steps or evidence of dislocation. The techniques most used for the observation of growth steps have been examination by reflection phase contrast microscopy or electron microscopy of crystal surfaces after growth has ended. These published methods did not reveal growth steps on sucrose crystals, the further refinements required for this and described here, could be applied to the growth of crystals of other substances.

2. Observation of growth steps in solution

Bunn (1945, 1949) observed layers on crystals of several substances growing from solution, but not on crystals of sucrose. He used microscopy with high power and in some cases dark ground illumination. Only when the growth steps are large and the crystal differs considerably in refractive index from the solution are the steps visible by transmitted light with an ordinary microscope. A more sensitive method is to view the crystal by reflected light, this was used by Forty (1951).

The optical properties of sucrose crystals and saturated solutions result in difficulties of observation of growth steps. These steps are of dimensions of the unit cell or multiples thereof. Beevers *et al.* (1952) gave the values $a = 10{\cdot}89$, $b = 8{\cdot}69$, $c = 7{\cdot}77$ Å, for the unit cell of the sucrose crystal. A solution of sucrose saturated at 30 °C. has a refractive index of 1·46 whilst for crystals of sucrose the refractive indices are about 1·56. The optical path difference for a step viewed by reflected light is therefore 30 times that for the same step viewed by transmitted light. This indicates that only steps which are several hundred

times the unit cell in height will be visible on a sucrose crystal in solution viewed by transmitted light. Growth steps must therefore be observed by reflection. As the intensity of the reflected beam from a face of a sucrose crystal in solution will only be 1/1000 of the incident beam, an intense light source must be used and stray light eliminated as effectively as possible.

Experimental procedure

Crystals of sucrose were grown in a microscope cell and examined by reflected light. Illumination along the microscope axis results in back reflections from the glass-air surfaces of the optical system which are more intense than the required image. This occurs even when all glass surfaces are bloomed. The incident beam was therefore inclined to the microscope axis and the crystal surface tilted until light was reflected from it into the microscope objective.

A microscope cell 3 mm. thick was used in which the crystals grew on a black surface. The cell rested on a table supported by three adjustable feet which were used to tilt the crystals relative to the microscope axis. A thermostat enclosed the cell and through the cell solutions of known concentration and temperature could be pumped. The tilting table rested on a rotating stage 9 inches in diameter.

Illumination was provided by a compact source lamp (Type ME/D, Mazda), a high pressure mercury arc. An image of the arc was focused on the crystal surface by means of a projection lens. The beam was reflected on to the crystal by a mirror placed close to the microscope objective. Optimum resolution was obtained by using a narrow pencil of light.

As the cell cannot be thinner than 3 mm. a microscope objective of long working distance was necessary and this limited the magnification. Growth steps were only visible at a supersaturation such that the step spacing was more than the resolving power of the microscope.

Observations

Examination of growing sucrose crystals using these methods led to the conclusion that growth proceeds by the movement of steps which result from the presence of dislocations in the crystals. Simple spirals resulting from single screw dislocations were sometimes visible and more frequently, complex systems of growth steps. A method of measuring the rate of crystal growth by observing step movement under known conditions was established.

On one crystal it was noted that at a low supersaturation, steps from a double spiral were pinned against a nearby cliff and a spiral centred on a single screw dislocation covered the face. The supersaturation was increased until the double spiral grew and owing to its greater activity, dominated the single spiral as shown on photograph 1. This behaviour was predicted by Frank (1949, 1951).

An example of a typical complex system of growth steps on the (010) face of a sucrose crystal is shown on photograph 2.

3. The removal of sucrose crystals from solution

For a complete study of the growth mechanism it was necessary to examine sucrose crystals whilst growing and after growth. The growth systems on dry crystals can be examined at high magnifications and step heights can be measured. When sucrose crystals are taken from solution the growth steps are obscured by a layer of viscous and highly concentrated solution. Two methods were developed for removing this layer without destroying the growth features. A small variation in temperature or in the concentration of the solutions during these processes can modify or destroy growth steps. Condensation of moisture on the crystals must be avoided.

