sodium dodecylsulfate, 4% lauryl alcohol and 82% water. The specimen was contained in a thin-walled capillary, and was taken without rotation. The structure was therefore not 'single-crystal', but was a strictly cylindrically oriented 'powder' specimen. This pattern type was predicted for structures *SSR*, RP_1S and RP_0S, and could occur for very special and unlikely rotations of SSP_1, $SS(RD)$, $SS(RP_1)$, $SS(RP_0)$, *DDR*, and DP_0R.

Conclusions

It can be seen from the above examples, that some information about the structure of mesomorphic phases can be derived from their diffraction patterns. It is obvious that one can be more specific about the classification of the structure if one can prepare both 'powder' and 'single-crystal' specimens. In addition, stationary and rotating sample techniques will help to distinguish similar structures. It is sometimes possible to orient mesomorphic systems by flow into a capillary tube, or by centrifuging in a capillary. Occasionally the container itself (e.g. a capillary) will influence the orientation of the specimen. Since many of the mesomorphic phases have high viscosities, time will usually aid the orientation process.

It is also obvious that a complete determination of the structure of any phase must include structure factor calculations so that predicted and observed intensities may be compared. As mentioned above, the present discussion completely ignores the molecular structure factor, and its influence on the intensity distribution in reciprocal space.

The help and encouragement of many colleagues at Procter and Gamble has been deeply appreciated. Special thanks are due Mr R. E. Kammann, who did the air-brush art work, and to Mr C. W. Hand, presently at Harvard University Graduate School, who helped with many of the drawings.

References

BERNAL, J. D. & FANKUCHEN, I. (1941–2). *J. Gen. Physiol.* **25**, 111.

BROWN, G. H. & SHAW, W. G. (1957). *Chem. Rev.* **57**, 1049.

HERMANN, C. (1931). *Z. Kristallogr.* **79**, 186, 337.

LUZZATI, V., MUSTACCHI, H. & SKOULIOS, A. (1957). *Nature, Lond.* **180**, 600.

LUZZATI, V., MUSTACCHI, H. & SKOULIOS, A. (1958). *Disc. Faraday Soc.* No. **25**, 43.

ROSEVEAR, F. B. (1954). *J. Amer. Oil Chem. Soc.* **31**, 628.

Acta Cryst. (1962). **15**, 1157

An X-ray Investigation of Wet Lysozyme Chloride Crystals. Preliminary Report on Crystals Containing Complex Ions of Niobium and Tantalum

BY ROBERT B. COREY, R. H. STANFORD, JR., RICHARD E. MARSH, YUEN C. LEUNG* AND LOIS M. KAY

Gates and Crellin Laboratories of Chemistry,† California Institute of Technology, Pasadena, California, U.S.A.

(*Received* 14 *February* 1962)

Egg-white lysozyme chloride when crystallized at pH 4·5 from solutions containing the ions $Ta_6Cl_{12}^{++}$ and $Nb_6Cl_{12}^{++}$ forms crystals containing these complex ions in the amount of approximately one ion per molecule of lysozyme. These crystals have the same space group ($P4_12_1$) and approximately the same cell dimensions as those of lysozyme chloride alone.

Two-dimensional and three-dimensional Patterson diagrams ($d_{min.} = 5$ Å) indicate that these ions occupy one set of eight-fold general positions in the unit cell and establish the coordinates of the ion centers. There is good evidence that the large complex ions cause a significant alteration in the configuration of the protein molecule. Some implications of these findings are discussed with respect to further investigations of the crystals.

1. Introduction

Egg-white lysozyme has many properties that recommend it as a subject for X-ray investigation, among them being its low molecular weight (about 14,700), its relative stability and homogeneity, its availability in highly purified form, and the ease with which it can be crystallized. Crystals of the chloride grown at pH 4·5 are especially stable and yield X-ray photographs with reflections extending to a minimum

* Deceased, November 11, 1958.

† Contribution No. 2812 from the Gates and Crellin Laboratories of Chemistry. The work described in this article was supported principally by a Contract (Nonr-220(05)) between the Office of Naval Research and the California Institute of Technology; it was also aided by research grant No. H-2143 from the National Heart Institute, Public Health Service, and by grants Nos. G-1265 and G-9467 from the National Science Foundation.

spacing of about 1·7 Å. Unfortunately, they have high symmetry (tetragonal) with 8 molecules in the unit cell, which is a disadvantage both in the collection of adequate quantitative X-ray data and in the interpretation of these data.

The first X-ray diffraction studies of egg-white lysozyme chloride were made by Palmer (1948), who has reported the following data for wet crystals grown at pH 4·5:

Tetragonal, $a=79\cdot1\pm0\cdot1$, $c=37\cdot9\pm0\cdot2$ Å,
$D_m=1\cdot233$, $Z=8$.
Space group $P4_12_1$ (or $P4_32_1$).

