would allow the disulfide exchange to occur more rapidly than if the molecules were forced apart by electrostatic repulsions.

The following sequence may be postulated for the heat denaturation of BSA: (1) hydrogen bonds are ruptured and rearranged, and the protein molecules aggregate and precipitate in the form of a metastable polymer of protein molecules linked by hydrogen and hydrophobic bonds, (2) a 30 sec. lag follows during which no extensive intermolecular disulfide exchange takes place and (3) after 30 sec. a very rapid intermolecular disulfide exchange occurs.

The addition of acetic acid will dissolve and disperse the polymer formed in (1) but will not break the covalent bonds of the polymer formed in (3). Mercuric ions tie up the sulfhydryl group and prevent the formation of the disulfide polymer in (3), thus the coagulum remains soluble in acetic acid. The structure of BSA is such that, when a solution of the material in (1) is lyophilized, the protein molecules are able to return to something similar to the native state.

BSA recovered after 15 sec. heating is obviously different from the native protein. The presence of a small amount of a dimer which was formed during the heating could explain the increase in the frictional properties as were observed with viscosity. It is difficult to say whether or not the dimer would have a lower optical rotation and increased susceptibility to digestion by trypsin. A complete separation of the monomer from the dimer would be necessary to see whether the monomer of recovered-BSA was identical to native BSA.

Markus and Karush[14] have found that one disulfide group of human serum albumin is more susceptible to reduction than are the rest. A single group, more reactive than the rest in BSA, would explain the appearance of the dimer before the rapid disulfide exchange had started and would also explain the frequent occurrence of small amounts of the dimer in preparations of BSA.

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[14] G. Markus and F. Karush, This Journal, **79**, 134 (1957).

SEATTLE, WASH.

[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND ANATOMY OF THE UNIVERSITY OF WASHINGTON]

The Crystal Structure of L-Cystine Hydrochloride[1]

By L. K. Steinrauf,[2] Juanita Peterson and L. H. Jensen

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The crystal structure of cystine hydrochloride has been solved by the method of superposition and refined by two-dimensional F_0 and ΔF syntheses. The configuration of the cystinyl group is the same as that in N,N′-diglycyl-L-cystine dihydrate but different from that in hexagonal cystine. Bond lengths and angles are near expected values and the angle between the planes determined by SS′C′ and by S′SC is 79.2°.

The importance of disulfide linkages in protein structure is well known and has been further demonstrated in recent work.[3] Since the cystinyl group occurs frequently in proteins and since the S–S interaction makes an important contribution to the vector space representation of proteins, its configuration is important in X-ray diffraction studies and in model building. It is therefore a matter of some importance to examine the configuration of the cystinyl group under a wide range of conditions.

Yakel and Hughes[4] have determined the configuration of the cystinyl group in a tripeptide N,N′-diglycyl-L-cystine dihydrate, and recently Oughton and Harrison have reported the structure of hexagonal cystine.[5] The present work is an investigation of the configuration of L-cystine as it crystallizes from aqueous hydrochloric acid.

Experimental

Crystals of L-cystine hydrochloride, HOOC–CH(NH_2·HCl)–CH_2–S–S–CH_2–CH(NH_2·HCl)–COOH, were prepared by allowing a hydrochloric acid solution to evaporate in a desiccator over phosphorus pentoxide. From oscillation and Weissenberg photographs of the needle-like crystals the following unit cell parameters were determined: a_0 = 18.61 Å., b_0 = 5.25 Å., c_0 = 7.23 Å., β = 103.6°. Systematic extinctions indicate the space group to be Cm, C2 or C2/m. The density observed by flotation is 1.520 g. cm.$^{-1}$, calculated, 1.515 g. cm.$^{-3}$ assuming 2 molecules per unit cell.

The intensities of the X-ray reflections were measured from unidimensionally integrated Weissenberg photographs with a microdensitometer tracing at right angles to the direction of integration by the camera. The areas under the peaks of the photometric tracings were measured with a planimeter and were assumed to be proportional to the integrated intensities.[6] Results indicate a precision of 2–3% in F_{rel} measured by this method.[7]

Determination of the Structure

The space groups Cm, C2 and C2/m cannot be distinguished by the systematic extinctions of the X-ray reflections. Since these three space groups have at least fourfold general positions, the two molecules of L-cystine hydrochloride must lie in special positions and have some symmetry element of the space group. Since the molecule of L-cystine cannot have symmetry m or i, the only one of the three space groups compatible with possible molecular symmetry is C2.[8] This agrees with that found by Srinivasan[9] and by Corsmit, Schuyff and Feil,[10] and is also the same as was found for N,N′-

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[1] Presented at the 4th International Congress of the International Union of Crystallography, Montreal, Canada, July, 1957.

