structure (Blake, *et al.*, 1966). The other bond angles at the Fe^{3+} ion are in the range 80·34 to 103·64°, which is about the same as that reported for $\alpha\text{-}Fe_2O_3$ (Blake, *et al.*, 1966).

The interlayer $Cl^-\text{-}Cl^-$ distance, 3·680 Å, closely approximates twice the van der Waals radius of chlorine.

The principal axes of the thermal motion probability ellipsoids are required by the crystal symmetry to be parallel to the crystallographic axes (as are the principal axes of the electric field gradient tensors, which were discussed in the *Introduction*). The root-mean-square components of thermal displacement along the three principal axes of the thermal motion probability ellipsoids are given in Table 5; these were computed with the Busing, Martin & Levy (1964) computer program *ORFFE*.

Table 5. *Thermal motion probability ellipsoids*

Root-mean-square component of thermal displacement* along principal axis parallel to

	a	b	c
Fe^{3+}	0·076 ± 0·005 Å	0·107 ± 0·005 Å	0·117 ± 0·005 Å
O^{2-}	0·090 ± 0·023	0·097 ± 0·023	0·116 ± 0·023
Cl^-	0·163 ± 0·007	0·112 ± 0·007	0·115 ± 0·008

* The limits of error are 3 × the estimated standard errors computed by the program *ORFFE*.

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The Relation Between γ- and α-Chymotrypsin. II.*
Direct Comparison of the Electron Densities at 5·5 Å Resolution

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Extensive comparisons have been made between 5·5 Å resolution electron density maps of the two molecules in the asymmetric unit of α-chymotrypsin, and between the α-chymotrypsin electron density and a 5·5 Å electron density map of γ-chymotrypsin. The transformations describing the relation between the respective pairs of molecules have been refined by a least-squares method. On the basis of these comparisons the respective electron density maps are shown to be identical, within experimental error. It is concluded that at this resolution there is no significant evidence of any conformational difference between the two α-chymotrypsin molecules or between α- and γ-chymotrypsin.

Introduction

α-Chymotrypsin (αCHT) and γ-chymotrypsin (γCHT) are the end products of the activation of chymotryp-

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sinogen A and are distinguished by their respective crystal forms (Kunitz, 1938; Desnuelle, 1960). αCHT crystallizes at pH 4·4 in a monoclinic space group with two molecules per asymmetric unit and the structure has been determined to atomic resolution (Matthews, Sigler, Henderson & Blow, 1967; Sigler, Blow, Matthews & Henderson, 1968). γCHT crystallizes at pH 5·6 in a tetragonal space group and a 5·5 Å resolution elec-

tron density map of the structure has been obtained (Cohen, Silverton, Matthews, Braxton & Davies, 1969). Corey, Battfay, Brueckner & Mark (1965) have shown that αCHT and γCHT may be slowly interconverted by proper control of the pH of the crystallizing solution. The enzymes are therefore chemically equivalent, and Corey et al. (1965) proposed that they represent different structural configurations of the same protein molecule.

A comparison of α- and γCHT made on the basis of points common to both crystal species suggested that the structures were very similar (Matthews et al., 1968). Nevertheless it was not possible to rule out the possibility of significant local conformational differences between the two molecules. To examine this possibility the transformation from γ- to αCHT was refined, and a direct point by point comparison was made of the 5·5 Å resolution electron-density maps of the two enzymes. At the same time it was of interest to make similar comparisons of the electron densities of the two molecules in the asymmetric unit of the αCHT crystals (Blow, Rossmann & Jeffery, 1964). The similarity of α- and γCHT, which are crystallized at a different pH and in different space groups, could then be compared with the agreement between the two αCHT molecules *in the same crystal*.

Method

The transformations from αCHT to γCHT (Matthews et al., 1968) and the transformation between the two molecules of αCHT (Blow, Rossmann & Jeffery, 1964) were refined using a least-squares method similar to that of Cox (1967) as used by Muirhead, Cox, Mazzarella & Perutz (1967). The approach used here differed from that of Muirhead et al. (1967) in that the electron density was transformed directly from one molecule to another rather than via an intermediate Cartesian system of axes. This leads to a reduction in computing time and avoids errors introduced by the repeated interpolation of the electron density. Also, to further reduce interpolation error, the electron density was calculated on grids of spacing approximately 1 Å, rather than the 2 Å spacings used by Muirhead et al. (1967).

Before refinement of the transformation relating any two electron density maps it is necessary that these maps be brought to a common scale. This was achieved by scaling the maps so that the root-mean-square electron densities within the respective boundaries of the molecules being compared are equal.

We found the r.m.s. difference in electron density to have a sharply defined optimum value. For example, in a preliminary comparison of the low resolution electron densities of the two molecules in the αCHT asymmetric unit, the root-mean-square difference in density for the inner part of the molecules was 0·129 electrons Å^{-3}. After translating one molecule with respect to the other by 0·33 Å the r.m.s. difference increased by 12% to 0·145 e.Å^{-3}.

