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The unit cells of four proteins.

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Introduction

In the search for more favourable protein crystals for X-ray crystallography a number have been examined roughly to see if they appear promising. All those described in this paper have more than one molecule in the asymmetric unit, so that no further work has been done on them. The results are published here mainly to prevent others spending time on unpromising material.

The protein crystals studied are the common forms of bovine pancreatic trypsin inhibitor, its compound with bovine trypsin, and two salts of chick lysozyme, the nitrate and the iodide. Details of preparation, and photographs of the first two of these protein crystals are collected together in Northrop, Kunitz & Herriott (1948), which will be referred to here as N.K.H. 1948.

All the crystals used were mounted 'wet' in sealed capillary tubes in the usual way. The X-ray photographs were taken with filtered copper radiation.

In some cases, where the crystal was rather small, a lead pinhole of about the same diameter as the crystal was used. This was mounted between the crystal and the X-ray source, and as near to the crystal as possible. In addition, a hanging back-stop was used, mounted a centimetre or so in front of the film, to catch the direct beam. The effect of these two precautions was to reduce the background scattering considerably, which is especially desirable when the crystal is small and the unit cell large.

In choosing space groups, those with mirror or glide planes have been excluded, on the grounds that since proteins are made up of laevo amino acids only, these symmetry elements will not occur.

Trypsin inhibitor

The bovine pancreatic trypsin inhibitor was very kindly supplied by Dr M. Kunitz (Kunitz & Northrop, 1936; N.K.H., 1948). As the crystals were very small they were recrystallized by slow evaporation. This was done as follows: Distilled water was added until all the crystals had dissolved. The flat-bottom tube containing a shallow layer of this solution was fitted with a cork, in which a short length of capillary tube had been fixed, so that evaporation could take place only very slowly. After some weeks at room temperature, crystals of a reasonable size (a few tenths of a millimetre in length) were found to have grown.

The crystals were short hexagonal prisms (see N.K.H., 1948, Fig. 60 for a photograph). They showed straight extinction, the slow ray being along the hexagonal axis. X-ray photographs were taken at film distances between 6 and 10 cm.

They confirmed the hexagonal symmetry and showed that the basal plane of the reciprocal lattice had mirror planes every 30°. The $000l$ reflexions were absent only for $l = 2n+1$. The space group is therefore probably $C6_32$. This has 12 general positions. The unit cell dimensions are approximately

$$a = 111, \quad c = 122 \text{ Å}.$$

This unit cell is most disappointingly large, since trypsin inhibitor is believed to have a molecular weight of 9000 (H. Gutfreund, personal communication). The dry dimensions were not determined, but no very extreme shrinkage was noticed when the crystals dried. If we assume that the crystal contains about 50% protein we find 60 molecules in the unit cell, that is 5 in each general position. This figure is only approximate, but it shows that the crystal is unlikely to be favourable for analysis.

The reflexions $20\bar{2}0$ and $22\bar{4}0$ are very strong. This suggests a pseudo-halving of the a axis in projection at very low resolution. As the c projection has a centre of symmetry, the amplitudes of these reflexions are real. With these two terms only four Fouriers projection are possible, but without further data it is not possible to decide the signs. The reflexion 0002 is also fairly strong.

Inhibitor-trypsin compound

This is the compound of bovine pancreatic trypsin and bovine pancreatic trypsin-inhibitor. It was prepared by the method of Kunitz & Northrop (1936). The crystals when first obtained were very small, and had to be recrystallized by slow evaporation. Even so, they were little more than 0·1 mm. in size, and X-ray photographs were taken using a film distance of about 3 cm.

The unit cell is monoclinic. It is convenient to describe it in terms of an *all face-centred* monoclinic cell, having the approximate dimensions

$$a = 113, \; b = 123, \; c = 112 \text{ Å}, \; \beta = 97°.$$

The crystals had the appearance shown in Fig. 61 of N.K.H. (1948). The longest dimension of these crystals is parallel to b, the shortest to c^*. Viewed perpendicular to c^* the slow ray is along b.

In order to describe the unit cell in the conventional form in which only one face is centred, we may choose

$$a = 113, \; b = 123, \; c = 75 \text{ Å}, \; \beta = 139°.$$

The space-group is then $A2$, which has four general positions.

Assuming a protein content of 50%, and a molecular weight of about 30,000 (H. Gutfreund, personal communication), we find 10 molecules in this unit cell, so that there are probably 2 or 3 molecules in the asymmetric unit. This, again, is disappointingly large.