[2] Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California.

[3] L. K. Steinrauf, Thesis, University of Washington, 1957.

[4] Yakel and Hughes, *Acta Cryst.*, **7**, 291 (1954).

[5] B. M. Oughton and P. M. Harrison, *ibid.*, **10**, 479 (1957).

[6] L. H. Jensen, This Journal, **76**, 4663 (1954).

[7] L. H. Jensen and E. C. Lingafelter, "Am. Cryst. Assoc., Abstracts," 1956, p. 21.

[8] L. K. Steinrauf and L. H. Jensen, *Acta Cryst.*, **9**, 539 (1956).

[9] R. Srinivasan, *ibid.*, **9**, 1039 (1956).

[10] A. F. Corsmit, A. Schuyff and D. Feil, *Proc. Roy. Soc. Amsterdam*, **59**, 470 (1956).

diglycyl-L-cystine dihydrate by Yakel and Hughes.[4]

The projection of the structure on the xz-plane was solved by the method of vector convergence as proposed by Beevers and Robertson.[11] As noted above, space group considerations require the molecule to have a twofold symmetry axis. Therefore the S–S bond, known to be present, must be parallel to the xz-plane. A length of 2.0 Å was assumed for this bond, and its orientation immediately determined by inspection of the Patterson projection on (010), Fig. 1a.

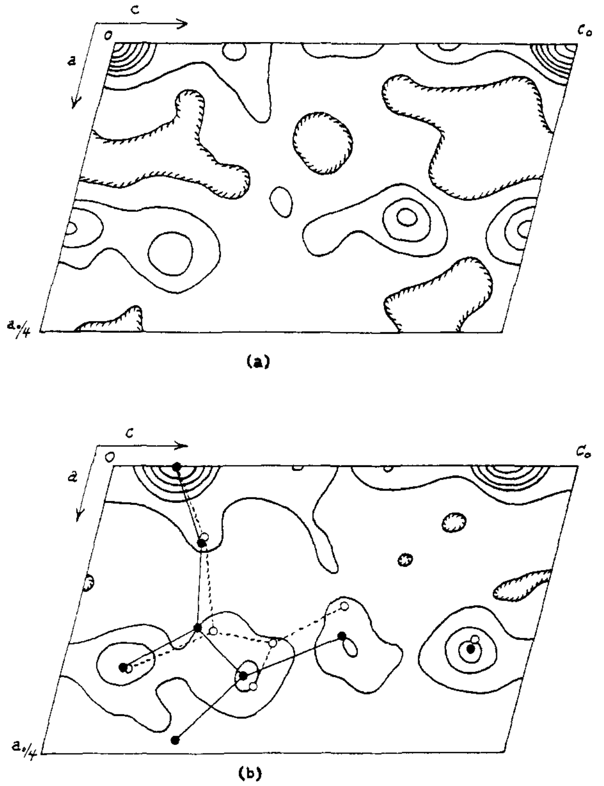

Fig. 1.—(a) $P(x, z)$, contours at arbitrary equal intervals of vector density. (b) Superposition function. Solid circles, coördinates assumed from superimposing molecular model; open circles, final atomic positions.

A superposition function was then calculated with the origin moved to each of the assumed S atom positions. The function calculated was

$$P_m(x,z) = \sum_{j=1}^{m} P(x_j, z_j)$$
$$= \frac{1}{v} \sum_{h} \sum_{l} [|F_{h0l}|^2 \sum_{j=1}^{m} \{\cos 2\pi(hx_j + lz_j)\}]$$

where P_m(x,z) is the sum of the superpositions of m identical Patterson functions with the origin at the points (x_1, z_1), (x_2, z_2), etc., and where $m = 2$ for the case in point.

The result of this superposition is shown in Fig. 1b. Although resolution is poor, the general form of the molecule is clear. A set of structure factors using the atomic positions indicated by the solid circles in Fig. 1b was calculated. The reliability index, R, was 48.0%. After one refinement cycle based on an F_o synthesis, R stood at 27.2%. The structure was further refined by successive F_o and ΔF syntheses[12] until R, decreased to 7.9%, the final value for the $h0l$ reflections.

The projection along [001] was solved by using the x-coördinates of the projection on (010) and approximate expected interatomic distances. The trial structure thus derived for the projection along [001] was refined by successive F_o and ΔF syntheses. The final value of R for the $hk0$ reflections is 7.9%. For both zones, final values of R were calculated neglecting unobserved and extinguished reflections.