He also described an air-dried form which is tetragonal and apparently has the same space group as the wet form, but smaller cell dimensions ($a=71\cdot2$, $c=31\cdot4$ Å). X-ray measurements of these air-dried crystals were used to derive a value ($13{,}900\pm600$) for the molecular weight of the anhydrous, chloride-free protein (Palmer, Ballantyne & Galvin, 1948). Later, complete three-dimensional intensity data extending to a minimum spacing of 5·7 Å were collected and used for the computation of the three-dimensional Patterson function of the air-dried crystals (Corey, Donohue, Trueblood & Palmer, 1952). Attempts to interpret these data led to no significant information about the structure of the protein molecule.

An investigation* of wet lysozyme chloride crystals was begun in 1951 in collaboration with Dr Palmer in the laboratories of the Western Utilization Research and Development Division, Department of Agriculture, Albany, California. In this investigation two sets of Cu $K\alpha$ intensity data were collected from oscillation photographs of wet lysozyme chloride crystals and were used to calculate a three-dimensional Patterson plot ($d_{\text{min.}}=4\cdot0$ Å) and a two-dimensional Patterson projection onto (001) ($d_{\text{min.}}=2.0$ Å). Both of these plots were similar in their general features to the corresponding plots derived from air-dried crystals; their wealth of additional detail was likewise uninterpretable in terms of the structure of the crystal.

The present investigation was begun in 1957; it comprises an attempt to determine the structure of egg-white lysozyme chloride by the use of isomorphous series of crystals containing heavy atoms. This paper is a brief report of the progress that has so far been made and of the course of the current work.

2. Experimental

(i) *The complex chlorides of tantalum and niobium*

The introduction of heavy atoms into proteins so as to form truly isomorphous series of crystals which are necessary for a direct solution of the phase-angle problem will depend for its success upon properties that are peculiar to each individual protein. In 1956 Prof. L. Pauling called to our attention the complex ions $Ta_6Cl_{12}^{++}$ and $Nb_6Cl_{12}^{++}$ which are identical in charge and so nearly identical in dimensions that crystals which differ only in containing one or the other of them might be expected to be precisely isomorphous throughout their structure. Preliminary experiments in which crystals of lysozyme chloride were grown in the presence of these ions showed that they were indeed incorporated into the crystals and produced striking and consistent changes in the X-ray patterns.

The configuration and dimensions of these complex ions of tantalum and niobium were determined by Vaughan, Sturdivant & Pauling (1950) by X-ray diffraction studies of concentrated alcoholic solutions of the chlorides $Ta_6Cl_{14}.7H_2O$ and $Nb_6Cl_{14}.7H_2O$. Both ions comprise six metal atoms situated at the corners of a regular octahedron and twelve chlorine atoms occupying peripheral positions along the radial perpendicular bisectors of the edges of the octahedron. The structure of these ions is shown in Fig. 1.

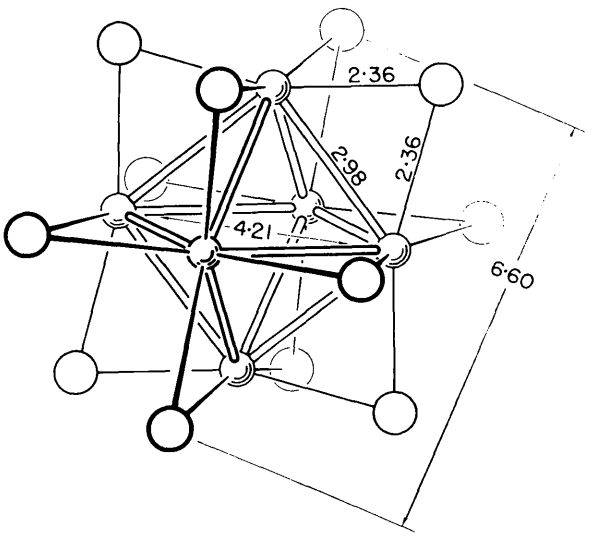


Fig. 1. A drawing showing the configuration and dimensions of the complex ions $Ta_6Cl_{12}^{++}$ and $Nb_6Cl_{12}^{++}$. Small shaded spheres represent the metal atoms at corners of an octahedron; unshaded circles represent chlorine atoms. Dimensions are in Ångström units.

Two batches of $Ta_6Cl_{14}.7H_2O$ were prepared for our use, one by Dr Herbert Segall and one by Mr Albert Hybl, both using the method of Lindner & Feit (1924). The compound was obtained in the form of microscopic crystals; in bulk it appears to be black, but separate crystals viewed under a microscope appear as tiny green hexagonal plates.

