

## Peptide–lanthanide interactions

### Crystal structure of a europium(III)–triglycine complex

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Crystals of  $\text{Eu}(\text{Gly-Gly-Gly})\cdot(\text{H}_2\text{O})_5\cdot(\text{ClO}_4)_3$  are triclinic, spacegroup  $P\bar{1}$  with  $a = 9.123$  (2),  $b = 11.185$  (5),  $c = 11.426$  (2) Å;  $\alpha = 90.79$  (2),  $\beta = 98.08$  (1),  $\gamma = 98.57$  (2)°;  $Z = 2$ . The europium cation is surrounded by four oxygens from three different peptide units and four oxygens from water molecules. The geometry around the metal is a distorted bi-capped trigonal prism. The peptide backbone conformation in this complex is compared with those in the free peptide and in various metal complexes. Considerable differences are observed between Eu(III) and Ca(II) complexes of triglycine. © Munksgaard 1994.

**Key words:** crystal structure; europium–peptide complex; glycyglycylglycine; peptide conformation; peptide–metal interactions

Lanthanide ions have been extensively used as spectroscopic probes of biological structures. Europium(III) and gadolinium(III) cations have been used as perturbing agents of chemical shifts and relaxation rates in biomolecules, in an approach to three-dimensional structure determination in aqueous solutions (1, 2). Terbium(III) is extensively employed as a fluorescent probe for metal binding sites in proteins (3). Studies of the interaction of lanthanides with biological systems acquire particular importance in view of the similarities in the sizes of the Ca(II) and Ln(III) ions and their preference for oxygen donors in complex formation (4). The use of lanthanide ions as a probe for Ca(II) binding sites has been widespread (1). A major prerequisite for this application is that binding to metal ions should not significantly perturb the structure being investigated. Systematic structural investigations of metal peptide complexes and a comparison with free peptide structures will permit an assessment of the conformational changes that result from metal binding to flexible peptide ligands. In this paper we report the structure of an europium triglycine complex, which shows pronounced differences from the structure of the calcium complex as well as that of free triglycine.

#### EXPERIMENTAL

A mixture of glycyglycylglycine (0.034 g;  $1.79 \times 10^{-4}$  mol) and europium perchlorate hexahydrate (0.1 g;  $1.79 \times 10^{-4}$  mol) was dissolved in 10 mL

of water. On slow evaporation of the solution at room temperature, colourless crystals of the complex  $\text{Eu}(\text{Gly-Gly-Gly})\cdot(\text{H}_2\text{O})_5\cdot(\text{ClO}_4)_3$  were obtained. The crystals were very hygroscopic. A suitable crystal with the dimension  $0.2 \times 0.75 \times 0.3$  mm was sealed in a Lindemann capillary for X-ray diffraction studies. The cell parameters are: Space group  $P\bar{1}$ ;  $a = 9.123$  (2),  $b = 11.185$  (5),  $c = 11.426$  (2) Å;  $\alpha = 90.79$  (2),  $\beta = 98.08$  (1),  $\gamma = 98.57$  (2)°;  $Z = 2$ . Intensity data were collected on a Enraf-Nonius CAD-4 diffractometer equipped with graphite monochromated Mo  $K_\alpha$  radiation ( $\lambda = 0.7107$  Å),  $\theta_{\text{max}} = 29^\circ$ , using the  $\omega/2\theta$  scan technique. The intensities of 6688 reflections were measured; of these 6064 were unique. For structure solution, 5729 reflections with  $F > 8\sigma(F)$  were used. The structure was solved by the Patterson heavy-atom method using the SHELXS 86 (5) program. Least-squares refinements were performed by the full-matrix method using SHELX 76 (6). One of the coordinated water molecules and a perchlorate ion showed disorder, hence they were refined isotropically; all other non-hydrogen atoms were refined anisotropically. The disordered water molecule was refined in two positions with partial occupancies. Most of the hydrogen atoms were located from the difference Fourier map. The remaining hydrogens were stereochemically fixed. All the hydrogen atoms were assigned 1.5 times the temperature factors of the non-hydrogen atoms to which they were attached. The hydrogen atom positions were not refined but were merely included in the structure factor

calculations. The structure was refined to an  $R$ -value of 0.057 ( $R_w = 0.061$ ,  $w = 1.0000/[\sigma^2(F) + 0.003078F^2]$ ). Fractional coordinates, along with isotropic thermal parameters, are listed in Table 1.

