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Conformation and Structure of α-L-Glutamyl-L-glutamic Acid

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Abstract

The crystal structure of the acidic dipeptide α-L-glutamyl-L-glutamic acid, α-L-Glu-L-Glu, $C_{10}H_{16}N_2O_7$, has been determined from three-dimensional X-ray diffractometer data. The dipeptide crystallizes in the space group $P2_1$ of the monoclinic system with two formula units in a cell of dimensions $a = 5{\cdot}343$ (2), $b = 7{\cdot}141$ (4), $c = 15{\cdot}944$ (6) Å, $\beta = 90{\cdot}11$ (3)° with $V = 608{\cdot}38$ Å^3, $D_c = 1{\cdot}508$, $D_o = 1{\cdot}51$ (1) g cm^{-3} and $\mu = 1{\cdot}38$ cm^{-1}. The structure was solved by direct methods and refined by least-squares techniques to a final value of the weighted R factor (on F) of 0·050 based on 1632 independent intensities with $I \geq 2\sigma(I)$. The dipeptide occurs as a zwitterion in the crystal, with the amino terminus protonated and the main-chain carboxyl group deprotonated. The two Glu side chains are on opposite sides of the backbone, and the structure is extended. The peptide linkage is significantly non-planar, the ω torsional angle being 167·6°. There is extensive intermolecular hydrogen bonding in the crystals, but no intramolecular hydrogen bonding.

Introduction

The role of acidic peptide residues, and especially of adjacent pairs of such residues, in the binding of calcium and magnesium in proteins has been the subject of intense recent biochemical study (Marsh, Scott, Hiskey & Koehler, 1979; Robertson, Koehler & Hiskey, 1979; Furie, Blumenstein & Furie, 1979; Nelsestuen, Resnick, Wei, Pletcher & Bloomfield, 1981; Kretsinger & Nelson, 1976). Of particular interest in this regard is the apparent enhanced selectivity for calcium over magnesium when pairs of glutamic acid (Glu) residues are replaced by γ-carboxyglutamic acid (Gla) residues (Williams, 1977; Nelsestuen & Suttie, 1973; Esmon, Suttie & Jackson, 1975; Stenflo & Ganrot, 1972); presumably, it is this selectivity which requires the presence of ten Gla residues (and no Glu residues) in the calcium-binding region of the blood protein prothrombin (Magnusson, Stottrup-Jensen, Petersen & Claeys, 1975).

In an attempt to provide a sound structural and molecular basis for an understanding of the roles of Glu, Gla and aspartic acid (Asp) residues in calcium-binding, we have undertaken a systematic examination of the structures of peptides containing these acidic residues. We felt that such a study was of particular value since, at the outset of our program, no crystallographic information on any linear peptide containing either Asp or Gla was available, and the only structural studies of Glu peptides involved the fully blocked dipeptide Z-(γ-ethyl)-L-Glu-(γ-ethyl)-L-Glu-ethyl ester (Benedetti, DiBlasio, Pavone, Pedone, Germain & Goodman, 1979) and the γ-glutamyl tripeptide glutathione (Wright, 1958). We have recently provided accounts of the crystal and molecular structures of the blocked Gla dipeptide Z-(γ-di-*tert*-butyl)-D,L-Gla-Gly ethyl ester (Valente, Hiskey & Hodgson, 1979), and the free dipeptides α-L-Asp-Gly.H_2O (Eggleston, Valente & Hodgson, 1981*a*), α-L-Glu-Gly (Eggleston, Valente & Hodgson, 1981*b*), and Gly-L-Asp.$2H_2O$ (Eggleston & Hodgson, 1981). We present here the structure of the first free peptide containing adjacent acidic peptide residues, α-L-glutamyl-L-glutamic acid, α-L-Glu-L-Glu, and compare this structure with that of the blocked analog.