Hydrogen atom coördinates were assigned by fitting a molecular model to the peaks of the appropriate ΔF maps. Hydrogen atoms were included in the structure factors for the last refinement cycles, but their positions were not refined.

Individual atom temperature factor parameters may be evaluated from the curvature of ΔF maps at the atomic positions.[12] A convenient method in practice is to change the parameters by arbitrary amounts between two ΔF syntheses and to extrapolate (or interpolate) from the resulting change of curvature. This method was used in the work reported here.

McWeeney scattering factors[13] were used for H, C, N and O atoms. Amsterdam values were used for the Cl^-.[14] Scattering factor values for the S atom were derived from Amsterdam and I.T.[15] values for Na and Cl^- and the I.T. values for the S atom by the interpolation formula

$$f_s = f_{Cl^-_{Am}} - \left[\frac{f_{Cl^-_{IT}} - f_{S_{IT}}}{f_{Cl^-_{IT}} - f_{Na_{IT}}}\right](f_{Cl^-_{Am}} - f_{Na_{Am}})$$

Of the 169 possible $h0l$ reflections, 24 were too faint to be observed and at least three were low due to secondary extinction. From these 142 usable reflections, 25 parameters (16 positional, 8 temperature factor and 1 scale) were determined giving 5.7 reflections per parameter.

Of the 68 possible $hk0$ reflections, 3 were too faint to be observed and 1 was low due to extinction. From the 55 usable $hk0$ reflections with $h \neq 0$, 11 parameters (8 positional, 2 temperature factor and 1 scale) were determined. Thus there are 5 reflections per parameter. Because there are fewer observed reflections per parameter and also because the projection of the structure along [001] is non-concentrosymmetric, we expect the y-coördinates to be less accurate than the x- or z-coördinates determined from the projection on (010).

Final values of F_c and F_o are listed in Table I.[16] Final ΔF syntheses are shown in Fig. 2. Atomic and temperature factor parameters corresponding to the final syntheses are given in Tables II and III, respectively. The more important bond lengths and bond angles are given in Table IV.

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(11) C. A. Beevers and J. H. Robertson, *Acta Cryst.*, **3**, 673 (1950).

(12) W. Cochran, *ibid.*, **4**, 408 (1951).

(13) R. McWeeney, *ibid.*, **4**, 413 (1951).

(14) J. Berghuis, *et al.*, *ibid.*, **8**, 478 (1955).

(15) "Internationale Tabellen zur Bestimmung von Kristallstrukturen," Vol. 2, 1935, p. 571.

(16) Table I has been deposited as Document Number 5541 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be had by remitting $1.25 for photoprints or for 35 mm. microfilm by check or money order payable to Chief, Photoduplication Service.

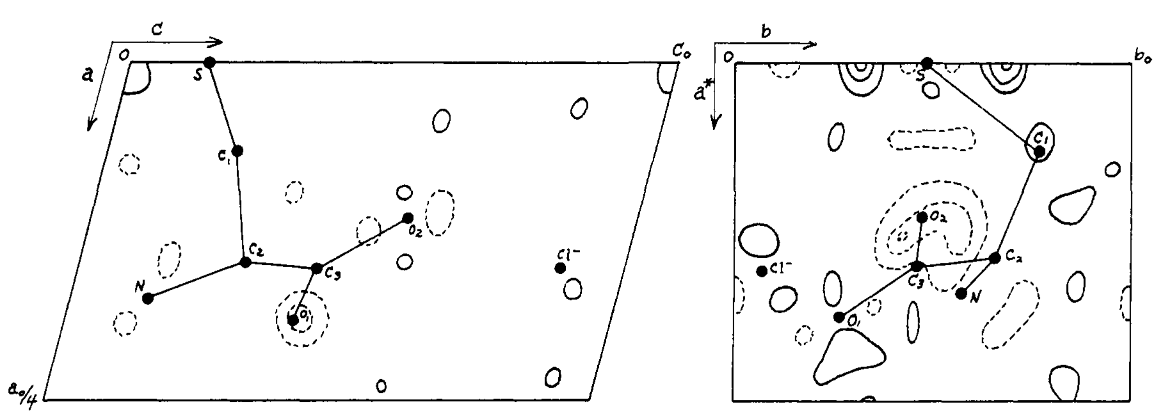

Fig. 2.—Final difference synthesis projected on (010) and along [001]. Contours at intervals of 0.5 e.Å.$^{-2}$, zero contour omitted, negative contours broken.