## RESULTS AND DISCUSSION

### Metal coordination

A view of the crystal structure is shown in Fig. 1. The coordination polyhedron around the metal is depicted

TABLE 1

Non-hydrogen atomic coordinates and isotropic thermal parameters ( $10^4 \text{ \AA}^2$ ) for  $\text{Eu}(\text{Gly-Gly-Gly}) \cdot (\text{H}_2\text{O})_5 \cdot (\text{ClO}_4)_3$

Atom	X/A	Y/B	Z/C	$U_{\text{eq}}$	$U_{\text{iso}}^a$
Eu	0.12358 (1)	0.13293 (1)	0.33312 (1)	269 (1)	
O1	0.2448 (4)	0.2890 (3)	0.2130 (3)	443 (11)	
O2	0.3615 (3)	0.0773 (3)	0.3132 (3)	408 (9)	
O3	0.8381 (4)	-0.0737 (4)	0.4767 (3)	457 (11)	
O4	0.8672 (3)	0.0526 (3)	0.3307 (3)	394 (9)	
N1	0.3518 (8)	0.5309 (5)	0.2468 (6)	675 (20)	
N2	0.4429 (4)	0.2559 (3)	0.1237 (3)	354 (10)	
N3	0.5729 (4)	0.0307 (3)	0.2576 (4)	381 (11)	
C1	0.3588 (5)	0.3265 (5)	0.1672 (4)	363 (15)	
C11	0.4101 (8)	0.4601 (5)	0.1611 (6)	593 (22)	
C2	0.4447 (4)	0.0752 (3)	0.2379 (4)	300 (10)	
C21	0.4047 (5)	0.1260 (4)	0.1168 (4)	363 (13)	
C3	0.7898 (4)	-0.0100 (3)	0.3955 (3)	288 (10)	
C31	0.6202 (5)	-0.0161 (5)	0.3715 (4)	418 (13)	
Ow5	0.0308 (4)	0.0639 (4)	0.1275 (3)	465 (11)	
Ow6	0.1077 (4)	-0.0877 (3)	0.3154 (4)	549 (13)	
Ow7	-0.0399 (5)	0.2766 (4)	0.2637 (6)	751 (19)	
Ow8 <sup>b</sup>	0.2762 (12)	0.3023 (9)	0.4579 (9)	800 (23) <sup>c</sup>	
Ow8' <sup>b</sup>	0.1882 (14)	0.3092 (11)	0.4642 (10)	646 (26) <sup>c</sup>	
Ow50 <sup>d</sup>	0.0410 (8)	0.5102 (6)	0.2049 (11)	1590 (51)	
Cl1	0.7659 (1)	0.2282 (1)	-0.0473 (1)	494 (4)	
Cl2	0.2497 (1)	0.2805 (1)	0.8096 (1)	519 (4)	
Cl3	0.6810 (3)	0.3606 (2)	0.4595 (2)	889 (7)	
O20	0.7118 (6)	0.3285 (4)	0.0057 (5)	715 (19)	
O21	0.7167 (6)	0.1225 (4)	0.0126 (5)	749 (19)	
O22	0.9246 (5)	0.2442 (6)	-0.0308 (5)	800 (21)	
O23	0.7119 (6)	0.2161 (6)	-0.1724 (5)	786 (21)	
O30	0.1212 (6)	0.2907 (5)	0.7220 (5)	795 (20)	
O31	0.2693 (7)	0.3759 (5)	0.8946 (4)	817 (20)	
O32	0.2241 (8)	0.1673 (5)	0.8601 (6)	914 (24)	
O33	0.3764 (7)	0.2885 (7)	0.7536 (9)	1237 (36)	
O40	0.6173 (16)	0.2791 (14)	0.5432 (13)	1908 (50) <sup>c</sup>	
O41	0.8093 (16)	0.4284 (14)	0.5154 (13)	1972 (51) <sup>c</sup>	
O42	0.5875 (18)	0.4389 (15)	0.4110 (15)	2219 (66) <sup>c</sup>	
O43	0.6835 (10)	0.2917 (8)	0.3541 (7)	1117 (22) <sup>c</sup>	

