Data collection: *DIF*4 (Stoe & Cie, 1992*a*). Cell refinement: *DIF*4. Data reduction: *REDU*4 (Stoe & Cie, 1992*b*). Program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997*a*). Program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997*b*). Molecular graphics: *XP* in *SHELXTL/PC* (Sheldrick, 1990). Software used to prepare material for publication: *SHELXL*97.

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: CF1307). Services for accessing these data are described at the back of the journal.

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The oxidized form of glutathione

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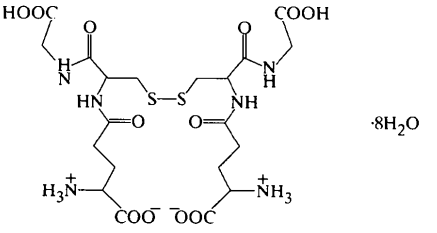
Abstract

The crystal structure of the oxidized form of the tripeptide glutathione (γ-L-glutamyl-L-cystylglycine octahydrate, $C_{20}H_{32}N_6O_{12}S_2\cdot 8H_2O$) has been determined at 100 K. The molecule was crystallized from aqueous solution in the presence of the precipitating agent polyethylene glycol by the vapor-diffusion method, which is commonly used in protein crystallization. The space group is $P2_12_12$ and the molecule contains a crystallographic twofold symmetry axis through the middle of the disulfide bridge. There are four water molecules per tripeptide, two of which are disordered. The S—S and S—C bond lengths are 2.0387 (9) and 1.8098 (19) Å, respectively, and the C—S—S bond angle is 105.03 (6)°.

Comment

The reduced form of glutathione (GSH) is present in animal cells and has various biological functions, notably the reduction of toxic peroxides. The SH group can be oxidized to form a disulfide bridge: 2GSH → GSSG + 2[H] (Metzler, 1977). The crystal structure of the reduced form of glutathione has been determined by Wright (1958) and redetermined by Görbitz (1987). The tripeptide glutathione (γ-L-glutamyl-L-cystylglycine) has a special peptide linkage as the glutamic acid residue is attached to the cystine residue through the γ-carboxyl group rather than through the α-carboxyl group.

The structure of $GSSG\cdot 8H_2O$ has been determined using orthorhombic crystals of space group $P2_12_12$. The crystal structure contains a crystallographic



GSSG

twofold symmetry axis through the center of the disulfide bridge (Fig. 1). There are four water molecules in the asymmetric unit and two of them are disordered. The stereochemical data for the side chain of the cystine residue and the disulfide bridge are given in Table 1. The glutathione peptide has a left-handed disulfide chirality [C—S—S—C = −97.0 (4)°], unlike cystine in the tetragonal (Chaney & Steinrauf, 1974) and hexagonal (Oughton & Harrison, 1959) crystal forms. The disulfide bridge length is 2.0387 (9) Å, which is the same, within experimental error, as the length found in tetragonal (2.043 Å) and hexagonal (2.032 Å) cystine crystals, and the mean value of 2.029 Å for 46 disulfides found in small molecules (Allen *et al.*, 1987). The C—S distance of 1.8098 (19) Å agrees also with the average value of 1.815 Å given in *International Tables for Crystallography* (Vol. C, 1992). The φ, ψ and ω

