this complex, the hydrogen atoms attached to the methyl groups of acetone were found to be in fixed positions, which are approximately those expected from the point of view of minimising the interaction of the hydrogen atoms of each methyl group with the oxygen atom and the other methyl group in the molecule. A similar result of fixation of hydrogen atoms in methyl groups has been reported by Goodwin, Przybylska & Robertson (1950) in 1:4 dimethoxybenzene.

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References

ALLEN, P. W., BOWEN, H. J. M., SUTTON, L. E. & BASTIANSEN, O. (1952). *Trans. Faraday Soc.* **48**, 991.
CRUICKSHANK, D. W. J. (1949). *Acta Cryst.* **2**, 65.
GOODWIN, T. H., PRZYBYLSKA, M. & ROBERTSON, J. M. (1950). *Acta Cryst.* **3**, 279.
HABERMANN, J. (1884). *Monatshefte für Chemie*, **5**, 329.
HOWELLS, E. R., PHILLIPS, D. C. & ROGERS, D. (1950). *Acta Cryst.* **3**, 210.
International Tables for Crystallography (1952). Birmingham: Kynoch Press.
KIMURA, M. & KURITA, Y. (1951). *J. Chem. Soc. Japan*, **72**, 396.
MCWEENY, R. (1951). *Acta Cryst.* **4**, 513.
PALIN, D. E. & POWELL, H. M. (1947). *J. Chem. Soc.* p. 208.
PALIN, D. E. & POWELL, H. M. (1948). *J. Chem. Soc.* p. 815.
PINNOCK, P. R., TAYLOR, C. A. & LIPSON, H. (1956). *Acta Cryst.* **9**, 173.
POWELL, H. M. (1948). *J. Chem. Soc.* p. 61.
ROMERS, C. & CREUTZBERG, J. E. G. (1956). *Rec. trav. chim. Pays-Bas*, **75**, 331.
SCHMIDLIN, J. & LANG, R. (1910). *Ber. dtsch. chem. Ges.* **3**, 2806.
WALLWORK, S. C. (1950). D. Phil. Thesis, Oxford University.
WALLWORK, S. C. & STANDLEY, K. J. (1954). *Acta Cryst.* **7**, 272.

Acta Cryst. (1959). **12**, 216

An X-ray Investigation of Lysozyme Iodide and Nitrate

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Cell dimensions and space groups have been determined for the isomorphous lysozyme nitrate and iodide in both wet and dry forms. All are monoclinic $P2_1$, the dry forms showing a halving of the wet cell along the *c* axis. The molecular weight of lysozyme is 13,900±300 and the iodide has 19 anions per protein molecule. This number of iodine atoms is too great to permit the use of the isomorphous-replacement method of structure determination.

Introduction

Lysozyme is a readily available globular protein of low molecular weight and as such should be suitable for exhaustive X-ray analysis. It has the added advantage that it is a very basic protein which forms crystalline salts with mineral acids. The chloride, which crystallizes in the tetragonal system, has been studied by Palmer, Ballantyne & Galvin (1948) and by Corey, Donohue, Trueblood & Palmer (1952). Carlisle (1949) has examined the bromide. Crick's (1953) examination of the iodide and nitrate showed them to be isomorphous with the bromide. The present work is concerned with the possibility of using the method of isomorphous replacement to study the structure of lysozyme by comparison of the iodide and the nitrate.

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Preparation of the crystals

The salts were prepared from isoelectric chick lysozyme supplied by Armour & Co. Ltd., following the method of Alderton & Fevold (1946). Although the iodide crystallized easily in a few days, it was found desirable to use a 0·5% protein solution instead of the recommended 2%. In order to obtain single crystals of the nitrate, it was necessary to reduce the potassium nitrate concentration from 5% to 2·5%, when reasonable crystals were obtained after a few weeks.

Wet crystals were preserved by mounting in sealed Pyrex capillary tubes together with a little mother liquor. Dry crystals were prepared by removing adhering liquid with filter paper and immersing the crystals in xylene which was then allowed to evaporate. Xylene-dried protein crystals have been found to give better X-ray diffraction patterns than air-dried crystals (Low & Richards, 1954).