Experimental

Colorless crystals of α-L-glutamyl-L-glutamic acid (Glu-Glu) were grown from aqueous dimethylformamide solution. A plate-like crystal of dimensions 1·00 × 0·75 × 0·30 mm was mounted on a glass fiber and placed on an Enraf–Nonius CAD-4 automatic diffractometer. The Enraf–Nonius routine *SEARCH* was employed to locate and center 25 reflections, which indicated that the crystals were in the monoclinic crystal system; a Delauney reduction showed that no cell of higher symmetry was present. ω scans of several axial reflections indicated that the crystal was of acceptable quality. The observed systematic absences are $k = 2n + 1$ for $0k0$ which are consistent only with space group $P2_1$. Accurate cell constants were obtained by least-squares refinement of the diffractometer settings for 25 reflections with $30° \leq 2\theta(\text{Mo}) \leq$

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35°. The density of the crystals as measured by flotation in a dichloromethane/iodomethane solution is 1·51 (1) g cm^{-3} which agrees well with the calculated value of 1·508 g cm^{-3} based on two molecules of dipeptide per unit cell and a molecular weight of 276·25.

Diffraction data were collected on the CAD-4 diffractometer equipped with molybdenum radiation [λ(Mo $K\alpha$) = 0·7107 Å] and a graphite monochromator. A unique set of data ($+h,+k,\pm l$) in the range $2\leq 2\theta \leq 60°$ was collected by the ω–θ scan technique. Reflections were scanned at a variable rate; a prescan was performed for every reflection to determine if a final scan was warranted and, if so, to select an appropriate scan rate. The maximum allowable time for a final scan was 60 s. Background counts were obtained by extending the final scan by 25% at each end. Intensity checks on three standard reflections were made after every 2 h of X-ray exposure time and orientation checks on three different standard reflections were made after every 200 reflections. No systematic variation in these standards was encountered throughout data collection.

Data reduction was carried out in the usual fashion. Raw intensities were calculated using the formula $I = S \times ATN(C - RB)$ where C is the total count of the scan, B is the total background count ($BH + BL$), R is the ratio of the scan time to the background scan time (t_s/t_b), ATN is the attenuator factor and is unity if the attenuator was not used and 17·42 if it was, and S is the scan rate. These intensities were assigned standard deviations according to the formula $\sigma(I) = [(S.ATN)^2(C + R^2B) + (pI)^2]^{1/2}$ where the quantities have the same definitions as above and the correction factor, p, was assigned a value of 0·01. The intensities and their standard deviations were then corrected for Lorentz–polarization effects but not for absorption. The absorption coefficient for this compound with Mo $K\alpha$ radiation (1·38 cm^{-1}) is not sufficient to warrant correction of the data. A total of 1905 independent reflections were processed of which 1632 had $I \geq 2\sigma(I)$. Only these latter data were considered observed and used in subsequent calculations.

Structure solution and refinement

The data were converted to normalized structure amplitudes (E) and the structure was discovered by use of the program *MULTAN* 78 (Main, Hull, Lessinger, Germain, Declercq & Woolfson, 1978), using 140 reflections with $E \geq 1·51$. An E map calculated from that set of phases having the highest combined figure of merit revealed the positions of all non-H atoms in the structure. The y coordinate of peptide O atom O_1 was arbitrarily fixed to define the origin of the cell. Isotropic, least-squares refinement of these positions gave values of the usual agreement factors $R = \sum ||F_o| - |F_c||/\sum |F_o|$ and $R_w = [\sum w(|F_o| - |F_c|)^2/\sum wF_o^2]^{1/2}$ of 0·121 and 0·131 respectively. All least-squares calculations were carried out on F, the function minimized being $\sum w(|F_o| - |F_c|)^2$ where the weights, w, are assigned as $4F_o^2/\sigma^2(F_o^2)$. In the calculation of the structure factors, F_c, the neutral-atom scattering factors were taken from *International Tables for X-ray Crystallography* (1974). The effects of the anomalous dispersion of all atoms were included. The values of f' and f'' were also taken from *International Tables for X-ray Crystallography* (1974). Anisotropic refinement of the 19 non-H-atom positions lowered the values of R and R_w to 0·083 and 0·100 respectively. Subsequent difference Fourier maps revealed the positions of all 16 H atoms. Examination of the model persuaded us that we had chosen the correct enantiomer. In subsequent least-squares calculations the non-H atoms were refined with anisotropic librational parameters while H atoms were refined isotropically. In the final cycle of least