Discussion

In the limit, ΔF syntheses show the difference between actual and assumed electron density and are, therefore, useful in showing electronic anisotropy. The observed anisotropy arises from several sources: inherent non-spherical electron distribution in the atom, bonding electrons, anisotropic thermal motion. We assume the latter to be the main cause of the electronic anisotropy in L-cystine hydrochloride, an assumption that can be verified only from data collected at two different temperatures.

TABLE II

ATOMIC COÖRDINATES

	x/a_0	y/b_0	z/c_0
C_1	0.0642	0.7660	0.2406
C_2	.1465	.6790	.3048
C_3	.1552	.4670	.4418
N	.1765	.6100	.1388
O_1	.1924	.2750	.4292
O_2	.1195	.4790	.5772
S	.0003	.5000	.1415
Cl^-	.1532	.0890	.8848
H_1	.067	.910	.100
H_2	.057	.900	.367
H_3	.188	.850	.377
H_4	.150	.460	.033
H_5	.172	.825	.033
H_6	.233	.460	.167
H_7	.133	.360	.667

TABLE III

TEMPERATURE FACTOR, $-\exp[B + C\cos^2\phi]\sin^2\theta/\lambda^2$

	B_{hk0}, Å.^{2}	C_{hk0}, Å.^{2}	ψ_{hk0},[a] deg.	B_{h0l}, Å.^{2}	C_{h0l}, Å.^{2}	ψ_{h0l},[a] deg.
C_1	2.45	0.00	..	2.45	0.00	..
C_2	2.45	.00	..	2.45	.00	..
C_3	2.45	.00	..	2.45	.00	..
N	2.45	.00	..	2.45	.00	..
O_1	2.45	.00	..	2.45	.00	..
O_2	5.00	.00	..	5.00	.00	..
S	1.30	1.60	0.00	1.30	2.20	14.4
Cl^-	2.75	0.00	..	2.25	1.50	−21.6

[a] ψ_{hk0} and ψ_{h0l} are the angles between the direction of maximum vibration and $+b^*$ or $+c^*$, respectively.

In refining the (010) projection of L-cystine hydrochloride, individual atom anisotropic temperature factors were determined although not used in the final F_c except for S and Cl^- because of the limited data. They were used, however, to evaluate an over-all isotropic temperature factor, $B = 2.45$, by using the relation

$$B = \frac{\sum_j N_j(B_j + \bar{C}_j)}{\Sigma N_j}$$

where

$$\bar{C}_j = \frac{\sum_0^{\pi/2} \cos^2\theta}{\sum_0^{\pi/2} \theta} C_j = \frac{\int_0^{\pi/2} \cos^2\theta d\theta}{\int_0^{\pi/2} d\theta} C_j = {}^1/_2 C_j$$

This is an average of the temperature factors of each atom (j) weighted according to the number of electrons (N) in that atom. Difference syntheses indicating departures from assumed spherical electron distribution with isotropic thermal motion corresponding to $B = 2.45$ are shown in Fig. 3.

TABLE IV

BOND LENGTHS AND BOND ANGLES

Bond, Å.		Angle, deg.	
S–S′	2.044	S′–S–C	103.8
S–C_1	1.865	S–C_1–C_2	112.8
C_1–C_2	1.561	C_1–C_2–C_3	111.5
C_2–C_3	1.474	C_1–C_2–N	111.2
C_2–N	1.482	C_2–C_3–O_1	122.7
C_3–O_1	1.238	C_2–C_3–O_2	118.1
C_3–O_2	1.307	N–C_2–C_3	110.9
Cl^-—H–O_2	2.98	O_1–C_3–O_2	119.1
Cl^-—H–N′	3.08		
Cl^-—H–N″	3.27		
Cl^-—H–N‴	3.25		

The electronic anisotropy of the sulfur atoms indicates maximum vibration essentially along the S–S bond. The oxygen atoms on the other hand appear to be vibrating maximally in a plane perpendicular to the O–C bond.

The configuration of the L-cystinyl group is the same as that in N,N′-diglycyl-L-cystine dihydrate[4] and in L-cystine hydrobromide[17] but different from that in hexagonal L-cystine.[5]

The angle between the SS′C'_1 and S′SC_1 planes is 79.2°, not very different from the corresponding angle in other derivatives of L-cystine. None of

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[17] To be submitted for publication.