Morphological and optical examination

Both salts crystallize as monoclinic sphenoidal laths elongated in the b direction. The crystals lie on {100} with the prism forms {001} and {$\bar{1}$01} also present. The habit is visibly polar, the laths terminating at one end in {0$\bar{1}$0} and at the other in general forms.

Optical examination of the dry nitrate showed the crystals to be biaxial negative with $\gamma \| b$ and α inclined 11° to c in the acute angle β. The refractive index β is 1·55 with the total birefringence less than 0·01. $2V$ is large.

The dry iodide has the same optic orientation as the nitrate. Its refractive indices were not measured.

Molecular weight of the protein

Cell dimensions were determined from b-axis oscillation photographs taken on a 3 cm. radius cylindrical camera. The values, which have an accuracy of ±0·5% are:

	a (Å)	b (Å)	c (Å)	β (°)	Z
Wet iodide	27·7	63·3	66·3	114·5	4
Wet nitrate	27·9	63·3	67·5	114·0	4
Dry iodide	25·8	57·5	32·2	117·4	2
Dry nitrate	26·1	57·7	32·2	117·2	2

a-axis oscillation photographs showed that $0k0$ reflections are present only when $k = 2n$ for both salts in wet and dry forms. As symmetry planes are not permissible, the space group must be $P2_1$ in all cases.

The density of the dry iodide was determined by floating the crystals in mixtures of benzene and carbon tetrachloride. After a mixture with approximately the same density had been found, crystals and liquid were transferred to a dilatometer. The density of the liquid was adjusted to that of the crystals by altering the temperature. The value obtained was 1·383 g.cm.$^{-3}$, the densities of several crystals being all within 0·001 g.cm.$^{-3}$ of this value.

The weight of half the contents of one unit cell of dry iodide calculated from these data is 17,675. Microanalysis of the xylene-dried iodide showed a loss in weight on drying at 100 °C. of 7·80% giving a value of 16,300±250 for the molecular weight of lysozyme iodide. The iodide content of the heat-dried iodide was found to be 14·8±0·3%. As some of this iodine might be in the form of potassium iodide from the original preparation, a potassium analysis was carried out but none was found. The molecular weight of the protein is thus 13,900±300 and the iodide contains 19·0±0·6 iodide ions per molecule.

This value for the molecular weight of the protein is the same as that found by Palmer *et al.* (1948) for lysozyme as the chloride although they found 11 chloride ions per molecule. The 19 iodide ions correspond with the basic groups found by Tanford & Wagner (1954) using titration methods and also with the chemical analyses by Monier, Gendron, Jutisz & Fromageot (1952).

Intensity distribution in the *h0l* zones

Multiple-film Weissenberg photographs of the $h0l$ zone of each of the four substances were taken with Cu K radiation on a 6 cm. radius camera. Intensities of reflections with spacings greater than 2 Å were estimated visually giving an intensity range of approximately 1000:1 and were corrected for Lorentz and polarization factors. Although the iodine contribution to the absorption coefficient of the iodide is 60 cm.$^{-1}$, giving rise to serious errors with the crystals used which had a cross-section of about 0·4×0·2 mm., absorption corrections were not made because of the difficulty of calculating them for a crystal of irregular hexagonal cross-section. Patterson projections of the four substances are shown in Fig. 1.

The projection of the wet nitrate (Fig. 1(*a*)), unlike that of the iodide (Fig. 1(*b*)), shows a pseudo-halving of the c axis. This suggests that the two protein molecules in the asymmetric unit are related by the translation $c/2$ while the corresponding sets of anions, which in the case of the iodide are responsible for more than half the scattering intensity, are not related in the wet cell but adopt identical orientations on drying.