Table 1. *Positional and thermal parameters in* α-L-*Glu*-L-*Glu*

	x	y	z	$U_{eq}{}^a/B^b$
O_1	−0·1119 (4)	0·8371	0·7276 (1)	0·180 (6)
O'	−0·0949 (4)	1·4081 (4)	0·7933 (1)	0·193 (5)
O''	0·2571 (4)	1·3479 (5)	0·7262 (2)	0·230 (8)
$O_1^{\varepsilon 1}$	−0·0005 (4)	0·8505 (4)	1·0508 (1)	0·220 (6)
$O_2^{\varepsilon 1}$	−0·0243 (4)	1·2789 (5)	0·4342 (1)	0·225 (6)
$O_1^{\varepsilon 2}$	−0·3507 (4)	1·0174 (4)	1·0533 (1)	0·214 (5)
$O_2^{\varepsilon 2}$	−0·4054 (4)	1·2420 (5)	0·3861 (1)	0·244 (6)
N_1	−0·5139 (5)	0·6639 (4)	0·7911 (1)	0·166 (4)
N_2	−0·3169 (4)	1·1134 (4)	0·7114 (1)	0·163 (5)
C_1^{α}	−0·5084 (5)	0·8729 (5)	0·7957 (2)	0·157 (5)
C_1^{β}	−0·4678 (5)	0·9396 (5)	0·8867 (2)	0·165 (5)
C_1^{γ}	−0·2253 (6)	0·8727 (6)	0·9251 (2)	0·188 (4)
C_1^{δ}	−0·2007 (6)	0·9241 (5)	1·0167 (2)	0·182 (10)
C_1'	−0·2923 (5)	0·9391 (5)	0·7400 (2)	0·156 (6)
C_2^{α}	−0·1091 (5)	1·2116 (4)	0·6720 (2)	0·161 (5)
C_2^{β}	−0·2032 (6)	1·3323 (6)	0·5992 (2)	0·192 (8)
C_2^{γ}	−0·3422 (6)	1·2170 (6)	0·5327 (2)	0·208 (10)
C_2^{δ}	−0·2648 (6)	1·2482 (5)	0·4434 (2)	0·182 (6)
C_2'	0·0317 (6)	1·3337 (5)	0·7365 (2)	0·170 (5)
H_1^1	−0·357 (5)	0·620 (4)	0·786 (2)	2·2 (6)
H_1^2	−0·606 (5)	0·622 (5)	0·745 (2)	2·1 (7)
H_1^3	−0·576 (6)	0·624 (5)	0·841 (2)	3·4 (8)
H_1^{α}	−0·682 (4)	0·921 (4)	0·774 (1)	1·1 (5)
$H_1^{\beta 1}$	−0·602 (5)	0·913 (4)	0·918 (2)	1·6 (6)
$H_1^{\beta 2}$	−0·468 (6)	1·086 (5)	0·891 (2)	3·3 (8)
$H_1^{\gamma 1}$	−0·088 (5)	0·900 (5)	0·896 (2)	2·8 (7)
$H_1^{\gamma 2}$	−0·204 (6)	0·716 (6)	0·923 (2)	5·3 (10)
$H_1^{\varepsilon 1}$	0·014 (8)	0·878 (9)	1·116 (3)	8·5 (13)
H_2	−0·469 (6)	1·166 (5)	0·718 (2)	3·6 (8)
H_2^{α}	0·020 (5)	1·121 (5)	0·651 (2)	3·5 (8)
$H_2^{\beta 1}$	−0·048 (6)	1·394 (6)	0·569 (2)	4·4 (9)
$H_2^{\beta 2}$	−0·324 (6)	1·420 (6)	0·620 (2)	5·0 (9)
$H_2^{\gamma 1}$	−0·517 (6)	1·221 (5)	0·538 (2)	4·1 (9)
$H_2^{\gamma 2}$	−0·289 (6)	1·096 (6)	0·539 (2)	5·1 (10)
$H_2^{\varepsilon 1}$	0·012 (5)	1·275 (5)	0·385 (2)	2·6 (7)

