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Calcium-Binding to α-Amino Acids: Crystal Structure of Calcium L-Glutamate Trihydrate

BY HOWARD EINSPAHR AND CHARLES E. BUGG

Institute of Dental Research and Department of Biochemistry, University of Alabama in Birmingham, University of Alabama Medical Center, University Station, Birmingham, Alabama 35294, *U.S.A.*

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Crystals of calcium L-glutamate trihydrate ($CaC_5H_7NO_4.3H_2O$) are trigonal, space group $P3_121$, with $a=8{\cdot}863$ (1), $c=20{\cdot}863$ (4) Å and six formula weights per unit cell. A trial structure was obtained by the heavy-atom method and refined by full-matrix least-squares calculations to a final R index of 0·021 for 954 absorption-corrected diffractometer data. Two of the water molecules in this structure exhibit disorder in hydrogen positions. The calcium ion is coordinated to three symmetry-related glutamate ions and to two crystallographically independent water molecules. Each glutamate ion, in turn, chelates three symmetry-related calcium ions, one through the two oxygen atoms of its α-carboxyl group, another through the two oxygen atoms of its γ-carboxyl group, and the third through its α-amino nitrogen atom in concert with one of its α-carboxyl oxygen atoms. The eight atoms of the calcium-coordination polyhedron assume a distorted square-antiprismatic arrangement; the Ca–N distance is 2·556 (2) Å, and the Ca–O distances range from 2·383 to 2·595 Å. Similar interactions probably account for the chelation of calcium ions by glutamate and aspartate residues of proteins and by free α-amino acids in aqueous solution.

Introduction

Protein–calcium complexes have been strongly implicated in a number of biological processes, among which are calcification of protein matrices, muscle contraction, nerve conduction, and the maintenance of protein conformation. Evidence suggests that glutamate and aspartate residues are the principal mediators of certain calcium–protein interactions, and these amino acids have been found at the calcium-binding sites of several crystalline proteins (Kretsinger & Nockolds, 1973; Matthews, Colman, Jansonius, Titani, Walsh & Neurath, 1972; Edelman, Cunningham, Reeke, Becker, Waxdal & Wang, 1972; Stroud, Kay & Dickerson, 1971; MacLennan & Wong, 1971; Keith, Padden & Giannoni, 1969).

Free amino acids also bind calcium ions, and it has been suggested that complexes thus formed play a part in certain biological processes (Ting-Beall & Wells, 1971; Mørch, Punwani & Greve, 1971). In aqueous solution, calcium complexes of α-amino acids exhibit larger association constants than calcium salts of simple carboxylic acids, which suggests that the complexes are not due solely to calcium interactions with the carboxyl groups of the amino acids. A model for calcium–amino acid complexes has been proposed (Davies, 1938), but no structural studies of these complexes have been reported.

To obtain information about structural factors controlling calcium interactions with proteins and with free amino acids, we are currently investigating the crystal structures of calcium complexes of amino acids and peptides. In this paper we describe the crystal structure of a hydrated calcium salt of L-glutamic acid.

Experimental

Colorless, transparent crystals of calcium glutamate trihydrate were obtained by slowly cooling a hot aqueous solution of an approximately equimolar mixture of calcium diglutamate and calcium bromide ($pH \simeq 10$). Weissenberg, oscillation and precession photographs indicate a trigonal lattice, Laue group $\bar{3}m1$. Systematic absences ($000l$, $l=3n$) indicate space groups $P3_121$ or $P3_221$.

The crystal selected for data collection approximated a truncated tetrahedron with a height of 0·2 mm and a base with sides of 0·3 mm. It was mounted on a Picker FACS-1 diffractometer with its c axis (normal to the base and the truncation face) slightly inclined to the φ axis of the diffractometer. Approximate cell parameters were calculated by a least-squares analysis of the angular settings for 12 high-angle Cu $K\alpha_1$ ($\lambda=1{\cdot}54051$ Å) reflections.

Intensity data were collected with the diffractometer by use of a scintillation counter, nickel-filtered Cu $K\alpha$ radiation and a θ–2θ scanning technique. Data were collected to $2\theta<128°$ at a scan rate of $1°\ \text{min}^{-1}$ and with a 20 s count time for each background. The intensities of three reflections, which were monitored periodically during the data-collection procedure, did not vary significantly throughout the experiment. All 954 intensity values thus obtained were assigned variances, $\sigma^2(I)$, according to counting statistics plus an additional term, $(0{\cdot}03S)^2$, S being the scan count, and were treated as observed in all subsequent calculations regardless of $I/\sigma(I)$ ratio. The intensities and variances were corrected for Lorentz and polarization effects; program *ORABS* (Wehe, Busing & Levy, 1962) was used to make absorption corrections. Transmission factors

range from 0·30 to 0·50. Structure factors and variances were scaled by means of a Wilson (1942) plot.