All comparisons made between αCHT and γCHT were based on 5·5 Å three-dimensional electron density maps of the tosylated enzymes. For tosyl-γCHT the electron density map was calculated using phases from the four heavy atom derivatives pipsyl-, iodinated-, HgI$_4$-, and PMMCMBS-γCHT described by Cohen et al. (1969). For tosyl-αCHT the phases were derived from three isomorphous derivatives, viz. pipsyl-, PtCl$_4$- and phenyl mercuric acetate-αCHT (Matthews et al., 1967). It may be noted that the coordinates of the heavy atoms of αCHT as used in the phase determination were not constrained to adhere to the local axis of symmetry. In subsequent discussion ϱ_{α_1} and ϱ_{α_2} will be used to define the electron densities of the two molecules in αCHT related by the local axis of symmetry. The 'average' αCHT electron density ϱ_α is obtained by superimposing ϱ_{α_2} on ϱ_{α_1} and then taking the mean of the two densities.

In calculating the criterion of agreement between the different molecules it was necessary to define a molecular boundary, since the transformation relating two molecules will in general apply only to the molecules under consideration, and not to the respective neighboring molecules in the crystal. While it would have been possible to make use of the known structure of αCHT to define a very detailed surface of the αCHT molecule, this would have made the calculations much more unwieldy, and seemed to be of marginal benefit. We therefore decided, for the purposes of the *refinement* of a given transformation, to define the electron density boundary by means of an ellipsoid. Once the optimum value of the transformation had been obtained the transformation could then be applied to any additional electron density points which might be outside the ellipsoid used. The ellipsoid chosen for these calculations enclosed most of the density ϱ_{α_1} of 'molecule 1' in the asymmetric unit of αCHT. Referred to the αCHT unit-cell, the ellipsoid was centered at $x = 0.275$, $y = 0.0$, $z = 0.768$ and had semi major axes of 23·5, 18·0, 16·5 Å parallel to **a***, **b** and **c** respectively. This ellipsoid was chosen to enclose as much of the αCHT molecule as possible, but to minimize the inclusion of regions where neighboring molecules come in close contact. The ellipsoid contains about 85% of the total molecular volume. In the γCHT cell the center of the ellipsoid is at $x = 0.140$, $y = 0.337$, $z = -0.318$. Preliminary calculations were also made with a smaller ellipsoid of semimajor axes 19, 13 and 13 Å. The transformation parameters did not change significantly in going from the smaller to the larger ellipsoid.

The different transformations were specified by the Eulerian angles φ, ψ, θ (*e.g.* see Coe, 1938) and the translational parameters d_1, d_2 and d_3, defined relative to the axes a^*, b and c of αCHT, and a, b, and c of γCHT (*cf.* Matthews et al., 1968). As a consequence of this choice of axes, a rotation of 180° about **a*** leads to a singularity in the rotation matrix. This accounts for the large standard deviations of φ and ψ in the α_1 vs. α_2 refinement (see Table 1). The standard deviations

were estimated from the elements of the inverse normal matrix and from the variance of the observations (*e.g.* see *International Tables for X-ray Crystallography*, 1959). Following Cox (1967) the variance of the observations $\sigma^2(m)$ was taken as $\dfrac{n}{l} \cdot \dfrac{n\Delta}{(n-f)}$ where Δ is the mean-square residual after fitting the electron density, f the number of degrees of freedom, n the number of grid points used for fitting, and l the number of these grid points that can be regarded as independent observations. Alternatively, we can write

$$n/l = N/L ,$$

where N is the number of grid points per asymmetric unit, and L the number of grid points per asymmetric unit which may be regarded as independent observations. N is readily determined, and we have assumed that the number of independent grid points per asymmetric unit is equal to the number of independent structure amplitudes used in calculating the electron density map. This leads to values of $n/l = 69$ and 78 grid points per independent observation for γCHT and αCHT respectively. The value used in calculating the variances of the α- to γCHT transformation was the mean of these two determinations. Cox (private communication) used a similar procedure to estimate (n/l), but assumed that for each structure amplitude there are *two* independent observations (corresponding to the A part and the B part of the Fourier coefficient), and in addition assumed that the structure amplitudes of the two structures being compared can be counted separately. (This procedure would lead to a reduction by a factor of two of the standard deviations quoted in Table 1.)

Results of refinement of transformations

The results of the different refinements are summarized in Table 1. In the Table the 'initial' values for the comparison between α_1 and α_2, the two molecules in the asymmetric unit of αCHT, are those obtained assuming that α_1 and α_2 are related by a local twofold axis of symmetry parallel to $\mathbf{a}^*$ and specified by the equation $y=0$, $z=0\cdot156x+0\cdot475$ (Blow *et al.*, 1965; Sigler, Jeffery, Matthews & Blow, 1966). Refinement of the transformation relating α_1 and α_2 gave no indication that the operation relating the molecules departed significantly from being a twofold axis parallel to $\mathbf{a}^*$.