(*a*) Calculated from the r.m.s. amplitudes in Å, where $U_{eq}^3 = U_1U_2U_3$. (*b*) For H atoms (in Å^2).

squares there were 1632 observations and 235 variables; no parameter shifted by more than 0·1 times its e.s.d., which is taken as evidence of convergence. The final values of R and R_w were calculated to be 0·055 and 0·050 respectively. Comparison of the values of $|F_o|$ and $|F_c|$ in the later stages of refinement indicated that no correction for the effects of secondary extinction was necessary, and none was made. A final difference synthesis contained no peak higher than 0·15 e Å^{-3}; it is noteworthy, however, that the top four peaks in this map were in positions approximately half way between covalently linked atoms, and may be attributable to bonding electron density. Similar features have been noted in other structures, of course (Beagley & Small, 1963, 1964; Delaplane & Ibers, 1969; Hodgson & Ibers, 1969). The atomic positional parameters derived from the last cycle of least squares, along with their standard deviations, as estimated from the inverse matrix, are presented in Table 1.*

Description of the structure

A view of a single molecule of the dipeptide, α-L-Glu-L-Glu, is shown in Fig. 1. The atom-labelling scheme used in Fig. 1 and throughout this paper is that recommended by the IUPAC–IUB Commission on Biochemical Nomenclature (1970). As can be seen in Fig. 1, the dipeptide exists as a zwitterion with the amino terminus protonated and the terminal (main-chain) carboxyl group ionized; the two glutamyl side-chain carboxyl groups are not ionized. As is also apparent in Fig. 1, the two glutamyl side chains are disposed on opposite sides of the backbone, which leads to a relatively extended structure. Evidently, this conformation would preclude the binding of both side chains to a single metal ion and, while at present we have no evidence to suggest that this conformation is present in metal complexes of Glu-Glu, it is apparent that major conformational changes would be necessary before such chelation would be possible.

The principal bond lengths and angles in the dipeptide are tabulated in Tables 2 and 3 respectively. Most of the bond lengths are very similar to the mean values found for a variety of dipeptides and recently summarized by Benedetti (1977). As we have noted elsewhere (Eggleston & Hodgson, 1981), the peptide carbonyl bond length (C'_1–O_1) is very sensitive to the extent of hydrogen bonding at O_1. When there is either no hydrogen bond or only a very weak interaction

* Lists of observed and calculated structure amplitudes and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36485 (14 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

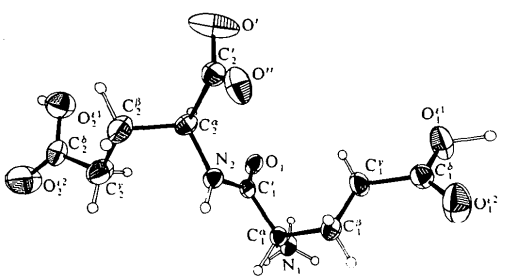

Fig. 1. View of a single molecule of α-L-Glu-L-Glu. Thermal ellipsoids are drawn at the 50% probability level, but H atoms are shown as spheres of arbitrary size.