Immediately after data collection, accurate cell parameters were obtained by a least-squares analysis of 2θ values of 22 high-angle Cu $K\alpha_1$ reflections ($\lambda=1{\cdot}54051$ Å) measured with the diffractometer at room temperature (23 ± 2°C). These parameters and other crystal data are listed in Table 1. The observed density was measured by flotation in a mixture of carbon tetrachloride, benzene, and ethylene dibromide. The standard deviation in the observed density is that for the average value of 9 independent measurements.

Table 1. *Crystal data*

Calcium L-glutamate trihydrate $CaC_5H_7NO_4.3H_2O$

Trigonal, $P3_121$	F.W. 239·2
$a=8{\cdot}863$ (1) Å	$\varrho_o=1{\cdot}679$ (1) g cm^{-3}
$c=20{\cdot}863$ (4)	$\varrho_c=1{\cdot}679$
$V=1419{\cdot}4$ Å^3	$Z=6$

μ(Cu $K\alpha$) = 57·8 cm^{-1}

Structure determination and refinement

Coordinates for the calcium ion were determined from a sharpened, three-dimensional Patterson map. Coordinates for several of the oxygen atoms in the calcium coordination-shell were then obtained from a three-dimensional sum-function superposition of sharpened Patterson maps translated to the calcium positions. Subsequent difference Fourier maps produced a complete heavy-atom trial structure in space group $P3_221$. Seven cycles of full-matrix least-squares refinement with isotropic temperature factors reduced the R index, $\sum||F_o|-|F_c||/\sum|F_o|$, to 0·082. After three cycles of refinement with anisotropic temperature factors ($R=0{\cdot}077$), examination of the glutamate moiety indicated that the incorrect D-isomer was represented. The correct enantiomer, which was obtained by inverting the asymmetric unit through the origin and converting to space group $P3_121$, produced an R index of 0·057. A difference electron-density map revealed trial positions for all except two hydrogen atoms, one each on water oxygen atoms O(6) and O(7), for which there were several possible peaks in the difference Fourier map. The R index was reduced to 0·024 by five cycles of refinement, including all positional parameters, anisotropic temperature parameters for the non-hydrogen atoms, isotropic temperature parameters for the eleven hydrogen atoms, and an isotropic extinction parameter g [after Zachariasen (1967) as formulated by Coppens & Hamilton (1970)]. The goodness-of-fit $\{[\sum w\Delta^2/(m-s)]^{1/2}$, where m is the number of observations and s is the number of parameters$\}$ was 1·71. At this stage, a difference Fourier map revealed interpretable positions for the two remaining hydrogen atoms. A portion of this map in the neighborhood of atoms O(6) and O(7) is shown in Fig. 1. We interpreted this map as evidence that hydrogen bonds between these water molecules are disordered in such a way as to produce two positions, H(11) and H(12), for the second

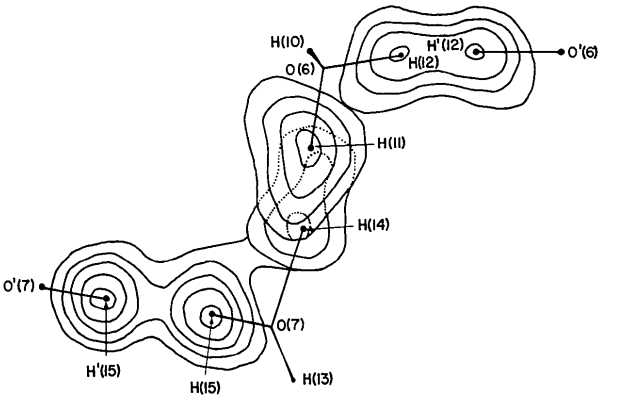


Fig. 1. A composite difference electron-density map in the region of O(6) and O(7). Contours are drawn at intervals of 0·05 e Å^{-3} beginning at 0·05 e Å^{-3}. $\sigma(\varrho_0)$ is approximately 0·04 e Å^{-3}. O(6) and O(7) are related to O'(6) and O'(7) by twofold axes parallel to **b** at $z=\frac{2}{3}$ and parallel to **a** at $z=\frac{5}{6}$, respectively.

