A Redetermination of the Crystal and Molecular Structure of Glutathione (γ-L-Glutamyl-L-cysteinylglycine) at 120 K

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The crystal and molecular structure of the tripeptide γ-L-glutamyl-L-cysteinylglycine (glutathione) has been redetermined at 120 K using 1628 reflections with $I > 2.5\sigma I$. The unit cell is orthorhombic, $P2_12_12_1$, with cell dimensions a = 5.622 (2), b = 8.781(3), c = 28.023(10) Å. The final R-factor was 0.056. The overall molecular geometry agrees with the first structure determination, but several large differences in bond lengths and bond angles are pointed out. The SH group is involved in a weak hydrogen bond. A very short intermolecular contact occurs betwen the C-terminal carboxylic group and the carbonyl group in the γ-glutamyl peptide bond (C···O 2.86 Å), accompanied by a substantial deviation from planarity for the peptide unit ($\omega_1 = -167.0°$).

Glutathione (GSH) is the tripeptide λ-L-glutamyl-L-cysteinylglycine. The most interesting chemical characteristics of this molecule are the γ-glutamyl linkage and the presence of a free SH group. The latter can be oxidized to form a disulfide bridge linking two GSH molecules [eqn. (1)].

$$2\ \text{GSH} \rightarrow \text{GSSG} + 2[\text{H}] \qquad (1)$$

GSH is present in an animal cells in high concentrations (1–5 mM)[1] and has various biological functions. It is conjugated enzymatically with a diverse array of xenobiotics, thus preventing reaction of these compounds with constituents of the cell.[2] It also acts as a reductant of toxic peroxides and has a specific role in the reduction of dehydroascorbic acid.

The crystal structure of GSH was determined by Wright in 1958.[3] The accuracy of this work is, however, low by present standards (R = 0.21). In order to obtain further knowledge of the structure of this molecule with possible hydrogen bonds involving the SH group, a reinvestigation of the crystal structure has been carried out.

Experimental

GSH is readily oxidized in aqueous solutions by oxidizing agents like NO, NO_2,[4] H_2O_2[5] and O_2 in the presence of Fe(III),[6] but it is stable to oxidation by atmospheric O_2 in the absence of heavy metals.[7] However, the compound exhibits limited stability in O_2-free water as non-enzymatic decomposition takes place. The main reactions are cleavage of the γ-glutamyl bond (with formation of pyroglutamic acid and cysteinylglycine) and

Table 1. Experimental conditions for data collection.

Instrument	Nicolet P3
Radiation	Graphite Crystal Monochromated MoK_α
Scanning mode	θ/2θ
Scan speed/° min^{-1}	2.0
Scan range/°	$2\theta_{\alpha1} - 1.0$ to $2\theta_{\alpha2} + 1.0$
Background count	For 0.35 of scan time at scan limits
Temperature/K	120
2θ range/°	6.0–70.0
Crystal dimensions/mm	0.50×0.20×0.05
No. of refl. measured	2388
No. of unique refl. $I > 2.5\sigma I$	1628

desulfurization,[8] of which the latter is of importance only at high pH. The apparent activation energy for both reactions is about 80 kJ mol^{-1}.[9] Decomposition turned out to be a major problem during crystallization, but a thin, flat crystal of reasonable size was eventually grown from an aqueous solution.

The conditions for data collection are summarized in Table 1. Cell parameters were determined by least-squares fit to the diffractometer settings for 25 general reflections. Standard deviations in the measured intensities were calculated as $\sigma I = [C_T+(0.02C_N)^2]^{1/2}$, where C_T is the total number of counts and C_N is the scan count minus the background count. The intensities were corrected for Lorentz and polarization effects, but not for absorption. The structure parameters determined by Wright were used as input, and all heavy atoms were refined isotropically by least-squares methods. The hydrogen atoms connected to carbon and nitrogen atoms were introduced in theoretical positions, while the carboxylic and the thiolic hydrogen atoms were located from a difference Fourier synthesis. Isotropic temperature factors were set equal to B_{eq} (heavy atom) and were given suitable increments of 0.2 and 1.2 for hydrogen atoms connected to C/S and N/O, respectively. All positional parameters and anisotropic temperature factors for heavy atoms were refined by least-squares methods, giving R = 0.056 and R_w = 0.050, with goodness of fit $S = [\Sigma w\Delta^2/(m-n)]^{1/2}$ = 1.93. The final parameters are given in Table 2. Atomic scattering factors for free heavy atoms and spherically bonded hydrogen atoms are taken from Ref. 10.

Table 2. Fractional coordinates for γ-L-Glu-L-Cys-Gly with standard deviations and equivalent isotropic temperature factors, B_{eq}, for non-hydrogen atoms.