Table 2. *Principal bond distances* (Å) *in* α-L-*Glu*-L-*Glu*

O_1–C'_1	1·225 (2)	C^{α}_1–C^{β}_1	1·541 (3)
O'–C'_2	1·250 (2)	C^{α}_1–C'_1	1·533 (3)
O''–C'_2	1·220 (2)	C^{α}_1–H^{α}_1	1·05 (2)
$O^{\varepsilon1}_1$–C^{δ}_1	1·309 (3)	C^{β}_1–C^{γ}_1	1·510 (3)
$O^{\varepsilon1}_1$–$H^{\varepsilon1}_1$	1·06 (3)	C^{β}_1–$H^{\beta1}_1$	0·90 (2)
$O^{\varepsilon1}_2$–C^{δ}_2	1·312 (3)	C^{β}_1–$H^{\beta2}_1$	1·05 (3)
$O^{\varepsilon1}_2$–$H^{\varepsilon1}_2$	0·81 (2)	C^{γ}_1–C^{δ}_1	1·512 (3)
$O^{\varepsilon2}_1$–C^{δ}_1	1·194 (3)	C^{γ}_1–$H^{\gamma1}_1$	0·89 (2)
$O^{\varepsilon2}_2$–C^{δ}_2	1·182 (3)	C^{γ}_1–$H^{\gamma2}_1$	1·12 (3)
N_1–C^{α}_1	1·494 (3)	C^{α}_2–C^{β}_2	1·529 (3)
N_1–H^1_1	0·90 (2)	C^{α}_2–C'_2	1·543 (3)
N_1–H^2_1	0·94 (2)	C^{α}_2–H^{α}_2	1·01 (2)
N_1–H^3_1	0·91 (2)	C^{β}_2–C^{γ}_2	1·534 (3)
N_2–C'_1	1·331 (3)	C^{β}_2–$H^{\beta1}_2$	1·05 (3)
N_2–C^{α}_2	1·457 (2)	C^{β}_2–$H^{\beta2}_2$	0·96 (3)
N_2–H_2	0·90 (2)	C^{γ}_2–C^{δ}_2	1·500 (3)
		C^{γ}_2–$H^{\gamma1}_2$	0·94 (2)
		C^{γ}_2–$H^{\gamma2}_2$	0·91 (4)