Table 2. *Positional and thermal parameters*

(*a*) Positional and thermal parameters for nonhydrogen atoms. Parameters for Ca are multiplied by 10^5; parameters for other atoms are multiplied by 10^4 with the exception of z, β_{33}, β_{13}, and β_{23}, which are multiplied by 10^5. Anisotropic temperature factors are expressed as: exp $\{-(h^2\beta_{11}+k^2\beta_{22}+l^2\beta_{33}+2hk\beta_{12}+2hl\beta_{13}+2kl\beta_{23})\}$. E.s.d.'s are in parentheses.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Ca	13917 (6)	52058 (6)	79031 (2)	533 (9)	587 (9)	120 (1)	252 (8)	5 (2)	1 (2)
O(1)	4138 (2)	5218 (2)	79988 (7)	64 (3)	85 (3)	123 (4)	36 (3)	34 (9)	39 (8)
O(2)	6811 (3)	6016 (3)	76846 (9)	67 (3)	114 (4)	214 (5)	55 (3)	69 (11)	164 (11)
O(3)	8466 (3)	12593 (3)	75163 (9)	95 (4)	85 (4)	206 (4)	51 (3)	−55 (11)	−87 (11)
O(4)	10830 (3)	12400 (3)	74706 (11)	64 (3)	74 (4)	315 (6)	25 (3)	−56 (11)	−104 (12)
O(5)	3271 (3)	7765 (3)	84971 (10)	101 (4)	102 (4)	206 (5)	62 (3)	−91 (12)	−147 (11)
O(6)	139 (3)	6792 (3)	73412 (8)	73 (4)	107 (4)	157 (4)	45 (3)	27 (10)	27 (10)
O(7)	12154 (3)	10306 (3)	76806 (10)	112 (4)	121 (4)	217 (5)	67 (3)	−61 (11)	−16 (12)
N(1)	3399 (3)	6513 (3)	69340 (10)	73 (4)	74 (4)	136 (5)	31 (3)	−72 (11)	3 (12)
C(1)	5384 (3)	6005 (3)	76182 (11)	60 (5)	53 (4)	136 (5)	26 (4)	−23 (13)	−30 (12)
C(2)	5247 (3)	7055 (3)	70594 (11)	67 (4)	72 (4)	116 (5)	30 (4)	9 (12)	25 (12)
C(3)	6237 (4)	9013 (3)	72116 (14)	68 (5)	71 (5)	184 (6)	33 (4)	−1 (15)	60 (14)
C(4)	8153 (4)	9752 (4)	73578 (14)	78 (5)	73 (5)	164 (6)	29 (4)	−38 (15)	−22 (15)
C(5)	9191 (4)	11701 (4)	74504 (11)	70 (5)	70 (5)	118 (5)	32 (4)	−45 (12)	−13 (13)

Table 2 (*cont.*)

(*b*) Positional and thermal parameters for hydrogen atoms. Assigned population coefficients are listed under the column heading *p*. Positional parameters are multiplied by 10^3. E.s.d.'s are in parentheses. Parameters not refined by least-squares calculations are indicated by (−). On the basis of grid spacings used in the difference map, e.s.d.'s of the *x*, *y*, and *z* parameters of unrefined atoms may be assigned values of 0·01, 0·01, and 0·005, respectively.

	p	*x*	*y*	*z*	*B* (Å²)
H(1)	1·0	335 (5)	743 (6)	673 (2)	5·3 (10)
H(2)	1·0	292 (5)	576 (5)	669 (2)	3·2 (8)
H(3)	1·0	582 (4)	687 (4)	668 (1)	2·0 (6)
H(4)	1·0	577 (4)	930 (5)	753 (2)	3·5 (8)
H(5)	1·0	603 (4)	957 (4)	682 (1)	3·2 (7)
H(6)	1·0	871 (4)	942 (4)	699 (2)	3·4 (7)
H(7)	1·0	832 (5)	920 (5)	774 (2)	3·9 (7)
H(8)	1·0	418 (5)	758 (5)	875 (2)	5·3 (10)
H(9)	1·0	292 (5)	822 (5)	867 (1)	2·9 (8)
H(10)	1·0	−78 (5)	657 (4)	743 (1)	2·6 (7)
H(11)	0·5	72 (−)	785 (−)	742 (−)	5·0 (−)
H(12)	0·5	16 (−)	675 (−)	689 (−)	3·5 (−)
H(13)	1·0	1192 (5)	1099 (6)	757 (2)	4·9 (10)
H(14)	0·5	1143 (−)	903 (−)	749 (−)	5·0 (−)
H(15)	0·5	1190 (−)	1016 (−)	803 (−)	3·5 (−)