The compound $Nb_6Cl_{14}.7H_2O$ is more difficult to prepare than the corresponding tantalum compound; it was first prepared and described by Harned (1913). The material used in our experiments was made by Prof. Harned during a visit to these Laboratories in 1956 (Harned, Pauling & Corey, 1960).

* This earlier investigation was supported in part by a grant from the John Simon Guggenheim Memorial Foundation to one of us (R. B. C.).

(ii) *Preparation of crystals*

The crystallization of lysozyme at pH 4·5 has been described by Alderton, Ward & Fevold (1945). Most of the lysozyme used in our investigations was isoelectric egg-white lysozyme purchased from Armour and Company; portions were checked for chromatographic homogeneity (Tallan & Stein, 1953) by Dr W. A. Schroeder in these Laboratories. Some X-ray data were taken from crystals prepared from lysozyme purchased from the Worthington Biochemical Corporation. Intensity data collected from crystals derived from the two sources showed excellent quantitative agreement.

Crystals of lysozyme chloride were grown from solutions containing 3% of the protein and 5% of sodium chloride; they were buffered at pH 4·5 with 0·09*M* acetate. Characteristic tetragonal bipyramidal crystals were obtained at room temperature.

Crystals of lysozyme chloride containing $Ta_6Cl_{12}^{++}$ were grown at pH 4·5 (0·09*M* acetate buffer) from solutions containing 3% of the protein, about 0·04 g.ml.$^{-1}$ of NaCl, and an amount of Ta_6Cl_{14} such that the molar ratio of complex ion to protein was 1:1. The crystals were green in color and resembled crystals of lysozyme chloride in face development. They grew readily at room temperature.

The crystals containing $Nb_6Cl_{12}^{++}$ did not grow readily at room temperature. After considerable experimentation, satisfactory crystals were obtained from solutions maintained at 5 °C. and pH 4·5 (0·09*M* acetate buffer) containing 2·5% of lysozyme, about 0·023 g.ml.$^{-1}$ of NaCl, and sufficient Nb_6Cl_{14} so that the molar ratio of complex ion to protein was 1:1. The crystals were light green in color and although they grew more slowly they were similar in size and habit to those containing $Ta_6Cl_{12}^{++}$.

(iii) *Isomorphism and unit-cell dimensions*

As a quantitative measure of the degree of isomorphism of the three types of crystals, the unit-cell dimensions were determined from three crystals of each type, powdered sodium chloride ($a_0 = 5{\cdot}6402$ Å; Swanson & Fuyat, 1953) being used as reference substance. Measurements on Weissenberg photographs of the positions of about 20 selected *h0l* reflections were used together with the measured positions of the superimposed sodium chloride lines to calculate the dimensions of the *a* and *c* axes of the unit cell by a least-squares procedure. The average values of the cell dimensions derived for the three types of crystals are listed below, together with their standard deviations calculated from the spread of values obtained from the three crystals of each type.

Crystal	a_0 (Å)	s.d. (Å)	c_0 (Å)	s.d. (Å)	V (Å^3)	s.d. (Å^3)
Lys HCl	79·11	0·13	38·21	0·05	$2{\cdot}391 \times 10^5$	$0{\cdot}006 \times 10^5$
Lys + $Ta_6Cl_{12}^{++}$	80·06	0·26	37·33	0·06	2·393	0·012
Lys + $Nb_6Cl_{12}^{++}$	80·07	0·09	37·15	0·07	2·382	0·006

(iv) *Crystal densities and the number of complex ions in the unit cell*

The densities of crystals of lysozyme chloride and lysozyme chloride containing the complex ions of tantalum and niobium were determined by the flotation method in mixtures of chlorobenzene and bromobenzene. The averages of six determinations of the density of each of the three types of crystals are listed, together with their standard deviations, in the following tabulation.

Crystal	Density (g.ml.$^{-1}$)	s.d.
Lys HCl	1·2320	0·0019
Lys + $Ta_6Cl_{12}^{++}$	1·3009	0·0046
Lys + $Nb_6Cl_{12}^{++}$	1·2751	0·0043

Based on the dimensions reported by Vaughan, Sturdivant & Pauling (1950) and an assumed van der Waals radius for chlorine of 1·8 Å, a sphere that would just contain the complex ion $Ta_6Cl_{12}^{++}$ would have the volume 557 Å^3, equivalent to the volume of 18·6 water molecules (volume of one water molecule taken at 30 Å^3). Accordingly, the presence in a unit cell of one $Ta_6Cl_{12}^{++}$ ion would be expected to cause an increase in weight equal to the weight of the ion minus the weight of 18·6 water molecules, namely, $0{\cdot}01956 \times 10^{-19}$ g. The increase in weight of the unit cell of a crystal containing $Ta_6Cl_{12}^{++}$ over that of a crystal of lysozyme chloride alone can be calculated from the volumes of the unit cells and the crystal densities; it is $0{\cdot}16651 \times 10^{-19}$ g., equivalent to 8·5 $Ta_6Cl_{12}^{++}$ ions. A corresponding calculation of the number of $Nb_6Cl_{12}^{++}$ ions in the unit cell of crystals containing the niobium complex yields the number 8·4. (The standard deviations associated with these numbers are 1·1 and 1·5, respectively.)