This can be seen most clearly by expressing the refined transformation from α_1 to α_2 as a screw operation, *i.e.* as the combination of a rotation and a translation. Using the expressions given by Cox (1967) it is found that the equivalent screw axis makes an angle of $0\cdot49°$ with $\mathbf{a}^*$ and passes through the point $x=0$, $y=0\cdot000$, $z=0\cdot474$. The angle of rotation about the screw is $179\cdot7°$ and the translation along the axis is $-0\cdot01$ Å. Assuming that the axis is strictly twofold and

Table 1. *Summary of transformation parameters*

Electron densities being compared		Eulerian angles φ (°)	ψ (°)	θ (°)	Translation components d₁ (Å)	d₂ (Å)	d₃ (Å)	Correlation c	R.m.s. difference (e.Å⁻³)
α,γ (inner ellipsoid)	Initial parameters	−74·3	56·9	−59·6	−37·8	29·5	−45·5		
	Refined parameters	−75·8	53·8	−61·0	−37·73	27·48	−44·81	0·829	0·114
	Standard deviations	0·7	0·7	0·6	0·19	0·56	0·52		
α,γ (full ellipsoid)	Initial parameters	As above							
	Refined parameters	−75·8	53·5	−61·0	−37·78	27·35	−44·73	0·775	0·123
	Standard deviations	0·4	0·4	0·4	0·13	0·34	0·33		
α_1,γ (full ellipsoid)	Initial parameters	As above							
	Refined parameters	−75·8	53·4	−60·6	−37·64	27·30	−45·18	0·725	0·142
	Standard deviations	0·5	0·5	0·4	0·14	0·38	0·37		
α_2,γ (full ellipsoid)	Initial parameters	105·8	123·1	−120·4	14·1	14·9	−13·9		
	Refined parameters	104·5	126·7	−118·8	15·27	13·88	−14·38	0·733	0·139
	Standard deviations	0·5	0·5	0·4	0·15	0·16	0·12		
α_2,α_1 (full ellipsoid)	Initial parameters	0	0	180	0	0	62·6		
	Refined parameters	50·0	50·5	179·6	−0·20	−0·05	62·47	0·770	0·130
	Standard deviations	45	45	0·4	0·12	0·16	0·11		

parallel to a^*, the equation for optimum agreement between the two molecules is $y=0$, $z=0.156x+0.474$, in close agreement with the equation quoted above which corresponds to the twofold axis giving the best agreement between pairs of 'related' heavy atom sites. For comparison, the equation of the local axis as determined initially from the 'rotation' and 'translation' functions is $y=0$, $z=0.156x+0.477$ (Blow et al., 1964; Blow, 1969).

In the refinement of the transformation between α, the 'averaged' αCHT molecule, and the γCHT molecule, the 'initial' values in Table 1 are those obtained using the transformation from α- to γCHT derived on the basis of seven points common to the two structures (Matthews et al., 1968). It may be noted that there are no large changes in any of the parameters. Also the r.m.s. discrepancy between the seven points described by Matthews et al. (1968) increased only slightly from 1.64 to 1.79 Å.

In its most convenient form the transformation relating αCHT and γCHT is expressed as a set of transformation equations which can be used to transform
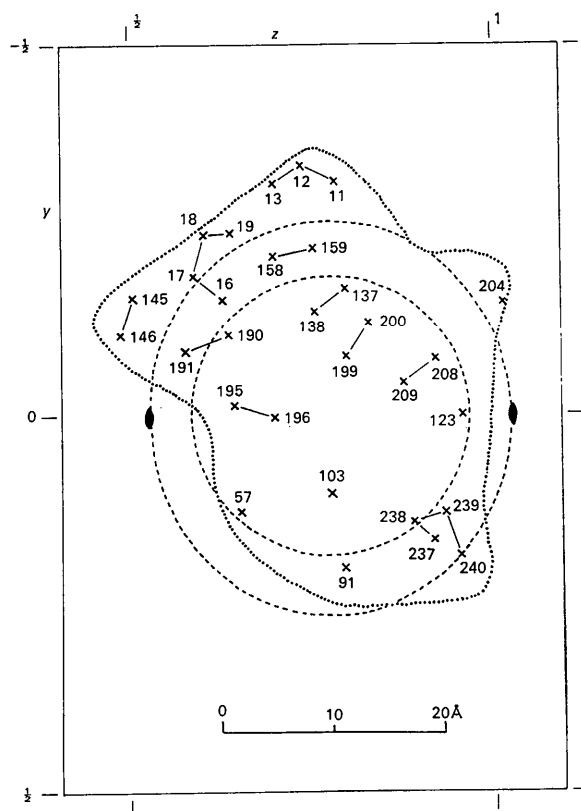