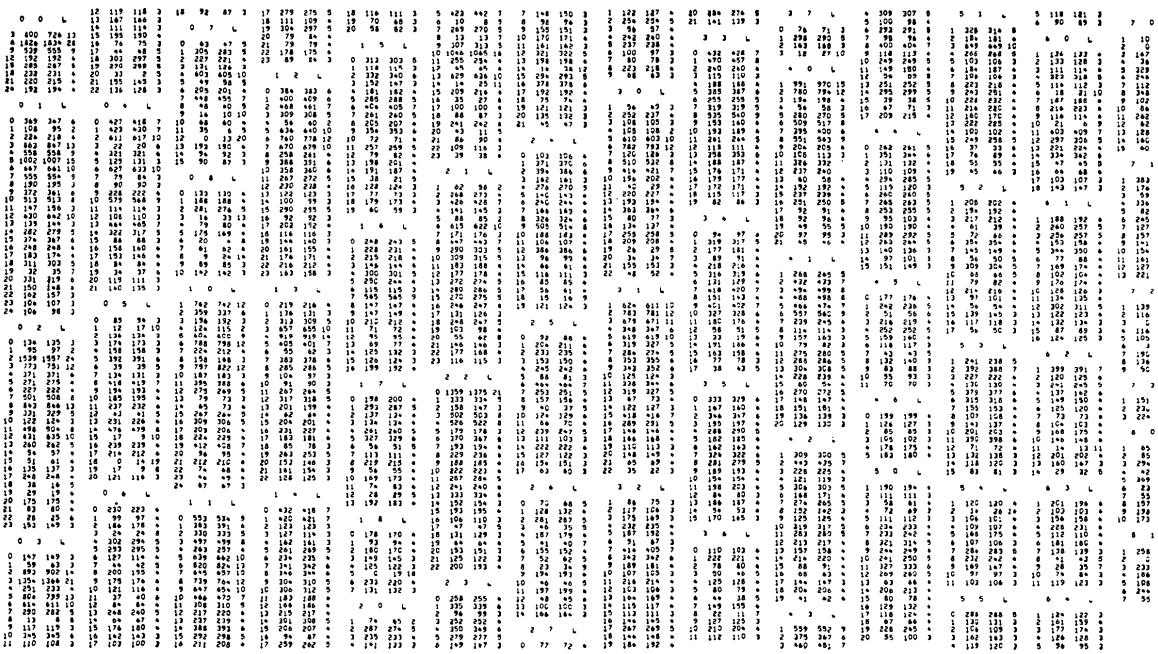
hydrogen atom bonded to O(6) and two positions, H(14) and H(15), for the second hydrogen atom bonded to O(7). We assigned to each of these positions a hydrogen atom with a population parameter of 0·5. Isotropic thermal parameters for partial hydrogen atoms were assigned according to peak breadths so as to be within the range of thermal parameters of the refined hydrogen atoms. After two more cycles of least-squares refinement, wherein partial hydrogen atoms were included in structure-factor calculations but not refined, a second difference Fourier map was calculated from which contributions of partial hydrogen atoms were omitted. This map was used to improve estimates of coordinates for the partial hydrogen atoms. The refinement in space group $P3_121$ was then completed by two cycles of least-squares calculations, during which all parameters, except those for the partial hydrogen atoms, were allowed to vary. The final *R* index is 0·021 and the goodness-of-fit is 1·37. In the final cycle, no parameter shifted more than $\frac{1}{6}$ of its standard deviation. Apart from several ripples of about 0·20 e Å^{-3} in the immediate vicinity of the calcium ion, no peaks or troughs with magnitudes exceeding 0·17 e Å^{-3} were present in a final difference Fourier map.

To confirm the expected absolute configuration of the glutamate ion (L-isomer) the final model was transformed and then refined in space group $P3_221$. During this refinement, no hydrogen-atom parameters were allowed to vary. After two cycles of least-squares calculations, the refinement converged at $R=0{\cdot}061$ and goodness-of-fit $=4{\cdot}24$. By use of the *R*-value ratio test (Hamilton, 1965), a comparison of the refinements of the two enantiomers indicates that the L-glutamate absolute configuration is correct, with a probable error of less than 0·5%.

A modified version of the full-matrix least-squares program *ORFLS* (Busing, Martin & Levy, 1962) was

Table 3. *Structure-factor table*

Listed are $|F_o|$, $|F_c|$, and $\sigma(F_o)$, all $\times 10$. $\sigma(F_o)$ values were obtained using the formula $\sigma(F_o)=[|F_o^2|+\sigma(F_o^2)]^{1/2}-|F_o|$. The final value of *g*, the isotropic secondary-extinction parameter specified above, is 0·004 (2).



used for all least-squares refinements. The quantity minimized was $\sum w(F_o^2 - F_c^2/k^2)^2$, where k is the scale factor and weights w are equal to $1/\sigma^2(F_o^2)$. Scattering factors for the nonhydrogen atoms (Ca^{2+}, O, N, C) were taken from *International Tables for X-ray Crystallography* (1962); anomalous dispersion correction factors for these atoms were taken from Cromer & Liberman (1970). The atomic scattering factors of Stewart, Davidson & Simpson (1965) were used for hydrogen atoms.

Table 2 lists the final parameters and their estimated standard deviations. Observed and calculated structure factor magnitudes are given in Table 3.