If, as indicated by the quality and reproducibility of the X-ray photographs, $Ta_6Cl_{12}^{++}$ and $Nb_6Cl_{12}^{++}$ fill definite positions in the crystals, the results of these calculations indicate that these complex ions may occupy one set of general eight-fold positions, corresponding to a molar ratio of the complex ion to lysozyme of 1:1.

(v) *Collection of intensity data*

For the collection of X-ray intensity data from protein crystals precession photographs often have advantages over Weissenberg photographs: indices of reflections are immediately obvious, specific patterns are more readily recognized, and the rectilinear arrangement of reflections makes possible the use of a microdensitometer for the measurement of intensities. Our first photographs of crystals of lysozyme chloride containing tantalum and niobium were precession

photographs taken with Cu $K\alpha$ radiation with a crystal-to-film distance of 9·0 cm. and a precession angle of 17° ($d_{\text{min.}}=2{\cdot}7$ Å). Precession photographs have continued to be useful for preliminary inspection and confirmation of the identity of crystals, and for obtaining quantitative data for zero-layer reflections. However, because of the 80 Å dimensions of the unit cell, we have not found precession photographs practicable for recording a significant body of general three-dimensional data. Most of our X-ray intensity data have been obtained from Weissenberg photographs as described in the following paragraphs.

Crystals about 0·3 to 0·7 mm. in diameter were sealed in thin-walled glass capillary tubes which also contained some mother liquor. They were photographed with Cu $K\alpha$ radiation in Weissenberg cameras with a crystal-to-film distance of 5·73 cm. Photographs of the $hk0$ and $h0l$ zones were taken with multiple films and the intensities of the reflections were measured visually by comparison with calibrated intensity scales prepared from a series of exposures of a single reflection from similar crystals.

The intensities of 632 non-equivalent $hk0$ reflections (minimum spacing, 1·8 Å) were obtained from two Weissenberg photographs made from two different crystals of lysozyme chloride, one grown from recrystallized egg-white lysozyme obtained from the Worthington Biochemical Corporation and the other from lysozyme obtained from Armour and Company. The intensities of both photographs were estimated by two or more independent observers. After appropriate correlation and scaling, the values of F^2 calculated from the estimates made from a particular photograph by different observers were in good agreement. Data from the two photographs also showed good agreement. Corresponding $hk0$ intensity data were obtained from crystals of lysozyme chloride containing the complex ions $Ta_6Cl_{12}^{+++}$ and $Nb_6Cl_{12}^{+++}$, respectively.

The intensities of 615 $h0l$ reflections from crystals of lysozyme chloride containing $Ta_6Cl_{12}^{+++}$ (minimum spacing, 1·7 Å) were estimated in a similar manner, as were corresponding $h0l$ reflections obtained from crystals of lysozyme chloride containing $Nb_6Cl_{12}^{+++}$. These data were also correlated and converted to average relative values of F^2 for the two types of crystals.

In a similar fashion, three-dimensional intensity data from crystals containing the ions $Ta_6Cl_{12}^{+++}$ and $Nb_6Cl_{12}^{+++}$ were collected from equi-inclination Weissenberg photographs of layer lines 1 to 7 about c. Values of F^2 for different levels in l were correlated by comparison with corresponding data from $h0l$ photographs.

We should remark here that crystals of lysozyme chloride grown from the same solution were frequently found to give rise to X-ray patterns that differed slightly in the relative intensities of certain reflections. Therefore, it was necessary to test all crystals which were to be used as a source of quantitative three-dimensional data, either of lysozyme chloride or of lysozyme chloride containing a complex ion, to make certain that they were of the form that would yield the common, or 'standard', X-ray pattern. Before taking an upper-level Weissenberg photograph, a preliminary zero-level photograph with the crystal oscillated over a selected range was always taken and compared with a 'standard' photograph. Crystals mounted for oscillation around the a axis were checked by taking preliminary $hk0$ precession photographs.