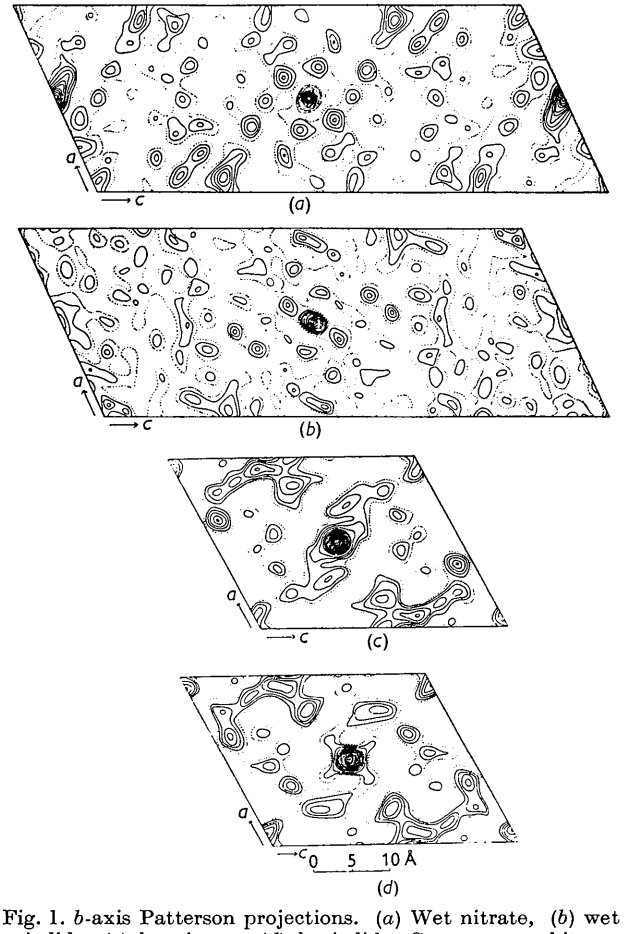

Fig. 1. b-axis Patterson projections. (*a*) Wet nitrate, (*b*) wet iodide, (*c*) dry nitrate, (*d*) dry iodide. Contours at arbitrary intervals.