<sup>a</sup>  $U_{\text{eq}} = 1/3 \sum_i \sum_j U_{ij} a_i^* a_j^*$ .

<sup>b</sup> Ow8 corresponds to a disordered water molecule; the site occupancy factors for Ow8 and Ow8' are 0.6 and 0.4, respectively. The three perchlorate ions Cl1, Cl2, and Cl3 bear the oxygen atoms labelled as (O20, O21, O22, O23), (O30, O31, O32, O33) and (O40, O41, O42, O43), respectively. Cl3 is a disordered perchlorate ion.

<sup>c</sup> Isotropically refined.

<sup>d</sup> Lattice water.

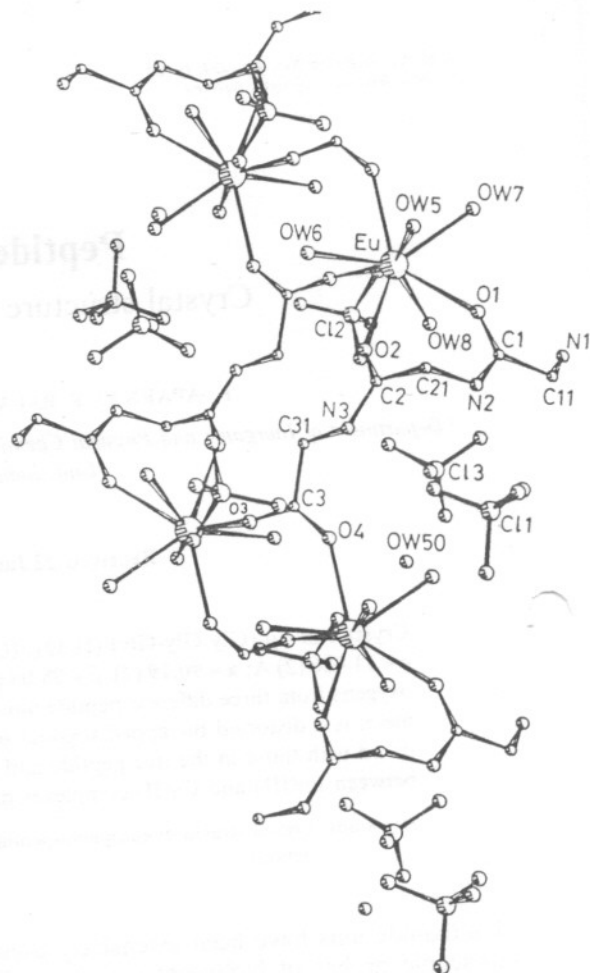


FIGURE 1

View of crystal structure of  $\text{Eu}(\text{Gly-Gly-Gly}) \cdot (\text{H}_2\text{O})_5 \cdot (\text{ClO}_4)_3$  along the  $Y$ -axis.

in Fig. 2. The europium cation is surrounded by four oxygens from three different peptide units and four oxygens from water molecules, making the coordination number eight. Selected Eu–O distances and O–Eu–O

TABLE 2

Selected bond distances ( $\text{\AA}$ ) and bond angles ( $^\circ$ ) in  $\text{Eu}(\text{Gly-Gly-Gly}) \cdot (\text{H}_2\text{O})_5 \cdot (\text{ClO}_4)_3$