3. Positions of the heavy-metal ions

(i) *Two-dimensional difference Patterson projections*

Because of striking differences between photographs of lysozyme chloride and those of lysozyme chloride containing the ion $Ta_6Cl_{12}^{+++}$, it was hoped that the positions of the complex ions would be readily found, even from preliminary two-dimensional data derived from the two types of crystals. However, inspection of several Patterson projections on (001) based on early precession photographs failed to define the x and y coordinates of the ions. This failure can probably be traced to the high symmetry of the crystals which tends to obscure the contribution of the heavy ions. The first information about the positions of the heavy-metal ions was obtained from a preliminary two-dimensional difference Patterson projection on the uv plane.

Although X-ray data from protein crystals do not meet the requirements necessary for the successful application of Wilson's statistics (Wilson, 1942), we nevertheless used this method in an attempt to bring data from Weissenberg photographs of crystals containing tantalum and niobium, respectively, approx-

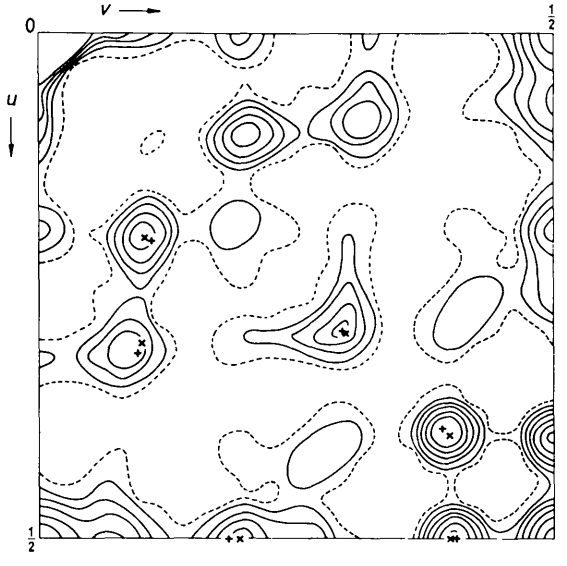


Fig. 2. A plot of a difference Patterson ($|F_{LTa}|^2-|F_{LNb}|^2$) projected onto (001); $d_{\text{min.}}=5$ Å.

imately to the same scale. Relative values of $F^2_{(hk0)}$ for 98 reflections extending to a minimum spacing of 5 Å were used. The resulting difference Patterson diagram, based on the coefficients $(|F_{LTa}|^2-|F_{LNb}|^2)$, could be interpreted as indicating the presence of eight heavy-metal octahedra with centers in the eight-fold general positions of $P4_12_1$, with $x=0{\cdot}342$, $y=0{\cdot}048$. Conspicuous maxima at $\frac{1}{2}$, 0 and $\frac{1}{2}$, $\frac{1}{2}$ at first suggested that additional ions might occupy a set of four-fold special positions with $x=y=\frac{1}{4}$, but this possibility was not supported by later determinations of the number of ions in the unit cell (see Part 2, above) and inspection of the three-dimensional Patterson diagram described below.

These tentative x and y coordinates were used as a basis for further adjustment of the relative values of $F^2_{(hk0)}$ for the crystals containing the different ions.