Fig. 1. Key to Figs. 2 and 3. For explanation see text. The numbered crosses indicate those α-carbon atoms of αCHT which lie within 2 Å of this section ($x=0.333$). The corresponding residues are as follows: Ser 11, Gly 12, Leu 13, Ile 16, Val 17, Asn 18, Gly 19, His 57, Asn 91, Ile 103, Leu 123, Val 137, Thr 138, Arg 145, Tyr 146, Ala 158, Ser 159, Ser 190, Cys 191, Ser 195, Gly 196, Leu 199, Val 200, Asn 204, Thr 208, Leu 209, Try 237, Val 238, Gln 239, Gln 240.

a point directly from the α- to the γCHT unit cell, or vice versa. If $(x_\alpha, y_\alpha, z_\alpha)$ are fractional coordinates for αCHT, and $(x_\gamma, y_\gamma, z_\gamma)$ fractional coordinates for γCHT, the equations to transform from γ- to αCHT are

$$x_\alpha = 0.755x_\gamma + 0.696y_\gamma - 1.419z_\gamma - 0.515$$
$$y_\alpha = -0.086x_\gamma + 0.880y_\gamma + 0.754z_\gamma - 0.045$$
$$z_\alpha = 1.013x_\gamma - 0.120y_\gamma + 0.498z_\gamma + 0.825$$

For the inverse transformation, the equations are

$$x_\gamma = 0.239x_\alpha - 0.080y_\alpha + 0.802z_\alpha - 0.542$$
$$y_\gamma = 0.365x_\alpha + 0.820y_\alpha - 0.203z_\alpha + 0.392$$
$$z_\gamma = -0.398x_\alpha + 0.360y_\alpha + 0.328z_\alpha - 0.459$$

Comparison of electron densities

By using the various transformations described above, it was possible to take the electron density of any chosen molecule and accurately superimpose this density on that of any other molecule, and so make direct point by point comparisons of the two molecules. The highest electron density of the averaged 5.5 Å resolution αCHT map is 0.65 e.Å^{-3} and that of the γCHT map 0.70 e.Å^{-3}. The points of highest density do not coincide exactly but both are within the α-helical region at the C-terminus of the respective molecules. The r.m.s. value of the electron densities is 0.18 e.Å^{-3}.

An overall measure of the similarity of any two electron density maps is given by the root-mean-square difference between them. The r.m.s. difference between the electron densities of the two αCHT molecules α_1 and α_2 is 0.130 e.Å^{-3}. The difference will depart from zero for two possible reasons; firstly because of real differences which may exist between the two molecules being compared, and secondly because of errors in the electron-density maps. In the high resolution studies of αCHT there is no indication within the chosen ellipsoid of a significant difference in the conformation of the two molecules in the asymmetric unit. Hence it may be assumed that the observed r.m.s. difference of 0.130 e.Å^{-3} is due only to errors in the low resolution αCHT electron-density map. This leads to an estimate of 0.092 e.Å^{-3} for the standard deviation of the unaveraged αCHT electron density (ϱ_{α_1} and ϱ_{α_2}), and 0.065 e.Å^{-3} for the standard deviation of the averaged density ϱ_α. The r.m.s. difference between the average density within the αCHT molecule and the density ϱ_γ of γCHT is 0.123 e.Å^{-3}; i.e. the agreement between ϱ_α and ϱ_γ is better than that between ϱ_{α_1} and ϱ_{α_2}. Therefore the αCHT and γCHT molecules must be very similar, if not completely equivalent. If the molecules are assumed to be identical then the r.m.s. difference quoted above and in Table 1 imply that the γCHT map has an r.m.s. error of about 0.104 e.Å^{-3}, i.e. only 10% higher than that of the unaveraged αCHT map. On the other hand the results would also be consistent with the interpretation that the γCHT map had a smaller error and that there was a small difference between the αCHT and γCHT molecules. On the basis of the above

criteria it is not possible to distinguish between these two possibilities.

Another factor which might influence the error estimates, and which has not been taken into account, is the use of an ellipsoid to approximate the molecular boundaries of αCHT and γCHT. In particular, in the crystals of αCHT, Arg 145 and Tyr 146 of one molecule enter the ellipsoidal boundary of the diad related molecule (see Fig. 1). To eliminate all possible errors due to 'edge effects' we recalculated the error in electron density using data for the 'inner ellipsoid', described previously, with semi major axes 19, 13 and 13 Å. In this case the r.m.s. difference between ϱ_{α_1} and ϱ_{α_2} is 0·128 e.Å^{-3}, and between ϱ_α and ϱ_α is 0·114 e.Å^{-3}. This leads to estimates of 0·090 e.Å^{-3} for the error in the electron density of the unaveraged αCHT density, and 0·094 e.Å^{-3} for the electron density of γCHT. One reason for obtaining these error estimates as accurately as possible, was to use them to test the expressions derived by Blow & Crick (1959), and by Dickerson, Kendrew & Strandberg (1961), which give the relation between the error in the electron-density map, and the 'figures of merit' of the phase angles as determined by

the isomorphous replacement method. Following Dickerson, Kendrew & Strandberg (1961), the mean-square error $\langle(\Delta\varrho)^2\rangle$ in an electron density map may be expressed as