Experimental procedure

METHOD 1. The crystals were washed with a solvent miscible with the saturated sucrose solution. An ideal solvent would mix so that the diluted solvent remained saturated down to low concentrations of sucrose. Few solvents are miscible with sucrose solution, however, ethylene glycol was found to be satisfactory. Figures for the solubility of sucrose in ethylene glycol–water mixtures given by Fey (1951) confirm this. Purified ethylene glycol, ethyl alcohol and acetone were saturated with sucrose at the same temperature as the growing crystals. This required stirring for 36 hr. When the crystals were to be removed, the solution used for growth was discarded and replaced by the glycol solution which was circulated vigorously over the crystals to ensure rapid mixing. The crystals were then washed several times with the alcohol solution and with acetone and dried with a current of dry air.

This process results in crystals having clean and highly reflecting faces which can be coated with a durable aluminium film for examination by the usual methods. Many crystals grown under known conditions have been isolated in this way, enabling the shape of the growth hills on the various crystal faces to be established. In some cases it was evident that modification of growth features during removal occurred, an example being the large nearly circular ring visible on photograph 3. An alternative method for which controlled conditions are only required for a short time was devised.

METHOD 2. In this method the sucrose solution was removed mechanically by circulating liquid paraffin over the crystals. An emulsifying agent was added to the liquid paraffin (S. G. 0·83–0·87) which was then brought to the same temperature as the crystals. A suitable emulsifying agent used was sorbitan monoleate. The crystals must be enclosed in a vessel such

Fig. 1. Growing sucrose crystal; domination of single spiral by double spiral. (100) face. ×60.

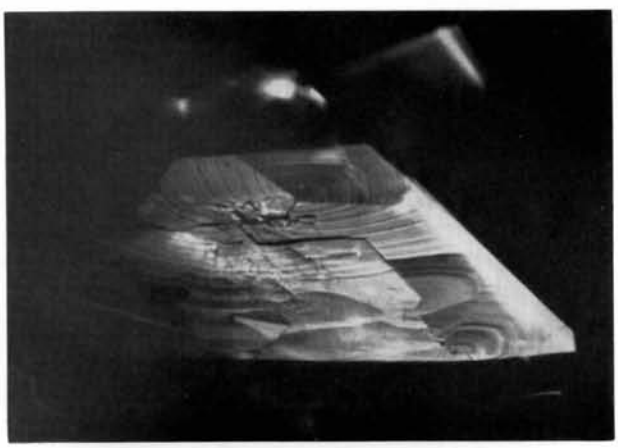

Fig. 2. Growing sucrose crystal; a typical step system. (010) face. ×60.

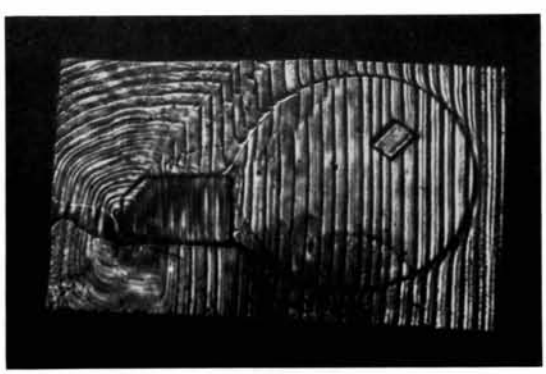

Fig. 3. Sucrose crystal isolated from pure solution by method 1. (100) face. ×60.

Fig. 4. Sucrose crystal isolated from solution containing dextrose by method 1. (100) face. ×60. Step height 4000 Å.