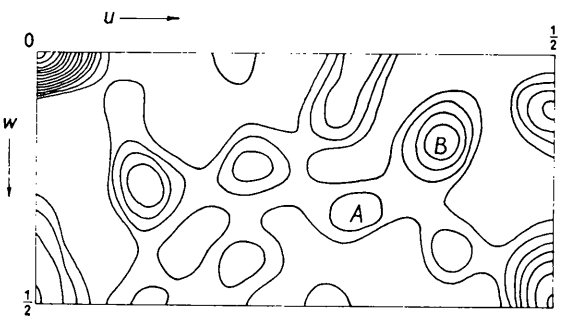
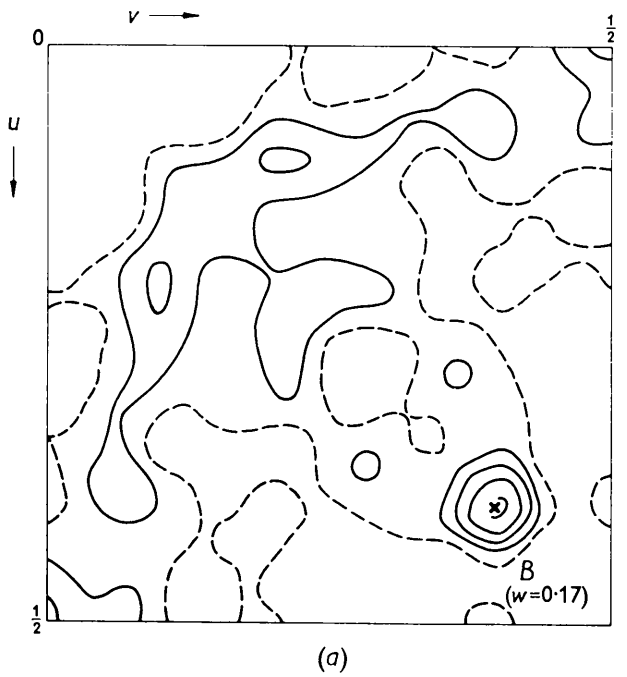
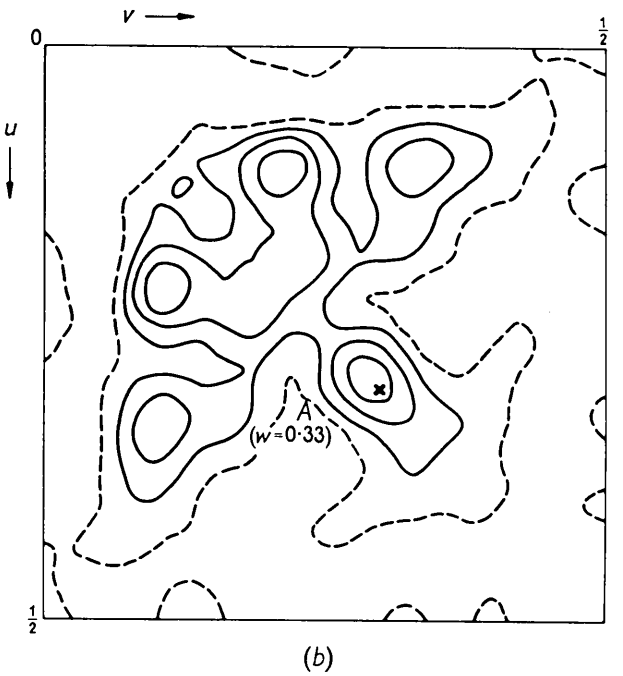


Fig. 3. A plot of the difference Patterson projected onto (010). The maxima labeled A and B are identified in the text.

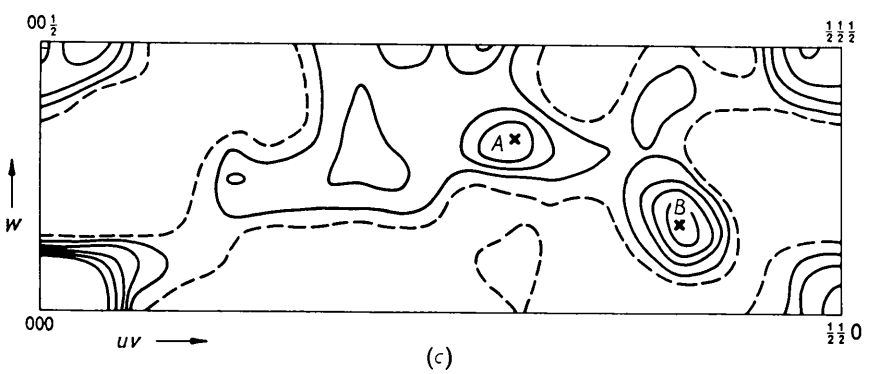
In the final stage of bringing these values close to the absolute scale, structure factors corresponding to $\Delta F^2_{(Ta-Nb)}$ were calculated for reflections with spacing



(a)



(b)



(c)

Fig. 4. Sections through the three-dimensional difference Patterson.
(a) Horizontal section $w=0{\cdot}20$; (b) section $w=0{\cdot}30$; (c) diagonal vertical section $u=v$.

greater than 5 Å. A specific orientation was assumed for the metal-atom octahedron; the scattering powers of the metal atoms were taken from the *Internationale Tabellen zur Bestimmung von Kristallstrukturen* (1935) and were corrected for anomalous dispersion (Dauben & Templeton, 1955). Scaling factors were then applied to the two sets of observed values of $F^2_{(hk0)}$ so as to fit their differences, ΔF^2_o, to the calculated values described above.

These new 'absolute' values of $F^2_{(hk0)}$ were used for a recalculation of the difference Patterson $P(u, v)$, a plot of which is shown in Fig. 2. In this figure the positions marked with a plus sign (+) represent vectors between ions situated in the eight-fold general positions with $x=0{\cdot}342$, $y=0{\cdot}048$, the coordinates derived from the original difference Patterson projection; vectors between corresponding ions with $x=0{\cdot}347$, $y=0{\cdot}050$ are represented by points marked with an (×), most of which appear to be situated closer to positions of maximum vector density.

Correspondingly adjusted values of F^2 for 80 $h0l$ reflections with a minimum spacing of 5 Å were used for the calculation of a difference Patterson projection on the uw plane (Fig. 3). Interpretation of this projection led to the assignment of the value $z=0{\cdot}335$ to the third coordinate of the ions in eight-fold general positions.

