The crystallographic studies of metal-peptide complexes

VI. DISODIUM TETRAGLYCYLGLYCINATOCUPRATE(II) 4.5-HYDRATE*

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SUMMARY

A violet-pink complex may be crystallized at high pH from a solution containing Cu2+ ions and the pentapeptide, tetraglycylglycine. A three-dimensional x-ray crystal structure analysis has shown that the complex is disodium tetraglycylglycinatocuprate(II) 4.5-hydrate, Na₂Cu(NH₂(CH₂CON)₂-CH₂CONHCH₂COO)-4.5H₂O. The crystals are triclinic, a = 7.306 Å, b = 10.902 Å, c = 12.012 Å, α = 91.73°, β = 103.64°, γ = 96.14°, Z = 2, Dm = 1.76 ± 0.02, Ds = 1.76 g⋅cm⁻³, space group P1. The intensities of 3003 x-ray reflections were recorded by counter methods. The structure was refined to a conventional residual R = 0.080.

The copper atom has coordination number four and a square planar configuration. It is bound to the peptide at the terminal N(amino) and the first three N(peptide) atoms. The amide protons are dissociated from the three metal-binding peptide groups. The fourth peptide group is not deprotonated. Neither it nor the terminal carboxyl group plays any part in the metal binding. The carboxyl group is so oriented that it accepts a weak hydrogen bond from the terminal amino group of the same peptide.

In the crystal, complexes related by centers of symmetry form dimers linked by two hydrogen bonds, from the free N(peptide) in each complex to an O(peptide) in the other. In each complex the O(peptide) which is involved in this hydrogen bond is also the atom which makes the closest nonbonding contact (3.17 Å) with the copper in the other half of the dimer.

The Na⁺ ions are surrounded by octahedra of oxygen atoms belonging partly to H₂O molecules and partly to peptide and carboxyl groups. The octahedra share edges to form blocks of four. The blocks are stacked on top of one another to form columns, and these column separate regions of the crystal in which the complex anions are stacked.

The crystal structure analyses of Cu(II) complexes of glycine (1), glyceylglycine (2), diglycylglycine (3), and triglycylglycine (4) have confirmed the existence of, and provided geometrical details for, metal-peptide interactions whose nature can be inferred from such properties of Cu(II)-peptide mixtures in solution as their potentiometric titration curves (6–11), their ultraviolet-visible (10, 11), infrared (12), and proton magnetic resonance (13) spectra, and their thermodynamic functions (14). The predicted formation of a chelate ring between the terminal N(amino) and the first O(peptide) atoms at low pH is found in the structure of Cu(Gly-Gly)Cl·1.5H₂O (3). The sequential loss of protons from the first, second, and third peptide groups as the pH is raised and the involvement of the corresponding N(peptide) atoms in metal binding are illustrated in the structures of Cu(Gly-Gly)-3H₂O (2), Na₂Cu(Gly-Gly-Gly)-H₂O (4), and Na₂Cu(Gly-Gly-Gly-Gly)-10H₂O (5), respectively. Similar interactions, in which the terminal N(amino) and two or three neighboring N(peptide) atoms are involved in the formation of tetradentate chelates, appear to occur when Cu(II)-containing solutions of oxytocin (15), lysozyme (16), the NH₂-terminal 24-residue fragment of bovine serum albumin (17), and the NH₂-terminal 5-residue fragment of sperm whale myoglobin (11) are titrated with alkali. Deaminoxytocin and N-acetyllysine vasopressin do not form similar complexes, at least at pH <12 (15, 16), leading to the conclusion that chelation via deprotonated N(peptide) atoms requires the terminal amino group to be available as a primary metal-binding locus. Studies of model compounds show that this rule may be broken when deprotonation of a peptide group enables the N(peptide) atom to participate in a chelate ring with another suitably placed functional group, e.g. a terminal carboxyl group (as in the structure of [Cu(Gly-Leu-Tyr)]₉·8H₂O·(diethyl ether) (18)) or a histidine side chain (as in the complexes of N-acetyl-(glycyl)-L-histidine peptides in solution (19)). It has also been shown that in the presence of Cu(II) ions the peptide bond between the
fourth and fifth residues of tetracyclic glycine is much more resistant to alkaline hydrolysis than the peptide bond between the fifth and sixth residues of pentaglycylglycine (20). A plausible explanation of this observation would be that a significant proportion of the pentapeptide complexes in solution have their NH2-terminal groups free and their ligands coordinated via four deprotonated N(peptide) atoms.

Formation and ionization constants for a 1:1 mixture of Cu(II) and tetracyclic glycine and the ultraviolet-visible spectra of the species present in solution at various pH values, have been reported (11). The potentiometric titration curves for the system have been interpreted without recourse to species in which more than three protons are displaced by the formation of metal-N(peptide) bonds. We here report the crystal structure analysis of disodium tetracyclic glycinatecuprate(II) 4.5-water. This is the first structure analysis of a metal-peptide complex in which the number of potential nitrogen donor atoms on the ligand exceeds the expected coordination number, four, of the metal atom.

**EXPERIMENTAL PROCEDURE**

Freshly precipitated copper(II) hydroxide (excess) was shaken with tetracyclic glycine (0.1 g) in water (5 ml). Unreacted Cu(OH)₂ was removed by centrifugation. The resulting blue solution turned pink when the pH was adjusted to 9 to 10 by the addition of 0.1 M NaOH. Pink crystals, with maximum dimensions of 0.3 mm, separated when ethanol was added to the solution dropwise at intervals spread over 2 to 3 weeks. Larger crystals, up to 1 mm in length, grew in 2 to 3 days from a solution which had been poured into an excess of a 2:1 ethanol-actone mixture. The crystal which was used for data collection had dimensions 0.25, 0.20, and 0.02 mm perpendicular to the well developed (100), (021) and (012) faces, respectively.