$$\langle(\Delta\varrho)^2\rangle = \frac{2}{V} \sum_{\substack{0 \\ h}}^{\infty} \sum_{\substack{-\infty \\ k}}^{\infty} \sum_{\substack{-\infty \\ l}}^{\infty} F_{hkl}^2 (1 - m_{hkl}^2), \qquad (1)$$

where the F_{hkl} are the structure amplitudes, m_{hkl} the corresponding 'figures of merit' of the phase determinations, and V the volume of the unit cell. For the 5·5 Å γCHT electron-density map there are 852 terms with mean figure of merit $\bar{m} = 0.87$. On taking proper account of the multiplicities of the various terms in the Fourier summation, the r.m.s. error in the electron-density map is estimated from (1) to be 0·077 e.Å^{-3} which agrees quite well with the value of 0·094 e.Å^{-3} as determined from comparisons of the electron densities within the interior of the molecules. This agreement tends to confirm the general applicability of equation (1), provided, of course, that the figures of merit have been correctly estimated. In the case of αCHT where there are 1375 independent terms with mean figure of
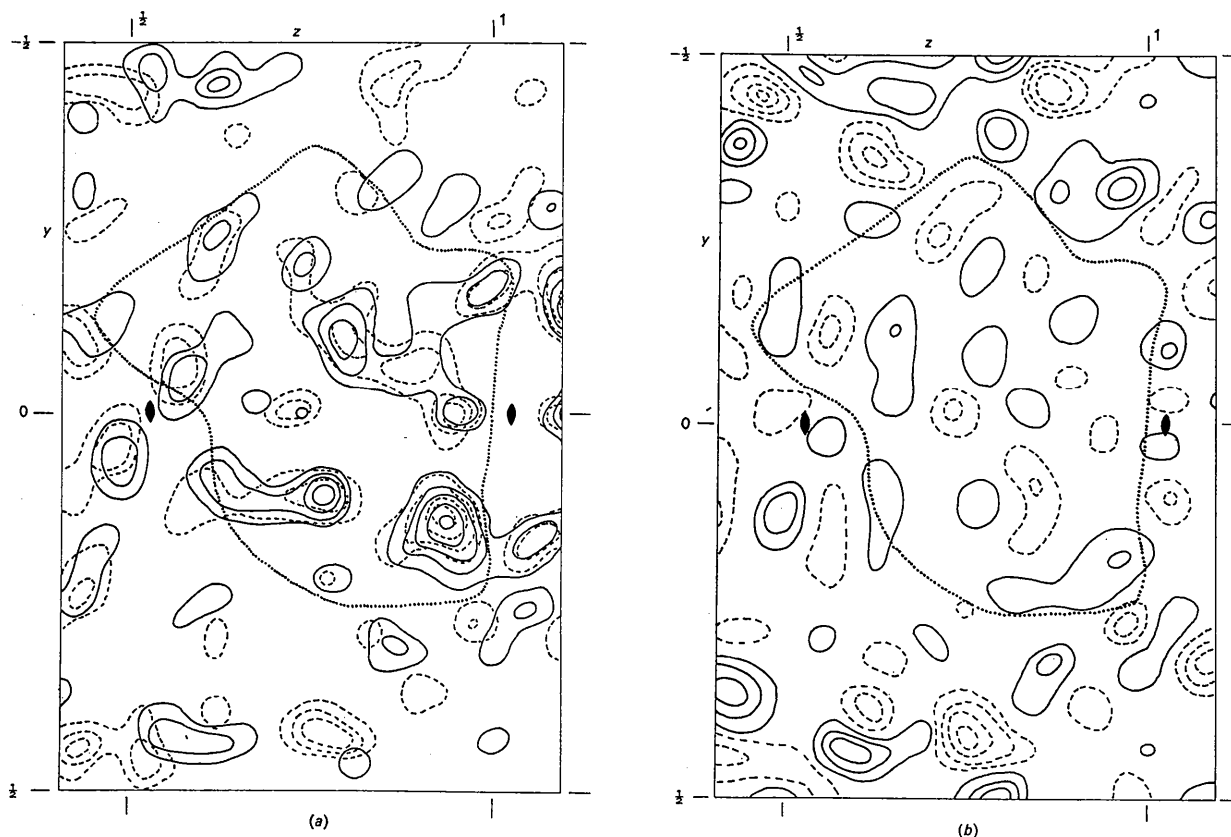

Fig. 2. Comparison of the electron densities of the two molecules in the asymmetric unit of αCHT at the section $x = 0.333$. The maps are contoured at intervals of 0·125 e.Å^{-3}. (a) Superposition of the electron density ϱ_{α_2} (broken contours) of molecule 2 of αCHT on the electron density ϱ_{α_1} (solid contours) of molecule 1. For clarity, the zero and negative contours have been omitted. (b) Difference electron density $\varrho_{\alpha_2} - \varrho_{\alpha_1}$. Positive contours drawn solid, negative contours broken, and zero contour omitted.

merit $\bar{m} = 0\cdot69$, the expression (1) leads to an estimated error of $0\cdot146$ e.Å^{-3}. This is higher than the value of $0\cdot090$ e.Å^{-3} obtained above, but the discrepancy is presumably due to the unusual method of calculating phases for αCHT (Blow & Matthews, unpublished), which leads to an underestimate of the figures of merit.