Atom	x	y	z	B_{eq}/Å^2
SG2	1.0475(3)	0.8948(2)	0.4599(1)	2.1
O11	0.1048(6)	0.2908(4)	0.4673(1)	1.7
O12	−0.0407(6)	0.5031(4)	0.4337(1)	1.8
OE1	0.4254(6)	0.7488(4)	0.3218(1)	1.7
O2	0.7695(7)	1.1479(4)	0.3737(1)	2.0
O′	1.0800(8)	1.1885(6)	0.2271(2)	5.5
O″	0.7327(8)	1.1212(6)	0.2566(1)	3.5
N1	0.5491(8)	0.3418(5)	0.4401(1)	1.3
N2	0.7020(7)	0.7348(5)	0.3794(1)	1.2
N3	0.9384(9)	0.9599(5)	0.3302(1)	1.4
CA1	0.3622(9)	0.4443(5)	0.4196(2)	1.1
CB1	0.3591(10)	0.4297(6)	0.3655(2)	1.5
CG1	0.5768(10)	0.4997(6)	0.3401(2)	1.3
CD1	0.5641(10)	0.6712(5)	0.3455(2)	1.3
C1	0.1212(8)	0.4065(6)	0.4422(2)	1.3
CA2	0.6667(9)	0.8919(7)	0.3941(2)	1.3
CB2	0.7311(10)	0.9107(7)	0.4468(2)	1.9
C2	0.7977(9)	1.0103(6)	0.3646(2)	1.3
CA3	1.0807(10)	1.0633(6)	0.3016(2)	1.9
C3	0.9647(11)	1.1293(6)	0.2578(2)	2.0
HS	1.093(9)	1.028(6)	0.454(2)	
HO″	0.702(11)	1.144(8)	0.229(2)	
HN11	0.656(10)	0.398(7)	0.445(2)	
HN12	0.580(10)	0.250(6)	0.424(2)	
HN13	0.512(10)	0.304(6)	0.467(2)	
HN2	0.800(9)	0.684(6)	0.394(2)	
HN3	0.959(10)	0.859(6)	0.325(2)	
HA1	0.393(9)	0.552(5)	0.432(1)	
HB11	0.208(9)	0.470(6)	0.353(2)	
HB12	0.349(9)	0.324(6)	0.360(2)	
HG11	0.558(9)	0.474(5)	0.305(1)	
HG12	0.727(9)	0.458(6)	0.356(2)	
HA2	0.522(9)	0.908(6)	0.391(2)	
HB21	0.653(9)	0.810(5)	0.460(2)	
HB22	0.686(9)	1.017(6)	0.457(2)	
HA31	1.217(9)	1.017(6)	0.292(2)	
HA32	1.120(9)	1.164(5)	0.322(2)	

Crystal data

γ-L-Glutamyl-L-cysteinylglycine (glutathione), $C_{10}H_{17}N_3O_6S$; orthorhombic, a = 5.622(2), b = 8.781(3), c = 28.023(10) Å, V = 1383.5(7) Å^3, M = 307.33, Z = 4, F_{000} = 648, space group $P2_12_12_1$, D_c = 1.475 g cm^{-3}.

Description and discussion

An ORTEP[11] drawing of the molecule is shown in Fig. 1 with atomic numbering, bond lengths and bond angles indicated. Hydrogen bond distances are given in Table 4. The bond angle CB2-SG 2-HS is 96(3)°. As usual for peptides, the molecule exists as a zwitterion, but unlike the structures of all other free peptides, the main chain carboxylic group is not ionized. The negative charge being located in the glutamyl moiety. This fact is supported by studies of acid-base properties of GSH by NMR,[12] giving for the two carboxyl groups pK_{a1}(glutamyl) = 2.3 and pK_{a2}(main chain) = 3.3.

The agreement between the bond lengths given in this work and those in the original paper by Wright is in general very good. Her estimate of