Table 3. *Bond angles* (°) *in* α-L-*Glu*-L-*Glu*

N_1–C^{α}_1–C^{β}_1	111·0 (2)	N_1–C^{α}_1–C'_1	107·1 (2)
N_1–C^{α}_1–H^{α}_1	107·2 (10)	C^{β}_1–C^{α}_1–C'_1	110·2 (2)
C^{β}_1–C^{α}_1–H^{α}_1	109·5 (10)	C'_1–C^{α}_1–H^{α}_1	111·9 (10)
C^{α}_1–C^{β}_1–C^{γ}_1	113·8 (2)	C^{α}_1–C^{β}_1–$H^{\beta1}_1$	110·6 (12)
C^{α}_1–C^{β}_1–$H^{\beta2}_1$	112·1 (13)	C^{γ}_1–C^{β}_1–$H^{\beta1}_1$	113·1 (12)
C^{γ}_1–C^{β}_1–$H^{\beta2}_1$	106·7 (13)	C^{β}_1–C^{γ}_1–C^{δ}_1	112·9 (2)
C^{β}_1–C^{γ}_1–$H^{\gamma1}_1$	115·2 (14)	C^{β}_1–C^{γ}_1–$H^{\gamma2}_1$	112·9 (14)
C^{δ}_1–C^{γ}_1–$H^{\gamma1}_1$	112·5 (14)	C^{δ}_1–C^{γ}_1–$H^{\gamma2}_1$	105·0 (14)
$O^{\varepsilon1}_1$–C^{δ}_1–$O^{\varepsilon2}_1$	124·7 (2)	$O^{\varepsilon1}_1$–C^{δ}_1–C^{γ}_1	111·9 (2)
$O^{\varepsilon2}_1$–C^{δ}_1–C^{γ}_1	123·3 (2)	O_1–C'_1–N_2	125·3 (2)
O_1–C'_1–C^{α}_1	120·3 (2)	N_2–C'_1–C^{α}_1	114·4 (2)
N_2–C^{α}_2–C^{β}_2	110·4 (2)	N_2–C^{α}_2–C'_2	110·8 (2)
N_2–C^{α}_2–H^{α}_2	111·0 (14)	C^{β}_2–C^{α}_2–C'_2	110·3 (2)
C^{β}_2–C^{α}_2–H^{α}_2	109·5 (13)	C'_2–C^{α}_2–H^{α}_2	104·7 (13)
C^{α}_2–C^{β}_2–C^{γ}_2	112·3 (2)	C^{α}_2–C^{β}_2–$H^{\beta1}_2$	108·7 (14)
C^{α}_2–C^{β}_2–$H^{\beta2}_2$	108·7 (16)	C^{γ}_2–C^{β}_2–$H^{\beta1}_2$	107·0 (13)
C^{γ}_2–C^{β}_2–$H^{\beta2}_2$	105·6 (17)	C^{β}_2–C^{γ}_2–C^{δ}_2	116·3 (2)
C^{β}_2–C^{γ}_2–$H^{\gamma1}_2$	114·1 (15)	C^{β}_2–C^{γ}_2–$H^{\gamma2}_2$	106·1 (19)
C^{δ}_2–C^{γ}_2–$H^{\gamma1}_2$	110·6 (14)	C^{δ}_2–C^{γ}_2–$H^{\gamma2}_2$	99·4 (19)
$O^{\varepsilon1}_2$–C^{δ}_2–$O^{\varepsilon2}_2$	122·8 (2)	$O^{\varepsilon1}_2$–C^{δ}_2–C^{γ}_2	113·7 (2)
$O^{\varepsilon2}_2$–C^{δ}_2–C^{γ}_2	123·5 (2)	O'–C'_2–O''	126·7 (2)
O'–C'_2–C^{α}_2	117·4 (2)	O''–C'_2–C^{α}_2	115·9 (2)
C^{δ}_1–$O^{\varepsilon1}_1$–$H^{\varepsilon1}_1$	113·1 (19)	C^{δ}_2–$O^{\varepsilon1}_2$–$H^{\varepsilon1}_2$	109·8 (15)
C^{α}_1–N_1–H^1_1	109·4 (15)	C^{α}_1–N_1–H^2_1	111·8 (15)
C^{α}_1–N_1–H^3_1	106·3 (17)	C'_1–N_2–C^{α}_2	121·5 (2)
C'_1–N_2–H_2	116·3 (17)	C^{α}_2–N_2–H_2	122·2 (17)

involving O_1 the C'_1—O_1 distance is relatively short (<1·22 Å) while strong hydrogen bonding gives rise to a larger value. This trend is followed in the present structure, in which there is a hydrogen bond involving O_1 (see below) and the C'_1—O_1 distance is 1·225 (2) Å.

In contrast to the other unblocked dipeptides previously referred to (Glu-Gly, Asp-Gly, Gly-Asp), the peptide linkage in Glu-Glu is significantly non-planar. The peptide 'plane' is defined by the six atoms C_1^α, C'_1, N_2, C_2^α, O_1 and H_2. The least-squares plane through these six atoms is tabulated in Table 4, and it is apparent that some of these atoms deviate from the plane by more than 0·12 Å. Moreover, the four atoms C_1^α, C'_1, N_2, and C_2^α which more narrowly define the peptide linkage are markedly non-coplanar, with deviations as large as 0·085 Å from the least-squares plane (see Table 4). These deviations can be contrasted with those in Glu-Gly, for example, where no atom deviates from the six-atom plane by as much as 0·04 Å (Eggleston & Hodgson, 1981).