In Table 1 we have quoted the value of the correlation coefficient c which was also used as a measure of the agreement of the respective electron densities. The correlation c_{12} between two electron densities ϱ_1 (xyz) and ϱ_2 (xyz) is defined by

$$c_{12} = \frac{\sum(\varrho_1 - \bar{\varrho}_1)(\varrho_2 - _2)}{\{\sum(\varrho_1 - \bar{\varrho}_1)^2 \cdot \sum(\varrho_2 - \varrho_2)^2\}^{1/2}},$$

where the summations are over the volumes being correlated, and the bars denote mean values of the electron density within this volume (e.g. see Fisher, 1958). The correlation coefficient has a value of unity when the two maps being compared are perfectly correlated, and zero if the maps are uncorrelated. The coefficient has the advantage that it is independent of the relative or absolute scales of the densities being compared, and therefore provides a convenient basis on which results from different structure comparisons may be compared.

The respective pairs of electron densities ϱ_{α_1} and ϱ_{α_2}, and ϱ_α and ϱ_γ were also compared by using the known transformations between them to bring all the maps to a common reference system (the αCHT unit-cell). The respective electron density maps could thereby be subtracted point by point and three-dimensional difference maps prepared. It was hoped that such difference maps would indicate specific localized differences between the molecules being compared. (The difference electron density evaluated here, e.g. $\varrho_\gamma - \varrho_\alpha$, corresponds to the difference between two distinct crystal species, and is not to be confused with the difference Fourier synthesis as used to compare isomorphous crystal structures.) From a careful inspection of the $(\varrho_{\alpha_1} - \varrho_{\alpha_2})$ and $(\varrho_\gamma - \varrho_\alpha)$ difference electron-density maps we conclude that at this resolution there is no significant evidence of a difference in structure either between α_1 and α_2 or between αCHT and γCHT. To illustrate the comparisons made between the various electron-density maps, Figs. 1 through 3 show a section of the molecule passing through the active site region. Fig. 1 is a key to the following diagrams. The sections are through the αCHT cell at $x = 0\cdot333$ and intersect molecule α_1. The electron-density maps corresponding to the other molecules have been transformed into this standard orientation by
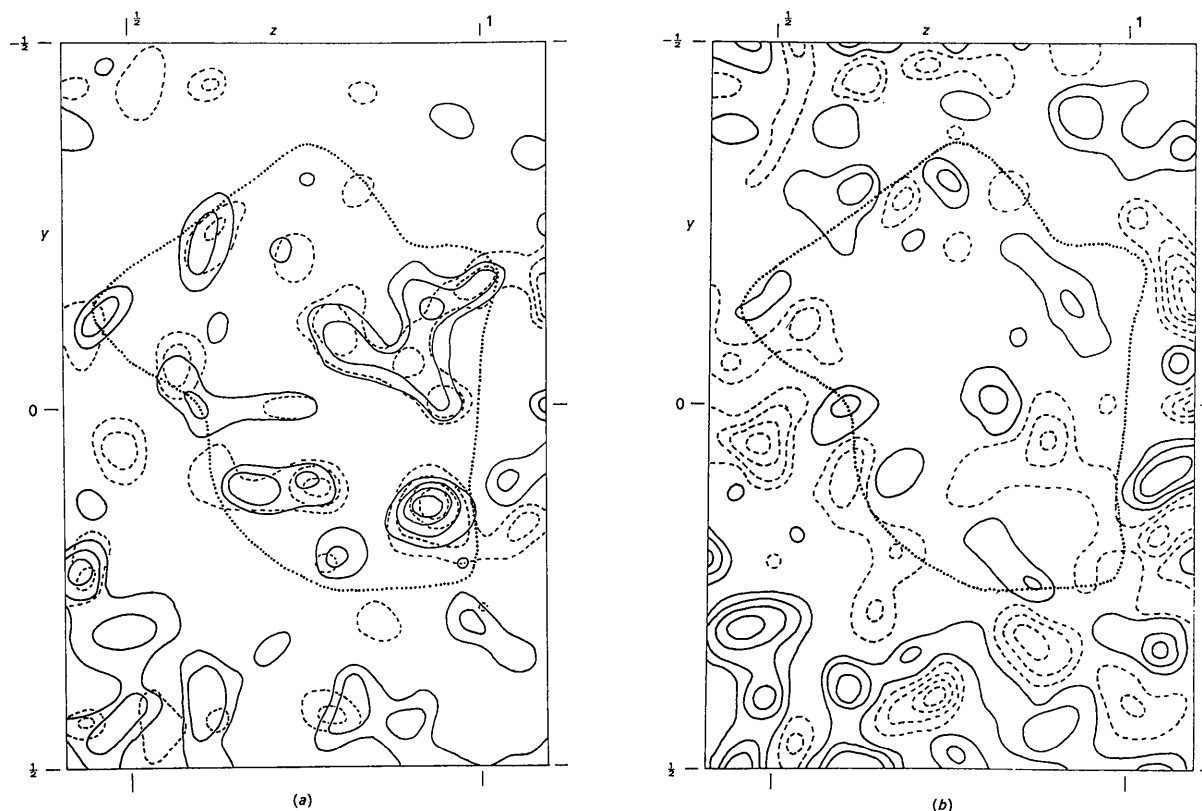