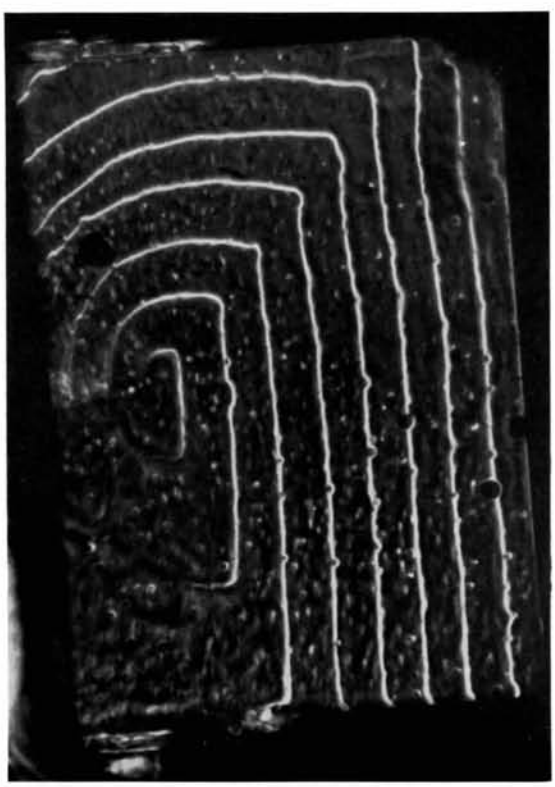

Fig. 5. Sucrose crystal isolated by method 2. (100) face. ×430. Step height 1700 Å. Phase contrast.

[*To face p.* 220

as a thin microscope cell. By means of a gear type pump the paraffin solution was circulated vigorously over the crystals, the direction of flow being reversed a few times. Pure liquid paraffin was then used to flush out the system, the crystals were washed with petroleum ether and dried with a current of dry air.

Examples of growth systems on sucrose crystals

The (100) face of a sucrose crystal grown from a pure solution is shown on photograph 3. This crystal was prepared by method 1 and shows a system of steps of varying heights, a feature often occurring on sucrose crystals.

Another example of a sucrose crystal on which is visible a growth hill having the asymmetric *D* shape characteristic on the (100) face is shown on photograph 4. The growth hill is remarkably even for such a large step height, this being 4000 Å. This crystal was grown in a solution containing 0·2% dextrose. On crystals grown in solutions of this composition, the step heights were about 10 times bigger than on crystals grown in pure solutions of sucrose.

The crystal shown on photograph 5 was isolated by method 2. This photograph also shows a (100) face, having a single spiral of step height 1700 Å on it. This crystal was removed from solution after being used for an experiment on the rate of growth.

Development of the methods of observation was assisted greatly by discussions with Mr K.W.Keohane on microscope techniques. We wish to thank the directors of Tate and Lyle Ltd. for their interest and support for the work described.

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The Crystal Structure of Ribonuclease Comparison of Three Dimensional Patterson Vector Maps of Crystals Grown from Ethyl and Tertiary Butylalcohol Respectively

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Three-dimensional Patterson vector maps have been calculated for monoclinic crystalline ribonuclease grown from ethyl alcohol using approximately 13,000 slightly sharpened F^2 terms.

An analysis of the vectors within 5 Å of the origin shows that it differs structurally from haemoglobin and myoglobin.

1. Introduction

This paper describes briefly the main results of our analysis of the three-dimensional vector maps of crystalline monoclinic ribonuclease grown from ethyl alcohol. These findings are in broad agreement with a similar independent analysis of crystalline ribonuclease (ribonuclease II) grown from tertiary butyl alcohol already published by Magdoff, Crick & Luzzati (1956). It is now clear that the most promising way of determining the structure of crystalline proteins of low molecular weight is by the use of the isomorphous heavy-atom technique that was started by Green, Ingram & Perutz (1954) for haemoglobin and which has now been so ably exploited by Kendrew *et al.* (1957) for myoglobin, where some knowledge has been gained about the manner of folding of the polypeptide chains of this molecule. We are also undertaking work of a similar nature on the isomorphously related crystalline complex ribonuclease parachloromercuribenzoate; this was started four years ago and though actively in progress will be a long task.

In the meantime for two reasons we consider it worth while publishing our observations on the Patterson vector distributions of ribonuclease. We find that there is almost a one-to-one correspondence between