Bond distances		Bond angles	
Eu–O1	2.464 (4)	O1–Eu–O2	74.1 (1)
Eu–O2	2.385 (3)	O1–Eu–O3	136.5 (1)
Eu–O3	2.275 (4)	O1–Eu–O4	130.8 (1)
Eu–O4	2.374 (3)	O2–Eu–O3	87.0 (1)
Eu–Ow5	2.455 (3)	O2–Eu–O4	142.8 (1)
Eu–Ow6	2.454 (4)	O3–Eu–O4	87.1 (1)
Eu–Ow7	2.419 (5)		
Eu–Ow8	2.478 (9)		

TABLE 3

Selected hydrogen bond distances ( $\text{\AA}$ ) and related angles ( $^\circ$ ) in  $\text{Eu}(\text{Gly-Gly-Gly}) \cdot (\text{H}_2\text{O})_5 \cdot (\text{ClO}_4)_3^a$ 

D-H...A	D-A	D-H-A
N1-H1...O23	3.08	137.5
N1-H1...O33	2.96	125.5
N1-H2...O42	2.96	134.4
N2-H6...O20	2.97	152.9
N3-H9...O21	3.36	164.8
Ow5-H14...O22	2.91	154.6
Ow5-H15...O32	3.23	134.2
Ow6-H12...O30	2.83	131.2
Ow6-H12...O32	3.38	133.8
Ow6-H13...O23	2.97	161.7
Ow7-H17...O43	2.88	129.6
Ow50-H19...O13	3.34	141.4

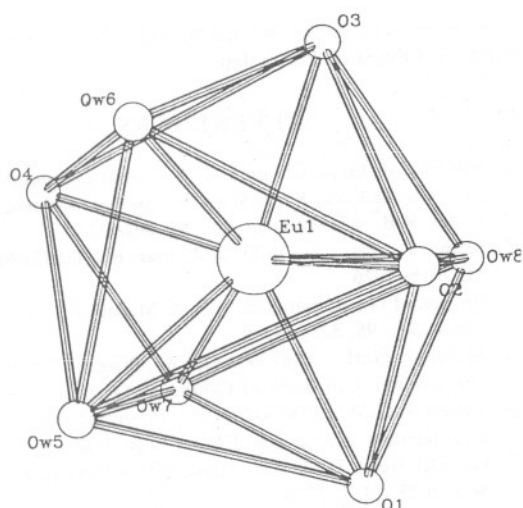
<sup>a</sup> D = donor; H = hydrogen; A = acceptor.

FIGURE 2

Coordination polyhedron around the europium metal ion.

angles are listed in Table 2. Several hydrogen bonds involving the tripeptide units, water molecules and the perchlorate ions have been identified in the crystal. Selected hydrogen-bond distances and related angles are listed in Table 3. One tripeptide carboxyl group bridges two metal ions, and the two carbonyl groups are chelated to another metal ion, thereby forming a polymeric network. The coordination polyhedron around the metal is a distorted bi-capped trigonal prism. The space between the polymeric chains is filled by one uncoordinated water molecule and three perchlorate anions per metal ion, which remain outside the coordination sphere of the metal.

The structure of the europium complex is unique and differs from those of other metal complexes of triglycine. It may be noted that in the calcium complex  $\text{CaCl}_2 \cdot (\text{Gly-Gly-Gly}) \cdot 3\text{H}_2\text{O}$  (7), the Ca(II) ion is bonded to seven oxygen atoms from four different pep-

ptide molecules and two water molecules. The major structural difference between these two complexes is that in the Ca(II) complex the carboxylate group of the tripeptide is chelated to a calcium ion and its carbonyl groups bridge two other calcium ions, whereas in the europium complex the carboxylate group bridges two different europium ions and the carbonyl groups are chelated to another europium ion. In the Cu(II) (8, 9), and Zn(II) (10) complexes, the tripeptide binds to the metal *via* one of its nitrogen atoms and a carbonyl oxygen atom. In the Ru(II) complex (11), only the two nitrogen atoms of the tripeptide are involved in bonding to the metal. It is also worth noting that in the neodymium perchlorate-diglycine complex (12), two Nd(III) centers are bridged by two bidentate and two tridentate carboxyl groups, and the coordination around each Nd(III) ion is completed by four more oxygen atoms, two from carbonyl oxygens and two from water oxygen atoms, making the coordination number nine.