Fig. 3. Comparison of the electron density, ϱ_γ, of γCHT [with the averaged electron density, ϱ_α, of αCHT at the section $x = 0\cdot333$. The maps are contoured at intervals of $0\cdot125$ e.Å^{-3}. (a) Superposition of ϱ_γ (solid contours) on ϱ_α (broken contours). Zero and negative contours omitted. (b) Difference electron density $\varrho - \varrho_\alpha$. Positive contours drawn solid, negative broken, and zero contour omitted.

using the refined transformations listed in Table 1. In Fig. 1 the dotted line indicates the boundary of the αCHT molecule as obtained from the interpretation of the 2 Å resolution electron-density map (Matthews et al., 1967; Sigler et al., 1968). The broken lines show the boundaries of the 'full molecule' and 'inner ellipsoid' as used in the various refinements. The numbered crosses indicate the position and residue number of those α-carbon atoms which lie within 2 Å of this section. The diad symbols on the line $y = 0$ indicate the positions of the local twofold axes of symmetry of αCHT. Fig. 2(a) illustrates the superposition of the section $x = 0.333$ of the 5.5 Å resolution electron densities of the molecules α_1 and α_2 of αCHT. It can be seen that the agreement is best within the molecular boundary and worst near $y = \frac{1}{2}$ and $y = -\frac{1}{2}$, i.e. away from the area of influence of the local diad axis relating α_1 and α_2. The section intersects the α-helical region of αCHT at about $y = 0.14$, $z = 0.94$ and it can be seen that both maps have high positive density at this point. The difference density $(\varrho_{\alpha_1} - \varrho_{\alpha_2})$ is shown in Fig. 2(b). This is a section with generally low undulating $\Delta\varrho$ within the molecular boundary, and higher features near $y = \pm \frac{1}{2}$. The numerically largest difference observed within the molecular boundary is -0.49 e.Å^{-3}, and is located on the section in Fig. 3(b). This large a difference only occurs at a few other points within the molecule.

The direct comparison between the electron density ϱ_γ of γCHT and the averaged density ϱ_α of αCHT is illustrated by Fig. 3(a) in which the respective electron densities are superimposed. Once again the agreement is clearly best within the molecular boundary, and the highest density of each section corresponds to the α-helical region of the molecule. The section of difference density $(\varrho_\gamma - \varrho_\alpha)$ corresponding to Fig. 3(a) is shown in 3(b). The negative region at about $y = 0.05$, $z = 0.90$ has a density of -0.45 e.Å^{-3}, the strongest feature within the molecule. In several other places the density approaches a similar value, and in addition larger differences are observed in the $(\varrho_{\alpha_1} - \varrho_{\alpha_2})$ map. Therefore we do not regard this feature as significant evidence of a structural difference at this point between αCHT and γCHT.

One region in which the structures of αCHT and γCHT might be expected to differ is in the neighborhood of Tyr 146. This residue is known to be accessible to iodination in crystalline γCHT, and takes up a slightly different conformation in iodinated γCHT to that observed in native αCHT (Sigler, 1967; Matthews et al., 1968). It is not known whether this conformation difference is associated with an intrinsic difference between αCHT and γCHT, or simply is a consequence of the iodination reaction in γCHT. The section shown in Fig. 3(b) passes close to Tyr 146 and there are features of 0.26 and -0.34 e.Å^{-3} near to this residue. These peaks are among the highest within the molecular boundary, and could correspond to a slight conformational difference between αCHT and γCHT in this region. On the other hand this region of the molecule also

contains two of the heavy atom sites used in the calculation of the electron density map of γCHT (i.e. the di-iodination sites of Tyr 146). Therefore the γCHT map might be expected to contain spurious features at these sites, so that an unusually large difference between ϱ_α and ϱ_γ in this region need not indicate any real structural difference between the two molecules. Another region where αCHT and γCHT may have a difference in conformation is in the vicinity of Ala 149, i.e. at the N-terminus of the C chain (Kraut, Wright, Kellerman & Freer, 1967; Wright, Kraut & Wilcox, 1968). In this case the $(\varrho_\gamma - \varrho_\alpha)$ map has a strong negative region of -0.44 e.Å^{-3} in the vicinity of Thr 151 and Pro 152, and an equally strong positive peak just outside the molecular boundary. However this is a region where the molecules in the crystals of both αCHT and γCHT come into close contact with their respective neighbours, and it is possible that the observed features, which straddle the molecular boundary, could be due simply to 'edge effects'.