The non-planarity of the peptide unit is also reflected in the ω torsional angle in Glu-Glu. The value of 167·6° found in Glu-Glu is significantly smaller than the values of 172·0–175·9° found in the other unblocked acidic dipeptides; in the fully protected Glu-Glu structure (Benedetti *et al.*, 1979) this angle is 173°. The φ/ψ torsional angles of −94·8 and 155·6° are within the range expected for an extended glutamyl structure (Anfinsen & Scheraga, 1975). The torsional angles χ_1^1 (around C_1^α—C_1^β) and χ_2^1 (around C_2^α—C_2^β) of 60·3 and −57·9° respectively, correspond to two of the three preferred positions noted by Ramachandran & Sasisekharan (1968) and are similar to the values tabulated by Benedetti *et al.* (1979). Similarly, the torsional angle χ_1^2 (around C_1^β—C_1^γ) of −174·5° is very similar to that of −173° in the protected analog. The torsional angle χ_2^2 (around C_2^β—C_2^γ) of −131·1°, however, is quite different from the expected *trans* conformation and from the value of −168° in the protected Glu-Glu structure. It is possible that this unusual, and presumably unfavorable, torsional angle comes about so as to allow a strong intermolecular hydrogen bond between $O_2^{\varepsilon 1}$ and the peptide O atom of a neighboring atom (see below).

Table 4. *Least-squares planes through the peptide linkage in α-L-Glu-L-Glu*

Plane 1[a]		Plane 2[b]	
Atoms	Δ (Å)	Atoms	Δ (Å)
C_1^α	−0·119 (3)	C_1^α	−0·065 (3)
C'_1	0·024 (3)	C'_1	0·053 (3)
N_2	0·021 (2)	N_2	0·085 (2)
C_2^α	−0·121 (3)	C_2^α	−0·073 (3)
O_1	0·077 (2)	O_1*	0·060 (2)
H_2	0·117 (32)	H_2*	0·215 (32)

(*a*) The equation of the six-atom plane is

$$-0{\cdot}3863X - 0{\cdot}3539Y - 0{\cdot}8518Z + 11{\cdot}8337 = 0$$

(*b*) The equation of the four-atom plane is

$$-0{\cdot}4153X - 0{\cdot}3285Y - 0{\cdot}8483Z + 11{\cdot}6060 = 0$$

* These atoms were not included in the calculation of the least-squares plane.