Results

Crystal packing

A complex set of calcium interactions and hydrogen bonds stabilizes the crystal structure. Fig. 2 shows the crystal packing and Table 4 lists hydrogen-bond distances and angles. All hydrogen atoms of water molecules, including those that are disordered, participate in

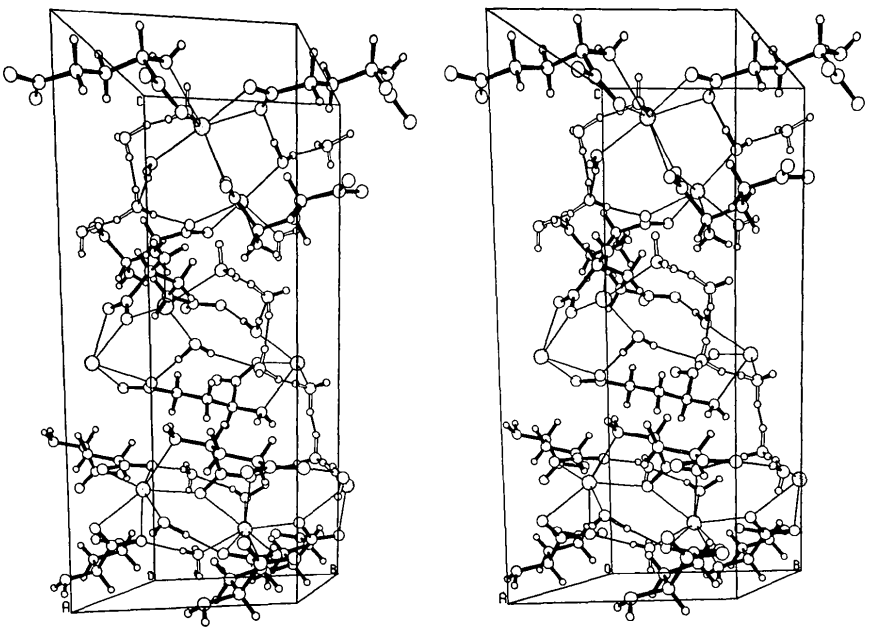


Fig. 2. A stereo-representation of the structure. Calcium ions are shown as dotted circles. The covalent bonds to disordered H atoms on O(6) and O(7) are undarkened. Hydrogen bonds joining O(6) and O(7) atoms are depicted by lines joining disordered H atoms. Hydrogen bonds joining N(1) and O(3) have been omitted. [This drawing, as well as those in Figs. 3–5, was prepared using program *ORTEP* (Johnson, 1965).]

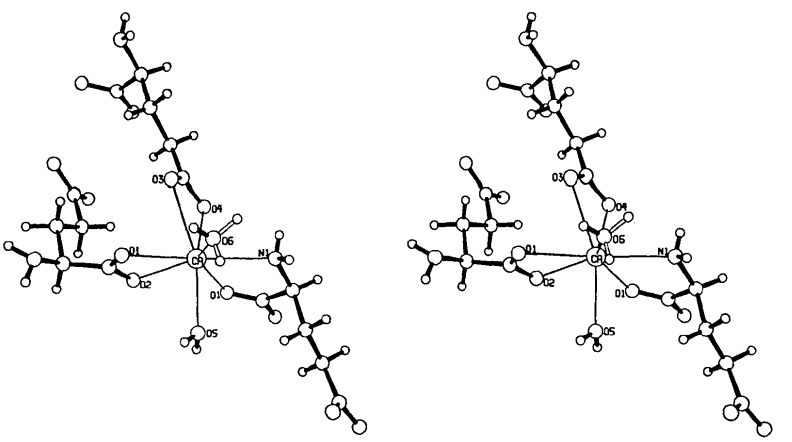


Fig. 3. A stereographic representation of the environment of the calcium ion. O(5) and O(6) are water oxygens. The bonds to disordered H atoms on O(6) are undarkened.

hydrogen bonding. One amino hydrogen atom, H(1), does not participate in hydrogen bonding, while the other forms a relatively weak hydrogen bond to atom O(3).

Fig. 3 shows the environment of the calcium ion, which is bound to three symmetry-related glutamate ions and to two crystallographically independent water molecules. One glutamate ion chelates the calcium ion through O(3) and O(4) – the oxygen atoms of the γ-carboxyl group; a second glutamate ion chelates the calcium ion through O(1) and O(2) – the oxygen atoms of the α-carboxyl group; and the third glutamate ion chelates the calcium ion through both O(1) and N(1), the nitrogen atom of the amino group. The calcium coordination shell is then completed by O(5) and O(6), the water oxygen atoms.