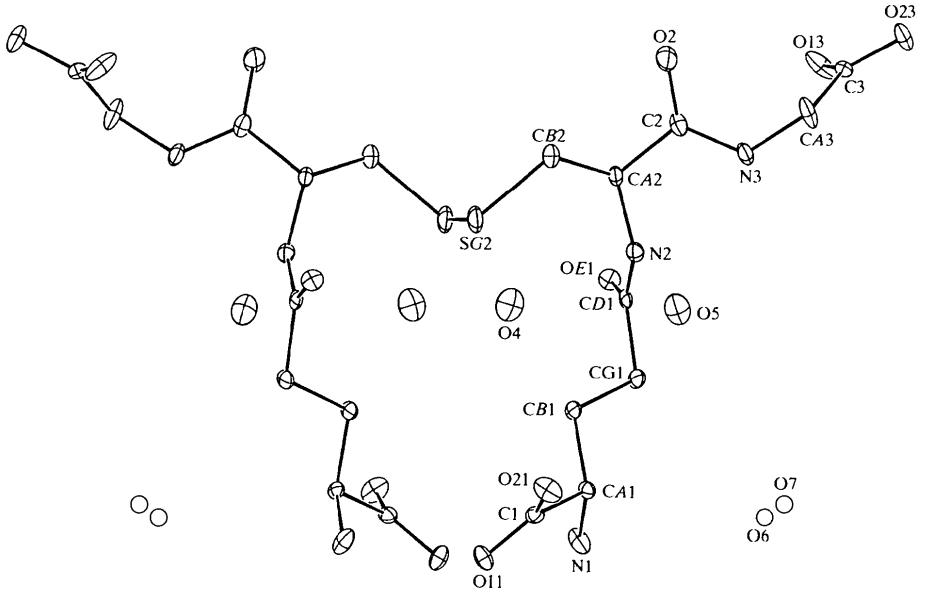


Fig. 1. Representation of the molecular structure and atom-numbering scheme of GSSG and the water molecules. The displacement ellipsoids are shown at the 50% probability level. The two disordered water molecules are shown as small circles corresponding to the sites with the highest occupancy factor.

dihedral angles of the main peptide chain are given in Table 1.

The glutathione molecule has two carboxyl groups (Fig. 1). An H atom (H23) was localized in the vicinity of the carboxyl O23 atom by difference Fourier synthesis. Moreover, the C3—O23 bond length is 1.323 (2) Å, which is clearly longer compared with the other carboxyl C—O distances (Table 1). The carboxyl moiety on the glycyl residue is thus neutral, whereas on the glutamyl residue it is negatively charged. The H atom protonating the O23 oxygen is involved in a hydrogen bond with the carboxylate group of a neighboring molecule (Table 2).

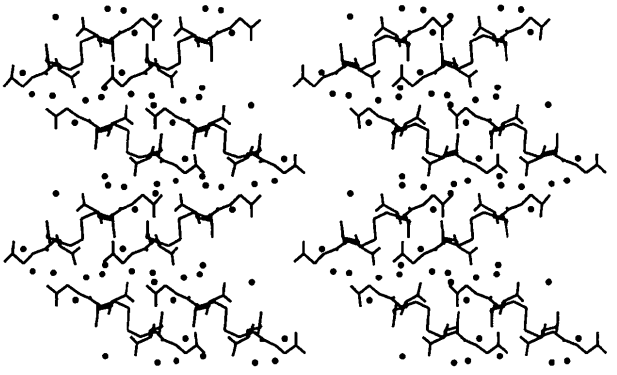


Fig. 2. A stereographic view of the GSSG crystal packing, with *a* horizontal and *b* vertical. Water molecules are represented as black dots and only one site for each of the two disordered water molecules is shown.

The amine group of the glutamyl residue is clearly positively charged, as the three H atoms are visible in the electron-density maps. The two amine and two carboxylate groups present in the GSSG peptide form two intramolecular head-to-tail salt bridges (N1—H21···O11) related by the diad axis. The amine moiety is also hydrogen bonded to the carbonyl O2 atom and to the disordered O6 water molecule (Table 2).

Two water molecules out of the four present in the asymmetric unit are disordered; this might be ascribed to the two solvent-channel networks in the GSSG·8H_2O crystals. The most visible in Fig. 2 is a straight channel parallel to the *a* axis, while the second one is a zigzag in the direction of the *b* axis. The presence of these channels explains why the crystals dry out after a few days of exposure to air.

Experimental

GSSG was purchased from SIGMA (St Louis, USA). Slow evaporation of water solutions of GSSG led to a viscous liquid without the formation of crystals. However, it was possible to obtain crystals in 3–4 d in the presence of the precipitating agent polyethylene glycol by vapor diffusion in a sealed volume at room temperature. The glutathione peptide was dissolved in an aqueous solution of polyethylene glycol (MW 8000) and drops of this solution were equilibrated against a concentrated solution of the polymer. This result shows that crystallization techniques employed for proteins can also be fruitful in the case of peptides. Since the crystals lose their water content over a period of a few days on exposure to air,

they were placed, after removal of the surrounding liquid, in a sealed quartz capillary. To avoid crystal drying, the capillary contained, on the side, a small amount of mother liquor. The stream of liquid nitrogen at 100 K, surrounded by a stream of dry air, was produced by an Oxford Cryosystems cooler (600 series; Cosier & Glazer, 1986). The diffractometer system was in a closed volume and no ice formation was observed on the capillary.