The above analysis shows that the α- and γCHT molecules have very similar overall conformations; at the resolution of this study there is no point at which the electron densities are significantly different. However since differences are most likely to occur on the surface of the molecule where the amino acid side chains have more freedom of movement, and since this is the most difficult region of the molecule to define precisely, such differences will be practically impossible to detect at this resolution.

Recent protein crystal structure determinations have demonstrated that there exist families of related proteins with closely similar conformations, e.g. the globins and the pancreatic proteolytic enzymes. While gross differences in conformation between such related proteins could be detected by a comparison at 5.5 Å resolution, this study indicates that detailed differences could only be detected at considerably higher resolution.

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Crystal and Molecular Structure of Cadmium Trihydrogenhexaoxoiodate(VII) Trihydrate

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Crystals of $Cd[H_3IO_6] \cdot 3H_2O$ are monoclinic, space group $P2_1/c$. The unit-cell constants are $a = 5.957$ (7), $b = 14.380$ (11), $c = 9.715$ (7) Å, $\beta = 120.8$ (2)°. The structure has been determined from three-dimensional data. The anion is single and nearly octahedral; the iodine–oxygen bonds can be subdivided into two groups: $I-O^*_{av} = 1.95$ Å and $I-O_{av} = 1.86$ Å. The cadmium atoms bridge two anions, forming bonds $Cd-O(2) = 2.25$ and $Cd-O(5') = 2.23$ Å; other short bonds are $Cd-OH_2(2) = 2.27$ and $Cd-OH_2(3) = 2.41$ Å. Other interactions between cations and a water molecule or anion range from 2.49 to 2.79 Å. Besides the bridging cadmium cations, the anions are bound to one another or to water molecules by several hydrogen bonds, some of which are fairly strong (2.62, 2.65, 2.70, 2.72 Å).

Introduction

Iodine(VII) can form several periodic acids, and the corresponding salts, namely hexaoxoiodic(VII), H_5IO_6, pentaoxoiodic(VII), H_3IO_5, tetraoxoiodic(VII), HIO_4, hendecaoxodiiodic(VII), $H_8I_2O_{11}$, decaoxodiiodic(VII), $H_6I_2O_{10}$, enneaoxodiiodic(VII), $H_4I_2O_9$, and tetra-caidecaoxotriiodic(VII), $H_7I_3O_{14}$. Two iso structural hydrates of the cadmium and calcium periodates have been prepared in this laboratory (Biagini Cingi, Emiliani & Guastini, 1967) to which, according to Siebert (1967), the formula $Ca_2H_2I_2O_{10} \cdot 8H_2O$ or $Cd_2H_2I_2O_{10} \cdot 8H_2O$ should have been assigned. This assignment was made by Siebert on the basis of the infrared spectra. However, other formulae could be assigned to these compounds, derived from some of the acids mentioned above. In order to assess which is the proper formula, we have undertaken the study of the crystal structure of the cadmium compound.

Experimental

Preparation

Crystals of the cadmium compound were prepared from aqueous solutions obtained by dissolving cadmium carbonate in periodic acid.

Crystal data

Compound: cadmium trihydrogenhexaoxoiodate-(VII) trihydrate, $Cd[H_3IO_6] \cdot 3H_2O$, F.W. 392·39. Crystal class: monoclinic, prismatic. Unit cell (Cu $K\alpha$, $\lambda = 1.5418$ Å), from rotation and Weissenberg photographs around [100] and [010]: $a = 5.957$ (7), $b = 14.380$ (11), $c = 9.715$ (7) Å, $\beta = 120.8$ (2)°, $V = 714.8$ Å^3, $Z = 4$, $D_x = 3.65$, $D_m = 3.61$ g.cm^{-3}. Space group $P2_1/c$ (No. 14–C^5_{2h}) from systematic absences. μ(Cu $K\alpha$) = 602·4 cm^{-1}.

Intensity measurement

Integrated reflexions $0kl$, $1kl$ ... $5kl$ and $h0l$, $h1l$ ... $h11l$ were recorded by an integrating camera; the intensities of 1447 observed independent reflexions out of the 1673 possible were measured by a micro-densitometer. Absorption corrections were applied as for cylindrical specimens ($\mu\bar{R}_{[010]} = 9.04$, $\mu\bar{R}_{[100]} = 10.84$). The atomic form factors of Berghuis, Haanappel, Potters, Loopstra, MacGillavry & Veenendaal (1955) were used for O, and those of Thomas & Umeda (1957) for Cd^{2+} and I.

The calculations were performed on the computer Olivetti Elea 6001/S of Centro di Calcolo Elettronico of the University of Parma.