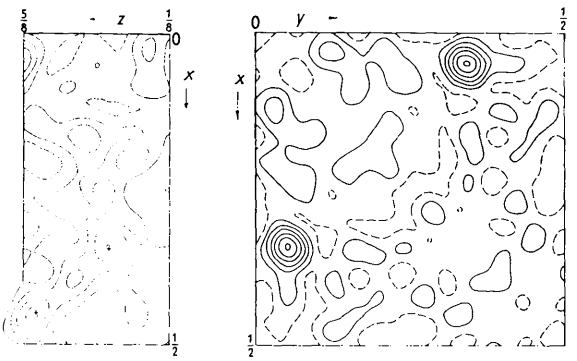
(ii) *Three-dimensional difference Patterson vector diagram*

General confirmation of the coordinates assigned to the centers of the complex ions was obtained from a three-dimensional difference Patterson ($|F_{LTa}|^2-|F_{LNb}|^2$) calculated from 625 reflections with a minimum spacing of 5 Å. The calculation was made over one-eighth of the unit cell at intervals $\Delta u=\Delta v=0{\cdot}02$, $\Delta w=0{\cdot}05$. Two typical horizontal sections, at $w=0{\cdot}20$ and at $w=0{\cdot}30$, and the diagonal vertical section $u=v$ are shown in Fig. 4. In Figs. 3 and 4 the maximum designated by A corresponds to the interaction $u=v=x-y$, $w=1-2z$, that designated by B to $u=v=x+y$, $w=2z-\frac{1}{2}$. Aside from the maxima associated with the positions of the ions, most of the vector density is situated near the corners of the cell octant where the horizontal, vertical, and diagonal mirror planes of the Patterson diagram intersect. Attempts to associate these maxima with additional ions in special or general positions in the crystal were unsuccessful. They probably represent residual protein-ion interactions accentuated by the symmetry of the Patterson diagram.

4. Two-dimensional Fourier projection of lysozyme chloride containing $Ta_6Cl_{12}^{++}$

Although the structure has no center of symmetry, the projections onto (001) and (010) are centrosymmetric, and accordingly the positions of the complex ions are sufficient to determine the signs necessary for electron density projections.

We first calculated values of ΔF_{Ta-Nb}—the difference between the structure factors of a $Ta_6Cl_{12}^{++}$ ion and a $Nb_6Cl_{12}^{++}$ ion—for all 102 $hk0$ reflections and 84 $h0l$ reflections out to a spacing of 5 Å. For this calculation the complex ions were placed in eight-fold general positions with their centers at $x=0{\cdot}347$, $y=0{\cdot}050$ and $z=0{\cdot}335$ and with their four-fold axes oriented parallel to the three crystallographic axes; an empirical temperature factor with B equal to 30·6 Å^2 was applied. The magnitudes and signs of ΔF_{Ta-Nb} thus calculated, when compared with the observed magnitudes of $|F_{LTa}|$ and $|F_{LNb}|$, served to establish the signs of F_{LTa} for the zonal data. Electron density projections onto 010 and 001 were then calculated; they are shown in Fig. 5. Like most other two-dimensional projections for protein crystals, these projections fail to provide any information about the protein molecule, and, at this resolution, they could not be expected to contain any information about the orientation of the complex ions.



(*a*) (*b*)

Fig. 5. Fourier projections of electron density of lysozyme chloride containing $Ta_6Cl_{12}^{++}$. (*a*) The projection onto 010. Contours are drawn at intervals of 4 e.Å^{-2} beginning with the 20-electron contour which is dashed. The crosses indicate the positions of the ions at (0·347, 0·050, 0·335) and (0·450, 0·847, 0·585). (*b*) The projection onto 001. Contours are at intervals of 2 e.Å^{-2} beginning with the 10-electron contour (dashed). The prominent peaks are ions at (0·347, 0·050, 0·335) and (0·050, 0·347, 0·665).

A quantitative comparison was made between the calculated differences ΔF_{Ta-Nb} and the corresponding observed differences in structure factors. With $|\Delta F|_o=||F_{LTa}|\pm|F_{LNb}||$, the discrepancy factor

$$R=\Sigma(|\Delta F|_o-|\Delta F|_c)/(\Sigma|\Delta F|_o)$$

is 0·26 for the 186 $hk0$ and $h0l$ reflections. Bearing in mind that the terms ΔF_o are differences between two observations, this factor is satisfactorily small and gives strong support to the conclusions that the crystals containing $Ta_6Cl_{12}^{++}$ and $Nb_6Cl_{12}^{++}$ ions are indeed isomorphous and that the centers of the complex ions have been correctly located.