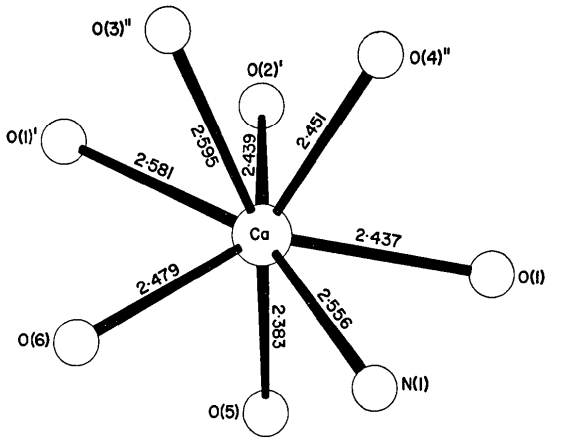


Fig. 4. The calcium ion coordination shell. Atoms O(1)′ and O(2)′ are generated from O(1) and O(2), respectively, by the symmetry operation $x-y, 1-y, \frac{5}{3}-z$. Similarly, O(3)″ and O(4)″ are generated from parent atoms by the operation $x-1, y-1, z$. The estimated standard deviation of a Ca–O or Ca–N distance (Å) is 0·002 Å.

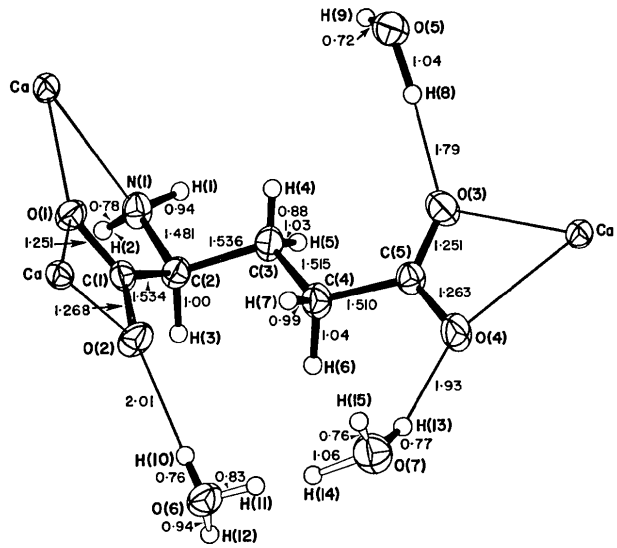


Fig. 5. The environment of the glutamate moiety. The thermal ellipsoids are drawn at the 50% probability level. H atoms are drawn as spheres of radius 0·09 Å. Distances are in Å. E.s.d.'s for lengths of bonds between nonhydrogen atoms are 0·004 Å; those for distances involving an H atom are 0·04 Å. The covalent bonds to disordered H atoms on O(6) and O(7) are undarkened. For these, e.s.d.'s are about 0·08 Å.

Table 4. *Hydrogen bond distances and angles*

D–H···*A*	*D*···*A*	H···*A*	*D*–H···*A*	H–*D*···*A*
O(5)–H(8)····O(3)*a*	2·830 Å	1·79 Å	175°	3°
O(5)–H(9)····O(7)*b*	2·777	2·08	167	9
O(6)–H(10)···O(2)*c*	2·767	2·01	179	1
O(7)–H(13)···O(4)	2·683	1·93	164	12
O(6)–H(12)···O(6)*d*	2·822	1·89	171	6
O(7)–H(15)···O(7)*a*	2·764	2·03	163	12
O(6)–H(11)···O(7)*c*	2·798	1·97	175	4
O(7)–H(14)···O(6)*e*	2·798	1·75	168	7
N(1)–H(2)····O(3)*f*	3·023	2·26	165	8

Code for symmetry-related atoms

(a)	$1+x-y$	$2-y$	$\frac{5}{3}-z$
(b)	$x-y$	$2-y$	$\frac{5}{3}-z$
(*c*)	$x-1$	y	z
(d)	$\bar{x}$	$y-x$	$\frac{4}{3}-z$
(e)	$1+x$	y	z
(f)	$1-x$	$y-x$	$\frac{4}{3}-z$

The geometry of the calcium-ion coordination polyhedron is shown in more detail in Fig. 4. The eight atoms that are bound to the calcium assume a distorted square-antiprismatic arrangement, with calcium–oxygen distances ranging from 2·383 to 2·595 Å, and a Ca–N(1) distance of 2·556 (2) Å; no other Ca–O or Ca–N distances are shorter than 4 Å. No hydrogen bonds exist between atoms within the calcium-ion coordination polyhedron and only those contacts within the polyhedron that are between atoms of the same glutamate moiety are shorter than sums of van der Waals radii. Each polyhedron is linked to its neighbors by five hydrogen bonds, with O(5) and O(6) serving as donors and O(2), O(3), and O(6) as acceptors. The shortest distance between calcium ions is 4·792 (2) Å. Each glutamate ion serves to link three calcium ions (Fig. 5). Atom O(1) is coordinated to two calcium ions, thus forming a vertex common to two adjacent coordination polyhedra; this linkage produces chains of polyhedra that lie along the 2_1 axes.