Crystal data

$C_{20}H_{32}N_6O_{12}S_2\cdot 8H_2O$
M_r = 756.76
Orthorhombic
$P2_12_12$
a = 9.4742 (2) Å
b = 16.6161 (4) Å
c = 10.8918 (7) Å
V = 1714.63 (12) Å^3
Z = 2
D_x = 1.466 Mg m^{-3}
D_m not measured

Ag $K\alpha$ radiation
λ = 0.56087 Å
Cell parameters from 57 reflections
θ = 3–22°
μ = 0.134 mm^{-1}
T = 100 (2) K
Rectangular prism
0.30 × 0.22 × 0.20 mm
Colorless

Data collection

Nonius Kappa–CCD diffractometer
Oscillation scans
Absorption correction: none
23 453 measured reflections
3430 independent reflections

3263 reflections with $I > 2\sigma(I)$
R_{int} = 0.051
θ_{max} = 25.6°
$h = 0 \rightarrow 13$
$k = 0 \rightarrow 25$
$l = 0 \rightarrow 16$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.038
$wR(F^2)$ = 0.100
S = 1.108
3430 reflections
261 parameters
H atoms treated by a mixture of independent and constrained refinement

$w = 1/[\sigma^2(F_o^2) + (0.0592P)^2 + 0.3916P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.034
$\Delta\rho_{max}$ = 0.394 e Å^{-3}
$\Delta\rho_{min}$ = −0.288 e Å^{-3}
Extinction correction: none
Scattering factors from *International Tables for Crystallography* (Vol. C)

Table 1. *Selected geometric parameters (Å, °)*

O11—C1	1.257 (2)	SG2—SG2^i	2.0387 (9)
O21—C1	1.251 (2)	C3—O13	1.2021 (19)
CA2—CB2	1.543 (2)	C3—O23	1.323 (2)
CB2—SG2	1.8098 (19)		
CA2—CB2—SG2	114.41 (12)	CB2—SG2—SG2^i	105.03 (6)
CG1—CD1—N2—CA2		−169.65 (14)	
CD1—N2—CA2—C2		−125.57 (16)	
CA2—CB2—SG2—SG2^i		−58.30 (13)	
CB2—SG2—SG2^i—CB2^i		−97.0 (4)	
N2—CA2—C2—N3		14.0 (2)	
CA2—C2—N3—CA3		−174.31 (14)	
C2—N3—CA3—C3		−85.2 (2)	

Symmetry code: (i) $-x, -y, z$.

Table 2. *Hydrogen-bonding geometry (Å, °)*

D—H···A	D—H	H···A	D···A	D—H···A
N1—H11···O6C^i	0.89	1.89	2.750 (3)	162
N1—H11···O6B^i	0.89	2.23	2.943 (3)	136
N1—H11···O6C	0.89	2.37	2.949 (3)	123
N1—H11···O6A^i	0.89	2.38	3.18 (3)	150
N1—H21···O11^{ii}	0.89	2.01	2.880 (2)	166
N1—H31···O2^{iii}	0.89	2.08	2.844 (2)	144
N2—H2···O5	0.86	2.02	2.871 (2)	169
N3—H3···OE1^{iv}	0.86	2.41	3.154 (2)	145
O23—H23···O11^{iv}	0.97	1.74	2.6707 (18)	162
O4—H14···OE1	0.950 (10)	1.848 (12)	2.7870 (19)	169 (3)
O4—H24···O21^v	0.956 (10)	1.739 (11)	2.691 (2)	173 (3)
O5—H15···O4^{vi}	0.949 (10)	1.845 (17)	2.743 (2)	157 (3)
O5—H25···O7B^{vii}	0.944 (10)	2.05 (2)	2.822 (4)	138 (3)
O5—H25···O6B^{vii}	0.944 (10)	2.14 (3)	2.840 (4)	130 (3)
O5—H25···O6A^{vii}	0.944 (10)	2.65 (5)	3.49 (4)	149 (3)

Symmetry codes: (i) $1-x, -y, z$; (ii) $-x, -y, z$; (iii) $x, y, 1+z$; (iv) $1+x, y, z-1$; (v) $\frac{1}{2}-x, y-\frac{1}{2}, -z$; (vi) $\frac{1}{2}-x, \frac{1}{2}+y, -z$; (vii) $x-\frac{1}{2}, \frac{1}{2}-y, -z$.

The absolute stereochemistry of GSSG is known (L-amino acids) and was thus used to fix the correct enantiomorph for refinement. All the H atoms could be located in difference Fourier maps, except for those bonded to the two disordered O6 and O7 water molecules. The H atoms of the GSSG molecule were constrained using the *AFIX* facility of *SHELXL*97 (Sheldrick, 1997). The water H-atom positions were refined using distance restraints. These disordered water molecules were split into three and two parts, respectively, with the sum of the occupancies restrained to 1.

Data collection: *COLLECT* (Nonius, 1998). Cell refinement: *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Data reduction: *DENZO* and *SCALEPACK*. Program(s) used to solve structure: *SIR*92 (Altomare *et al.*, 1994). Program(s) used to refine structure: *SHELXL*97. Molecular graphics: *ORTEP* (Johnson, 1970).

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: SX1077). Services for accessing these data are described at the back of the journal.

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