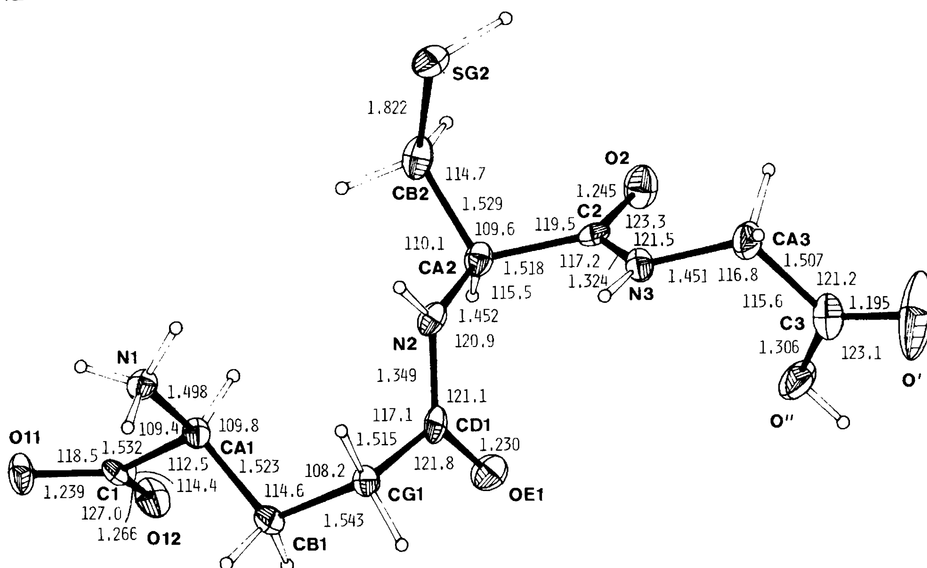

Fig. 1. ORTEP drawing of the molecule with numbering, bond lengths and bond angles indicated. The e.s.d.'s are 0.006 Å and 0.4°, respectively.

the standard deviation in bond length is 0.028 Å, and all but three differences in corresponding bond lengths in the two structures fall within this limit. Wright's values for the remaining three are (Δ compared to this work in parentheses) N2–CD1 1.31 Å (−0.04 Å), CA3–C3 1.57 Å (+0.06 Å) and CA1–C1 1.49 Å (−0.04 Å). The agreement between the two structures as reflected by bond angles is not quite so good. In the carboxyl moieties, four differences are particularly large: O′-C3-CA3 115.6° (−5.6°), O″-C3-CA3 121.5° (+5.9°), C3-CA3-C3 109.4° (−7.4°) and O11-C1-O12 121.6° (−5.4°).

The molecule is roughly S-shaped. The principal torsion angles are (in ° with e.s.d.'s in parentheses):

θ^1 (N1,CA1,CB1,CG1) = −70.8(5),
θ^2 (CA1,CB1,CG1,CD1) = −68.7(5),
θ^3 (CB1,CG1,CD1,N2) = 100.4(5),
θ' (O11,C1,CA1,N1) = −9.7(6),
ω_1 (CG1,CD1,N2,CA2) = −167.0(4),
φ_2 (CD1,N2,CA2,C2) = −86.9(6),
χ_2^1 (N2,CA2,CB2,SG2) = 69.9(5),
χ_2^2 (CA2,CB2,SG2,HS) = 89(4),
ψ_2 (N2,CA2,C2,N3) = −2.9(6),
ω_2 (CA2,C2,N3,CA3) = −176.8(4),
φ_3 (C2,N3,CA3,C3) = −87.9(6),
ψ_T^2 (N3,CA3,C3,O″) = 18.0(8).

The glutamic acid residue is attached to the cysteine residue through the γ-carboxyl group rather than the α-carboxyl. This gives a special peptide linkage, but there are no extraordinary values for bond lengths or bond angles concerning the peptide bond. The CD1–N2 bond (1.349 Å) is just slightly longer than the α-L-Glu-L-Xaa peptide bond in other short peptides (1.324–1.340 Å). However, the deviation from 180° for ω_1 (Δ = 13.0°) is among the largest observed for a peptide linkage. Data for least-squares planes through the peptide unit are given in Table 3.

A consequence of the occurrence of the γ-peptide bond is the lack of a regular side chain, since what would have been the L-Glu side chain in a regular peptide now constitutes an elongated

Table 3. Displacements (Å) from least-squares planes through the γ-L-Glu-L-Cys peptide linkage.

CG1	0.069	CG1	0.073
CD1	−0.038	CD1	−0.066
N2	−0.090	N2	−0.073
CA2	0.086	CA2	0.079
OE1	−0.009	OE1[a]	−0.099
HN2	−0.15	HN2[a]	−0.29

[a]Atoms not included in the least squares plane calculation.

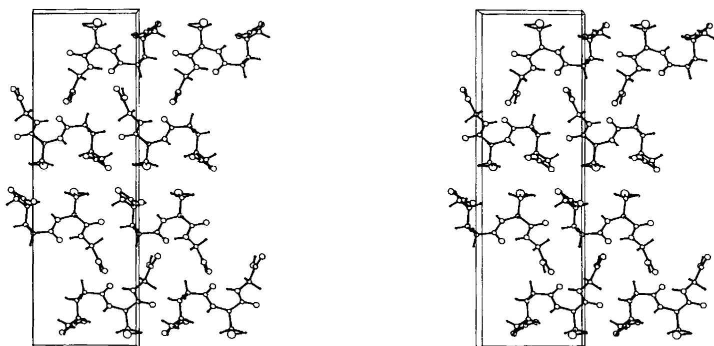

Fig. 2. Molecular packing of glutathione in the crystal viewed along the *a* axis.