Disordered water molecules

As described in Table 4, the hydrogen bonds formed by disordered hydrogen atoms are O(6)–H(12)··· O(6)*d*, O(7)–H(15)···O(7)*a*, O(6)–H(11)···O(7)*c*, and O(7)–H(14)···O(6)*e*. In the first two, the donor and acceptor atoms are related by twofold crystallographic axes, and the hydrogen-atom positions, H(12) and H(15), correspond to one of the two symmetry-equivalent positions across these twofold axes (see Fig. 1). Atoms H(11) and H(14) correspond to the two positions found for the hydrogen atom that forms the hydrogen bond between O(6) and O(7). It might be

suggested that the space group is actually $P3_1$, and that the apparent disorder is simply an artifact of the higher symmetry imposed by space group $P3_121$. While this hypothesis could account for the disordered hydrogen atoms between water molecules that are related by twofold crystallographic axes, it cannot explain the fact that two symmetry-independent positions are observed for the hydrogen atom linking O(6) and O(7). Consequently, it appears that the disorder is real and that the proper space group is $P3_121$.

The disordered hydrogen bonds link water molecules to form a continuous helix that extends along the *c* axis and is centered on the 3_1 axis that passes through the origin (Fig. 2). Two simple models can account for the disorder. One interpretation, which we consider the less likely, is that the water molecules are extensively disordered within a given chain. This model would require numerous disruptions of the chain at regions where the direction of hydrogen bonding is reversed. The second interpretation, which we believe is more feasible, holds that hydrogen bonds are ordered over extended regions of the individual chains, but that the direction of hydrogen bonding may vary from chain to chain. This model should lead to a more stable configuration, since it permits continuous hydrogen bonding between water molecules, without the disruptions required by disorder within a chain.

Geometry of the glutamate ion

The conformation of the glutamate moiety, covalent bond lengths, and thermal ellipsoids for nonhydrogen atoms are depicted in Fig. 5. Bond angles are listed in Table 5. Torsion angles for the glutamate ion are compiled in Table 6, where values for the present structure are compared with those from the crystal structures of copper glutamate dihydrate (Gramaccioli & Marsh, 1966) and zinc glutamate dihydrate (Gramaccioli, 1966).

Table 5. *Valence angles*

E.s.d.'s of angles formed exclusively by nonhydrogen atoms are 0·2°; those of angles involving H atoms are 3–4°. For angles involving disordered atoms, e.s.d.'s are 5–6°.

O(1)–C(1)–O(2)	122·3°	C(3)—C(4)–C(5)	115·5°
O(1)–C(1)–C(2)	120·3	C(3)—C(4)–H(6)	108
O(2)–C(1)–C(2)	117·4	C(3)—C(4)–H(7)	111
C(1)–C(2)–C(3)	110·8	C(5)—C(4)–H(6)	108
C(1)–C(2)–N(1)	110·3	C(5)—C(4)–H(7)	108
N(1)–C(2)–C(3)	108·5	H(6)—C(4)–H(7)	105
C(1)–C(2)–H(3)	108	C(4)—C(5)–O(3)	121·7
N(1)–C(2)–H(3)	112	C(4)—C(5)–O(4)	117·3
C(3)–C(2)–H(3)	108	O(3)—C(5)–O(4)	121·0
C(2)–N(1)–H(1)	109	H(8)—O(5)–H(9)	114
C(2)–N(1)–H(2)	116	H(10)–O(6)–H(11)	103
H(1)–N(1)–H(2)	102	H(10)–O(6)–H(12)	106
C(2)–C(3)–C(4)	114·5	H(11)–O(6)–H(12)	103
C(2)–C(3)–H(4)	113	H(13)–O(7)–H(14)	118
C(2)–C(3)–H(5)	104	H(13)–O(7)–H(15)	105
C(4)–C(3)–H(4)	108	H(14)–O(7)–H(15)	102
C(4)–C(3)–H(5)	113		
H(4)–C(3)–H(5)	104		

Table 6. *Torsion angles for the glutamate moieties in calcium glutamate trihydrate, copper glutamate dihydrate* (*Gramaccioli & Marsh*, 1966) *and zinc glutamate dihydrate* (*Gramaccioli*, 1966)

E.s.d.'s are 0·3° for the Ca structure and 0·5–0·6° for the Cu and Zn structures. Signs of the angles reflect the convention of Klyne & Prelog (1960). The designation of atoms O(3) and O(4) in the Ca structure is the reverse of that for the Cu and Zn structures.