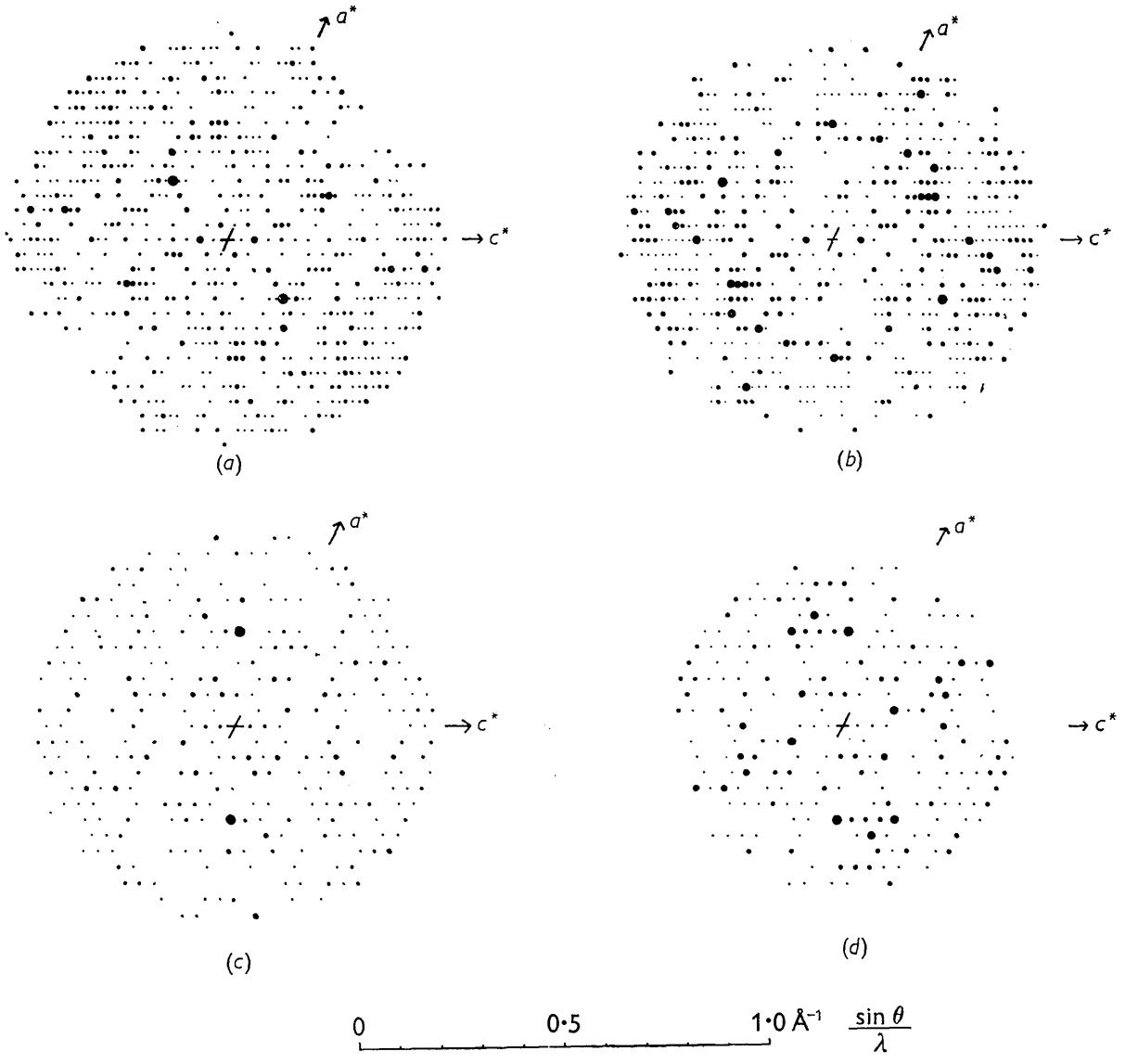

Fig. 2. Weighted reciprocal lattices for the $h0l$ zones. (*a*) Wet nitrate, (*b*) wet iodide, (*c*) dry nitrate, (*d*) dry iodide.

Direct comparison of the projections of the two salts is not valid because of the very different absorption errors.

The effects of temperature factor and scattering factor were eliminated from the intensity data by plotting $\log I$ versus $\sin^2\theta$ and finding a sharpening factor from the slope of the best straight line through the points. For the wet compounds and the dry nitrate all values out to $2\sin\theta = 0{\cdot}8$ were used. For the dry iodide the limit was $2\sin\theta = 0{\cdot}65$. Beyond these limits the number of observed reflections fell off sharply and they were not considered to be representative of the intensity distribution.

The sharpened intensity data are shown in the form of weighted reciprocal lattices (Fig. 2). Although a considerable number of intensities was measured beyond the limiting value of $2\sin\theta$ they are not shown because the sharpening factor in this region is uncertain and also because the minimum observable intensity is greater than the average sharpened value.

The intensity distributions resemble those of other small proteins, such as ribonuclease, in that the greatest weight of intensity is in the 4 Å region and that there is negligible scattering in the 10 Å region.

Conclusion

Although it had been hoped to make use of the isomorphism of iodide and nitrate to help in establishing the structure of lysozyme, no evidence has been obtained for the positions of the iodine atoms. Even to locate the heavy atoms would probably require a full

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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References

ALDERTON, G. & FEVOLD, H. L. (1946). *J. Biol. Chem.* **164**, 1.
CARLISLE, C. H. (1949). Personal communication.
COREY, R. B., DONOHUE, J., TRUEBLOOD, K. N. & PALMER, K. J. (1952). *Acta Cryst.* **5**, 701.
CRICK, F. H. C. (1953). *Acta Cryst.* **6**, 221.
LOW, B. W. & RICHARDS, F. M. (1954). *J. Amer. Chem. Soc.* **76**, 2511.
MONIER, R., GENDRON, Y., JUTISZ, M. & FROMAGEOT, C. (1952). *Biochim. et Biophys. Acta*, **8**, 588.
PALMER, K. J., BALLANTYNE, M. & GALVIN, J. A. (1948). *J. Amer. Chem. Soc.* **70**, 906.
TANFORD, C. & WAGNER, M. L. (1954). *J. Amer. Chem. Soc.* **76**, 3331.

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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