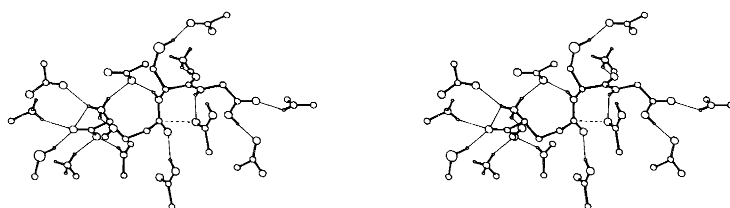

Fig. 3. The surroundings of a single molecule; all hydrogen bonds are lined. The carboxyl-carbonyl interaction has been dotted.

main chain. The θ^1 and θ^2 torsion angles are both ÷gauche, giving the rather folded conformation of the molecule. Investigations on GSH by NMR methods[13] show this to be one of the stable conformations in solution. It can be seen from Fig. 1 that there is no kind of interaction between the two ionized N-terminal groups and the CD1–OE1 carbonyl group. This is in accordance with the fact that an intramolecular hydrogen bond has not been observed in any of the previously investigated crystal structures of free peptides. The θ^3 torsion angle has adopted a value of 100.4°, which is a common value for the ψ torsion angle in regular peptides.

NMR investigations indicate equal populations for all three staggered conformations at the C_α–C_β bond in the cysteine residue. However, in all but one instance (six different crystal environments), χ^1 is about 65° in crystal structures with free SH groups,[14–17] which might suggest a preference for the +gauche conformation in the solid phase for the cysteine side chain.

The crystal packing with the unit cell is illustrated in Fig. 2. There is a complicated three dimensional hydrogen bond network. The geometries of the hydrogen bonds are given in Fig. 3 and Table 4. The carboxyl O12 atom forms a hydrogen-bonded bridge between N1 and N2, and

Table 4. Hydrogen bond distances (Å) and angles (°).[a]

D	H	A	D–H	D···A	H···A	D–H···A
SG3	HS	O11	1.21	3.499	2.33	161
O″	HO″	OE1	0.81	2.620	1.85	160
N1	HN11	O12	0.81	2.712	1.95	157
N1	HN12	O2	0.94	2.810	1.98	147
N1	HN13	O11	0.85	2.862	2.09	151
N1	HN13	O11[b]	0.85	2.650	2.29	106
N2	HN2	O12	0.82	2.923	2.14	161
N3	HN3	O′	0.90	2.876	2.10	144

[a]C–H distances are 0.83–1.07 Å (mean 0.98 Å); e.s.d.'s for X–H bond lengths 0.05 Å. [b]Intramolecular bond.

the OE1···O″ hydrogen bond is seen to be very short for such a bond to a peptide carbonyl (2.620 Å). To the author's knowledge, the intramolecular hydrogen bond N1–HN1···O11 is the first of this kind to be observed in a free peptide, although a corresponding arrangement is seen in the crystal structure of the peptide sweetener aspartame (α-L-Asp-L-Phe methyl ether).[18] It may further be noted that the sulfur atom acts as a donor in a weak hydrogen bond to a carboxyl oxygen atom at $1+x, 1+y, z$. Clear evidence of sulfur as a hydrogen bond donor has previously been seen only in the structure of *N*-acetyl-L-cysteine.[17] In the latter compound, the sulfur atom also accepts in a weak hydrogen bond. No such contact is seen in the present structure, the shortest intermolecular S···H distance being 3.26 Å (SG2···HN12).

Fig. 3 also shows a short intermolecular C···O contact of 2.857 Å. This may be described as an O···C=O interaction with the oxygen atom as a weak nucleophile.[19] For C···O distances of between 2.8 and 2.9 Å the O···C=O angle (α) is statistically expected to be 90–110°, and in the RR′C=O carbonyl group the carbon atom is displaced a distance Δ = 0.01–0.03 Å from the RR′O plane towards the oxygen atom.[19] In the present structure, values of $\alpha = 91.7°$ and Δ = 0.026 Å are found. This interaction is at least partly responsible for the large deviation from planarity for the peptide unit described earlier.

Lists of structure factors and anisotropic thermal parameters are available from the author on request.

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