	Ca	Cu	Zn
O(1)–C(1)–C(2)–N(1)	−18·2°	−3·6°	−0·4°
O(2)–C(1)–C(2)–N(1)	163·7	178·5	180·4
O(1)–C(1)–C(2)–C(3)	101·8	119·4	124·4
O(2)–C(1)–C(2)–C(3)	−76·2	−58·6	−54·8
C(1)–C(2)–C(3)–C(4)	58·2	−54·5	−56·8
N(1)–C(2)–C(3)–C(4)	179·4	67·8	67·1
C(2)–C(3)–C(4)–C(5)	174·6	182·9	183·4
C(3)–C(4)–C(5)–O(3)	12·3	−172·3	−170·2
C(3)–C(4)–C(5)–O(4)	−169·1	6·7	7·8

The glutamate ion in the crystal structure of calcium glutamate trihydrate consists of two approximately planar segments: the α-amino acid portion – atoms C(1), O(1), O(2), C(2), and N(1); and the remainder of the anion – atom C(3), C(4), C(5), O(3), and O(4) – as well as atoms C(2) and N(1). Within each portion, the atoms are coplanar to ±0·15 Å, and the dihedral angle between the two planes thus defined is about 69°. The conformation about the C(2)–C(3) bond is such that C(3)–C(4) is *gauche* to C(1)–C(2) and *trans* to N(1)–C(2). This conformation of the anion contrasts with that found in the crystal structures of copper glutamate dihydrate and zinc glutamate dihydrate, where C(3)–C(4) is *gauche* to both C(1)–C(2) and N–C(2). The conformation of the carbon backbone about C(3)–C(4) is *trans* in all three examples. Overall, bond distances and angles in the three structures are in good agreement. The only significant differences arise within the α-carboxyl group, which chelates the calcium ion but not the copper and zinc ions. Reviews of the structural properties of glutamate moieties in other crystal structures have been presented by Hirokawa (1955) and Ashida, Sasada, & Kakudo (1967).

Discussion

Calcium ions are chelated at three different sites on the glutamate anion (Fig. 5). Two of these sites – those involving the paris of oxygen atoms from the α- and γ-carboxyl groups – display closely related patterns of chelation; more detailed geometry of these sites is depicted in Fig. 6. In both cases, the calcium ion is coordinated to the two oxygen atoms of the carboxyl group, resulting in a four-membered nonplanar ring system (the calcium ion is displaced 0·54 Å and 0·50 Å from the planes of the α- and γ-carboxyl groups, respectively). Corresponding distances and angles within these rings are in remarkable agreement for the two sites. A noteworthy feature of the calcium–

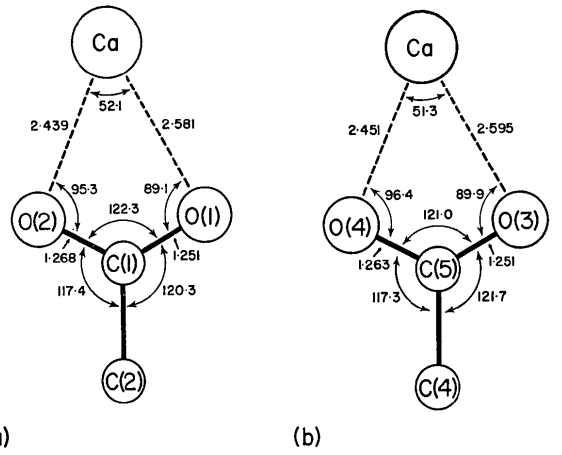


Fig. 6. Comparison of the calcium chelation geometries of (*a*) the α-carboxyl and (*b*) the γ-carboxyl groups of the glutamate moiety. Distances (in Å) have e.s.d.'s of 0·002–0·004 Å. Angles (°) have e.s.d.'s of 0·2°.

carboxyl interactions is the disparity in the lengths of the Ca–O distances. In both carboxyl groups, one Ca–O distance is about 2·45 Å, and the other is about 2·60 Å; the shorter distances are those to the carboxyl oxygen atoms that display the longer C–O bond distances. Calcium–carboxyl interactions similar to those in Fig. 6 are probably of major importance in the binding of calcium ions to the carboxyl groups of proteins.

The third calcium chelation site utilizes the amino-nitrogen atom in concert with one of the oxygen atoms from the α-carboxyl group. This mode of chelation is probably of general importance in the binding of calcium ions to free α-amino acids. A number of α-amino acids, including glycine, have been shown to bind calcium ions in aqueous solution. In general, the dissociation constants for α-amino-acid–calcium-ion complexes are approximately an order of magnitude smaller than those for calcium salts of simple carboxylic acids. To explain this phenomenon, Davies (1938) presented a model for calcium complexes of α-amino acids. Our results concur with Davies's prediction that α-amino acids would use the nitrogen atom of the α-amino group and one oxygen atom from the carboxyl group to chelate calcium ions. This mode of calcium chelation is similar to that observed in calcium salts of α-hydroxycarboxylic acids, where the oxygen atom of the α-hydroxyl group joins with an oxygen atom of the carboxyl group to bind calcium ions (Cook & Bugg, 1973; and references therein).

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