Intermolecular hydrogen bonding in α-L-Glu-L-Glu is extensive with all available H atoms participating in the bonding. A drawing of the complete hydrogen-bonding network is included as Fig. 2. The peptide carbonyl acts as an acceptor from a side-chain carboxyl of a molecule related by the screw along **c** with $O_1\cdots O_2^{\varepsilon 1}$ and $H_2^{\varepsilon 1}\cdots O_1$ distances of 2·713 (2) and 1·92 (2) Å and an $O_2^{\varepsilon 1}$—$H_2^{\varepsilon 1}\cdots O_1$ angle of 165 (3)°. The amide N_2 acts as a donor along **a** toward O″ of the ionized carboxyl terminus with $N_2\cdots O''$ and $H_2\cdots O''$ distances of 2·836 (2) and 1·96 (3) Å and an N_2—$H_2\cdots O''$ angle of 163 (3)°. The ionized carboxyl also acts as an acceptor through O′ from the protonated amino group at N_1 of a molecule along **b** with $N_1\cdots O'$ and $H_1^1\cdots O'$ distances of 2·890 (2) and 2·07 (2) Å and N_1—$H_1^1\cdots O'$ angle of 152 (2)°. The protonated amino group N_1 is also a donor along **c** to the side-chain carboxyl of a screw-related molecule with $N_1\cdots O_1^{\varepsilon 2}$ and $H_1^3\cdots O_1^{\varepsilon 2}$ distances of 2·790 (2) and 1·90 (2) Å and N_1—$H_1^3\cdots O_1^{\varepsilon 2}$ angle of 170 (2)°. The remaining H atom of the protonated amino group apparently participates in a bifurcated hydrogen bond involving both O″ of a molecule one unit cell away along **a** and **b** and $O_2^{\varepsilon 2}$ of a screw-related molecule one unit cell away along **a** and **c**. The relevant distances are $N_1\cdots O''$ and $H_1^2\cdots O''$, 2·767 (3) and 2·11 (2) Å; $N_1\cdots O_2^{\varepsilon 2}$ and $H_1^2\cdots O_2^{\varepsilon 2}$, 2·911 (3) and 2·26 (2) Å. The angles involved are N_1—$H_1^2\cdots O''$, 126 (2), and N_1—$H_1^2\cdots O_2^{\varepsilon 2}$, 126 (2)°. The hydrogen-bonding scheme is completed by the donation from $O_1^{\varepsilon 1}$ along **c** to the ionized carboxyl of a screw-related molecule with $O_1^{\varepsilon 1}\cdots O'$ and $H_1^{\varepsilon 1}\cdots O'$ distances of 2·569 (2) and 1·52 (2) Å and an $O_1^{\varepsilon 1}$—$H_1^{\varepsilon 1}\cdots O'$ angle of 168 (3)°. It is interesting to note that both O atoms of the ionized carboxyl group act as acceptors in two hydrogen bonds.

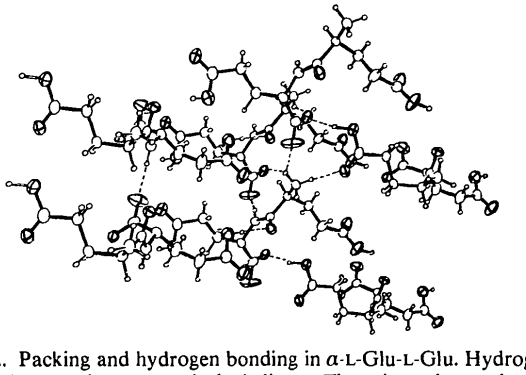

Fig. 2. Packing and hydrogen bonding in α-L-Glu-L-Glu. Hydrogen bonds are drawn as dashed lines. The view shown has **a** horizontal and **c** vertical.

It is again noteworthy that in this structure, as in the other acidic dipeptides which have been examined, there is no evidence of any intramolecular hydrogen bonding; such bonding has been inferred from solution spectroscopic data in L-Arg-L-Glu (Lancelot, Mayer & Helene, 1979).

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La Structure du Disuccinate de *trans*-Cyclohexylène-1,4 et de Diméthyle*

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Abstract

The crystal structure of 1,4-*trans*-cyclohexylene dimethyl disuccinate, $C_{16}H_{24}O_8$, $M_r = 344{\cdot}37$, has been solved by direct methods and the final R value is 0·046 for 1332 observed reflections. The crystals (m.p. 360 K), which have a triclinic unit cell of dimensions $a = 6{\cdot}169$ (3), $b = 7{\cdot}276$ (3), $c = 10{\cdot}360$ (4) Å, $\alpha = 89{\cdot}32$ (3), $\beta = 84{\cdot}30$ (5) and $\gamma = 73{\cdot}84$ (5)°, belong to the space group $P\bar{1}$ [$V = 444$ Å^3, $F(000) = 184$, $Z = 1$, $d_o = 1{\cdot}28$, $d_c = 1{\cdot}287$ Mg m^{-3}, μ(Cu $K\alpha$) = 0·834

* Note: les noms 'cyclohexylène-1,4' et 'cyclohexanediyl-1,4' (Brisse & Palmer, 1982; Brisse & Rémillard, 1982) désignent le même radical bivalent $-C_6H_{10}-$.

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