Lysozyme nitrate and iodide

The isoelectric chick lysozyme was obtained from Messrs Armours. The nitrate and the iodide were crystallized by the method of Alderton & Fevold (1946). Lysozyme crystallizes extremely easily by comparison with most proteins. An amorphous precipitate will often change overnight into large well-formed crystals.

The crystals were usually tabular, with straight extinction when viewed perpendicular to the shortest dimension, the slow ray being along the largest dimension,

which is in fact the b axis of the monoclinic cell. There were occasional hollow or semi-hollow tabular crystals, probably due to twinning.

X-ray pictures of the nitrate were taken using film distances between 4 and 8 cm. The unit cell is monoclinic, probably $P2_1$. A reasonable choice of axes is

$$a = 27{\cdot}9,\ b = 63,\ c = 66{\cdot}3\ \text{Å},\ \beta = 114\tfrac{1}{4}^\circ,$$

as this makes all three axes parallel to the faces of the crystal, b being parallel to the longest dimension, and a to the shortest. Moreover, there is a marked tendency for low-order reflexions to be weak or absent for l odd.

The volume of the unit cell is close to half that of the wet tetragonal lysozyme chloride (of which a few pictures were taken) which is known (Palmer, Ballantyne & Gavin, 1948) to contain eight molecules, so that there is little doubt that there are four molecules in the unit cell, and therefore two molecules in the asymmetric unit. The absences mentioned above suggest that if we place one molecule at the origin there is a second one roughly half way along the c axis.

The crystals of lysozyme iodide were similar to the nitrate, though hollow forms were not observed. The few X-ray photographs taken suggest that its X-ray diffraction pattern is very similar to if not identical with that of lysozyme nitrate. It is understood from Dr C. H. Carlisle (personal communication) that lysozyme bromide is also very similar to the nitrate, and that the dry dimensions confirm that there are four molecules in the wet cell.

No further work has been done on these two lysozyme crystals, but they are more promising than the other crystals described in this paper and might perhaps repay further study.

It is a pleasure to acknowledge the help and advice of Dr M. F. Perutz and Dr J. C. Kendrew in teaching me to take X-ray photographs of proteins. I should also like to thank Dr C. H. Carlisle and Dr H. Gutfreund for allowing me to quote unpublished material.

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The polarization factor for inclined-beam photographs using crystal-reflected radiation.

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The polarization factor for unpolarized incident radiation (*Internationale Tabellen*, 1935, vol. 2, p. 560), namely

$$P = \tfrac{1}{2}(1+\cos^2 2\theta), \qquad (1)$$

is independent of the geometrical features of the diffraction experiment, since it is a function of the Bragg angle only. When the incident radiation has been monochromatized by crystal reflexion, however, it is partially polarized. The polarization factor is therefore more complicated, and depends on the relative orientation of the reflecting planes of the monochromator crystal and those of the specimen, as well as on the Bragg angle of reflexion at each crystal. In the special case when the incident and reflected rays at both the monochromator and the specimen all lie in a plane, it is well known that the factor reduces again to the simple form

$$P = \frac{1+\cos^2 2\alpha \cos^2 2\theta}{1+\cos^2 2\alpha}, \qquad (2)$$

where α is the Bragg angle for the reflexion at the monochromator. This formula is well known, since it applies to the equatorial plane of a suitably arranged powder- or single-crystal camera, and it is in such applications that crystal-reflected radiation has been predominantly employed. It is the purpose of this communication to derive the polarization factor in the general case, and to express it in terms of simple film co-ordinates to facilitate its application to inclined-beam photographs recorded on cylindrical films.

Let the diffracting crystal be located at O, the origin of orthogonal co-ordinates OX, OY, OZ such that the plane XOY contains the ray incident at O and also the ray incident on the monochromator crystal M. After diffraction, the ray proceeds along OP which makes an angle χ with the plane XOY, and its projection OQ, on this plane, makes an angle Υ_0 with OY (Fig. 1).

Consider a plane polarized ray incident on the monochromator crystal at the Bragg angle α and with its

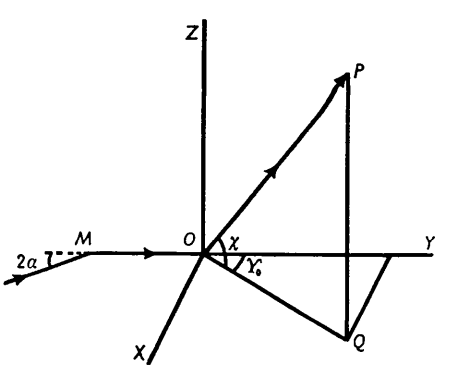

Fig. 1.