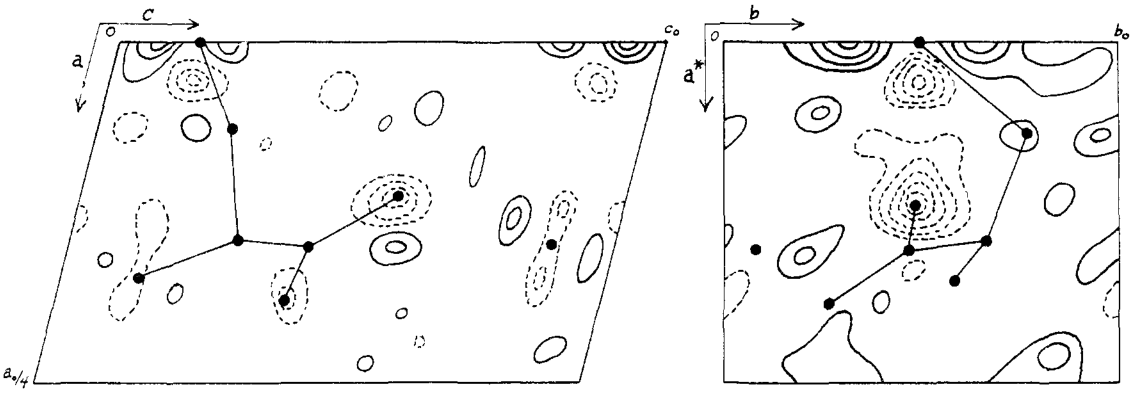

Fig. 3.—Difference synthesis showing electronic anisotropy. Contours at intervals of 0.5 c.Å.$^{-2}$, zero contour omitted, negative contour broken.

the bond lengths differs significantly from expected values, and all bond angles are close to expected values.

The hydrogen bond arrangement is of particular interest. Difference syntheses and C–O bond lengths confirm the expectation of a neutral carboxyl and a charged amino group inferred from the fact that the crystals were grown from a highly acid solution. One H atom is bonded to the O_2 atom and three to the N atom. Each of these H atoms is involved in a hydrogen bond to Cl^- and together make essentially a tetrahedral matrix of H atoms about each Cl^-.

A Cl^- which is hydrogen bonded to three appropriately spaced charged amino groups may have some significance in protein structures. Three such groups would strongly attract chloride ions and bind them more tightly than widely spaced groups.

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SEATTLE, WASHINGTON

[CONTRIBUTION FROM THE INSTITUTE OF PHYSICAL CHEMISTRY OF THE UNIVERSITY OF BASLE]

A Generalization of the Frit Method for the Measurement of Diffusion Coefficients

BY F. GRÜN AND CHR. BLATTER

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The frit method for determining diffusion coefficients, which was originally developed for cylindrical frits with sealed edges, applies for frits of any shape.

1. Wall, Grieger and Childers have described a simple method for measuring diffusion coefficients of substances in liquid solutions.[1] It consists of soaking a circular disc of porous material with sealed edges (or a thin, non-sealed disc) in the solution of the diffusing substance and then suspending the disc in a bath of pure solvent. The apparent weight of the suspended disc is determined at various times. The change of weight corresponds to the loss of solute by diffusion. For large t the amount of solute in the disc, $Q(t)$, decreases exponentially with t, *i.e.*

$$\log Q(t) \sim -\alpha Dt + \beta \qquad (t \to \infty) \qquad (1)$$

where the constant α depends on the thickness and porosity of the disc used. It is obvious that from such an experiment the value of D can be calculated after determining α by a calibration experiment.

2. As indicated in Wall's paper, the theoretical basis of this method is as follows. For "one-dimensional diffusion" out of a homogeneous cylindrical disc we have (see *e.g.*[2])

$$Q(t) = Fdc_0 \frac{8}{\pi^2} \sum_{\nu=0}^{\infty} \frac{1}{(2\nu + 1)^2} \exp[-Dt(2\nu + 1)^2\pi^2/d^2] \qquad (2)$$

where $Q(t)$ is the amount of diffusing substance in the disc at time t as before, F and d are area and thickness of the disc, respectively, and c_0 is the uniform initial concentration of the diffusing substance in the disc.

It is safe to assume that (2) applies to a porous disc as well, if only d is replaced by the "effective thickness" d' and similarly F by F'. Then from

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[1] F. T. Wall, P. F. Grieger and C. W. Childers, THIS JOURNAL, **74**, 3562 (1952); see also F. T. Wall, *et al.*, *ibid.*, **75**, 3550, 6340 (1953).

[2] P. Frank, R. v. Mises, "Differential- und Integralgeichungen der Physik," Vol. II, M. S. Rosenberg, New York, N. Y., Reprint, 1943, p. 557f.