TABLE 4

Comparison of structural parameters<sup>a</sup> in metal complexes of Gly-Gly-Gly (GGG)

Compound	$\psi_1$	$\omega_1$	$\phi_2$	$\psi_2$	$\omega_2$	$\phi_3$	$\psi_3$	$\psi'_3$	Coordinated atoms
GGG	-149.9	-176.8	177.6	-171.5	-178.5	172.8	-172.6	6.9	-
Eu-GGG	158.1	176.5	78.2	99.3	-178.3	154.7	163.3	-13.7	O1, O2, O3, O4
Ca-GGG	-163.4	179.1	98.4	3.3	-177.2	-110.5	-167.1	8.5	O1, O2, O3, O4
GGG·HCl	-164.8	-172.1	153.4	-159.7	178.5	80.1	-172.7	4.8	-
GGG·Li	-147.1	-176.6	62.5	-141.0	-175.0	145.1	179.1	-1.9	O2, O3, O4
Zn-GGG	-164.7	-171.1	-91.8	-160.0	-175.3	109.5	-169.4	2.0	O1, O3, N1
Cu-GGG	-167.1	-171.5	-114.2	-134.9	178.5	-84.5	-175.2	6.8	O1, O3, N1
Cu-GGG-(Na)	6.2	179.7	-178.8	12.3	-173.1	-131.5	-160.9	-58.8	O4, N1, N2, N3
Ru-GGG	11.8	-175.0	-100.1	-5.5	-175.8	77.4	-171.0	-9.2	N1, N2

<sup>a</sup>  $\phi$ ,  $\psi$  and  $\omega$  defined according to ref. 16.

*Peptide backbone conformations: a comparison of metal complexes*

The backbone dihedral angles for free triglycine and its various metal complexes reported in the literature are listed in Table 4. Since all the complexes [with the exception of the Li(I) complex (13)] crystallize in centrosymmetric space groups, the sign of the backbone torsional angles has been chosen by selecting the enantiomorphic conformation which gives the best superposition on the arbitrarily chosen enantiomer of the free triglycine. For the Li(I) complex, the reported coordinates produced the best superposition. With the exception of an early structure *viz.* CuGGG(Na) (9), refined to only a moderate accuracy ( $R = 13.2$ ), in all other cases the  $\phi, \psi$ -angles of the central glycine residue lie within the sterically allowed limits. This observation suggests that metal coordination does not energetically over-ride local steric constraints.

Complexation of triglycine to Eu(III) leads to significant differences in the backbone conformations, particularly at Gly(2), as seen by changes of  $\phi, \psi$ -values (Table 4). In free triglycine (14) and its hydrochloride (15), the two carbonyl groups of Gly(1) and Gly(2) are oriented in opposite directions along the peptide backbone. In the europium complex, because of the chelate effect, the two carbonyl groups are turned to one side to form a seven-membered ring. Backbone conformational changes are also observed upon Ca(II) complexation to triglycine, but the details of these structural changes are different, as shown by the data in Table 4.

The present work on the structure of the Eu(III)-triglycine complex clearly shows that the use of lanthanides as 'models' for Ca(II) in biological systems must be viewed with caution. The assumption of isomorphism between Ca(II) and Eu(III) complexes requires a more detailed examination. It is also worth noting that large conformational changes have been observed between uncomplexed and metal-complexed cyclic peptide ionophores such as valinomycin and antamanide (17).

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