If crystals of plain lysozyme chloride are truly

isomorphous with those containing the complex ions, it should be possible to make a similar comparison between calculated and observed contributions of the heavy ions, and to assign signs to the zonal reflections of lysozyme. Accordingly, attempts were made to adjust the scale of the lysozyme hydrochloride data so as to bring the magnitudes of $|F_L|$ into approximate average agreement with the magnitudes of $F|_{LTa}|_o \pm |F_{Ta_6Cl_{12}^{++}}|_c$. These attempts were unsuccessful. Crystals of lysozyme hydrochloride, thus, are not isomorphous with those containing the complex ions, the ions apparently causing a significant alteration in the configuration of the protein molecule. The unit-cell dimensions listed in Part 2 above seem to give some confirmation to this conclusion.

5. Discussion

The work thus far seems to have established certain facts about crystals of lysozyme chloride containing the ions $Ta_6Cl_{12}^{++}$ or $Nb_6Cl_{12}^{++}$, and to have left several fundamental questions in doubt.

We have strong evidence that the configuration of the molecule of lysozyme in crystals of the hydrochloride differs significantly from its configuration in crystals containing the large tantalum and niobium ions. These two types of lysozyme crystals, those that contain tantalum or niobium complex ions and those that do not, are therefore to be regarded as structurally different species.

From the first type of crystals, information may be sought by two procedures. Since the use of only a single pair of isomorphous crystals gives rise to ambiguities in the phase angles, a logical approach would be the introduction into these crystals of additional ions of other heavy elements in order to resolve the ambiguities. Thus far, we have not been able to obtain crystals containing other ions in addition to those of tantalum or niobium. In the meantime, we have been exploring the possibility of obtaining information about the configuration of the protein molecule directly from the single pair of isomorphous crystals. The results are already somewhat encouraging. It is clear that any studies of these crystals must be concerned with the specific orientation, or lack of orientation, of the complex ions and that this question will become more and more important as attempts are made to derive the positions of the atoms of the protein molecule from high-resolution data.

Investigations of the second type of crystals, those of lysozyme chloride alone, will require the incorporation of other simpler heavy atoms or ions. Much effort has been spent in attempts to achieve the desired conditions; recent experiments have been encouraging, but more work will be necessary before definite statements can be made as to their usefulness.

The authors wish to express their thanks to Prof. Linus Pauling for suggesting the use of the tantalum and niobium complex ions and for his interest in these investigations. Most of the crystals were grown and photographed by Mrs M. L. Lawrence, Mrs W. B. Kamb, Miss M. E. Hovanessian, and Miss S. W. Williams; density measurements and chemical analyses were made by Mrs Lawrence and Mrs P. Clauser. X-ray intensities were measured by Mrs Y. Meier, Mrs L. Samson, and Mrs J. Stroll. Many of the calculations were made by Dr D. M. Burns, Dr F. J. Ewing, Mr R. E. Long, and Mr H. D. Nathan. Mr Crellin Pauling assisted in the preparation of the niobium complex. Miss L. Casler prepared the Fourier plots and made the illustrations. Miss A. Kimball assisted with the data processing and the preparation of the manuscript.

References

ALDERTON, G., WARD, W. H. & FEVOLD, H. L. (1945). *J. Biol. Chem.* **157**, 43.

COREY, R. B., DONOHUE, J., TRUEBLOOD, K. N. & PALMER, K. J. (1952). *Acta Cryst.* **5**, 701.

DAUBEN, C. H. & TEMPLETON, D. H. (1955). *Acta Cryst.* **8**, 841.

HARNED, H. S. (1913). *J. Amer. Chem. Soc.* **35**, 1078.

HARNED, H. S., PAULING, C. & COREY, R. B. (1960). *J. Amer. Chem. Soc.* **82**, 4815.

Internationale Tabellen zur Bestimmung von Kristallstrukturen (1935). Berlin: Borntraeger.

LINDNER, K. & FEIT, H. (1924). *Z. anorg. Chem.* **137**, 66.

PALMER, K. J. (1948). Abstracts of Papers, First Congress of the I.U.C., 17–18; *Structure Reports* **11**, 729 (1947–48).

PALMER, K. J., BALLANTYNE, M. & GALVIN, J. A. (1948). *J. Amer. Chem. Soc.* **70**, 906.

SWANSON, H. E. & FUYAT, R. K. (1953). *Standard X-Ray Diffraction Powder Patterns*, National Bureau of Standards. Circular 539, **II**, 41.

TALLAN, H. H. & STEIN, W. H. (1953). *J. Biol. Chem.* **200**, 507.

VAUGHAN, P. A., STURDIVANT, J. H. & PAULING, L. (1950). *J. Amer. Chem. Soc.* **72**, 5477.

WILSON, A. J. C. (1942). *Nature, Lond.* **150**, 151.