**Crystal Data**

C₁₀H₁₅O₆N₅Cu₂Na₂.4.5H₂O F.W. = 490.9

Triclinic, a = 7.306(4), b = 10.902(3), c = 12.012(3) Å

α = 91.73(2)°, β = 103.64(4)°, γ = 96.14(4)°

Dₐ = 1.76 ± 0.02, Dₜ = 1.76 g·cm⁻³, U = 924.3 Å³

Z = 2, μ = 27.4 cm⁻¹, F(000) = 504

λ(CuKα) = 1.5406 Å, λ(CuKα) = 1.5443 Å

Space group P1 is compatible with structure analysis. No physical or statistical tests for centrosymmetry were carried out.

All x-ray diffraction data were recorded on a manually operated Buerger-Supper equi-inclination diffractometer (21), with nickel-filtered CuKa radiation and a scintillation counter with pulse height discrimination. The unit cell dimensions were fitted by least squares to the values of 4 sin2θ/λ² of 41 reflections for which θ ≥ 45°. The numbers in parentheses represent the statistical standard deviations in the least significant digits of the unit cell parameters. The density, Dₐ, of the crystals was determined by flotation in CCl₄-BrCH₂CH₂Br.

The reflection intensities were recorded by an ω-scan procedure. Details of the method have been described elsewhere (22). Background measurements were made at the beginning and end of each scan. The intensities were corrected for the Lorentz and polarization factors and for absorption (23). An expression due to Hoard and Jacobson (24) was used to assign standard deviations σ(F) to the structure amplitudes |F(obs)| with allowance for systematic as well as statistical (counting) errors. (The constants Cᵣ, Cₛ, and Cₛ in this expression were given values 0.02, 0.05, and 0, respectively.) A total of 3003 independent reflections were recorded for the layers Hkl (0 ≤ H ≤ 8). Of these reflections, 869 were unobservably weak (i.e. the integrated peak counts differed from the respective background counts by less than 3 S.D.).

**STRUCTURE ANALYSIS AND REFINEMENT**

The position of the copper atom was established by a three-dimensional Patterson synthesis. The positions of all of the other atoms were then readily found in a three-dimensional electron density map based on phases calculated from the copper atom alone. The only equivocal feature of the map was a pair of peaks close to and related by, the symmetry center at (0.5, 0.5, 0). If the peaks were merely artifacts of the data, then the structure was left with an improbably large cavity in which a water molecule could easily be accommodated. If one of these peaks represented a water molecule, then the symmetry-related peak lay too close to it to represent anything other than a vacant site. Accordingly, the oxygen O(11) of a water molecule was included in the structure with occupancy 0.5 on the assumption that it was randomly distributed over the two sets of sites.

Three cycles of full matrix least squares refinement with isotropic vibrational parameters reduced the residual R, defined as Σ ||F(obs) - s| - |F(calc)|| / Σ |F(obs)|, to 0.123. Refinement was continued with anisotropic vibrational parameters for all atoms except O(11). One cycle in which only the vibrational parameters and the over-all scale were refined further reduced R to 0.087. The total number of variable parameters now exceeded the capacity of the available combination of program and computer. The atoms were therefore divided into two overlapping groups, all of whose parameters were refined in alternate cycles. (With the list numbers from Table I, the first group consisted of atoms 1 to 14, 23, 25 to 27, 29, and the second group of atoms 1, 2, 10 to 24, 27, 28. In this way each cycle dealt with a maximum number of variables between which interactions were likely to occur.) Convergence was reached when R = 0.080. A (F(obs) - F(calc))-synthesis was calculated after the completion of the refinement. It contained no significant peaks, and hydrogen atom positions could not be deduced from it. The final atomic positional and thermal parameters are listed in Table I. Since correlating data from intersecting layers were not used to show that the intensity data were recorded on a single scale (21), we do not interpret the anisotropic temperature parameters in terms of thermal vibration ellipsoids.

The function minimized in the least squares refinement was \( \Sigma w \left( |F_{\text{obs}}| - |F_{\text{calc}}| \right)^2 \) where \( w = \sigma^{-2}(F) \). Standard atomic scattering factors were used for C, N, O (25) and for Cu⁺ (26). The curve for Cu⁺ was corrected for the real part of the anomalous scattering by subtracting 2.1 electrons over the whole sin@ range (27). The refinement calculations were made with the program ORFLS (28).

* The complete list of observed and calculated structure amplitudes may be ordered as NAPS Document 01156 from ASIS National Auxiliary Publications Service, c/o CCM Information Corporation, 909 Third Avenue, New York, New York 10022, remitting $2.00 for microfiche and $5.00 for photocopies.
DESCRIPTION OF STRUCTURE

The atoms of the ligand molecule have been labeled as follows:

\[
\text{NH}_2-\text{CH}_2-\text{C}-\text{NH}_2-\text{CH}_2-\text{C}-\text{NH}_2-\text{CH}_2-\text{C}-\text{NH}_2-\text{CH}_2-\text{C}-\text{NH}_2-\text{CH}_2-\text{C}-\text{O}
\]

The pentapeptide acts as a tetradentate ligand via the N(1)(amino) atom and the three deprotonated N(peptide) atoms N(2), N(3), and N(4). The fourth peptide group and the terminal carboxyl group are not involved in metal binding. The coordination number of the copper atom is four, and the coordination is square planar. Fig. 1 is a stereoscopic diagram of a single complex.

**TABLE I**

Atomic parameters in Na₂[Cu(Gly-Gly-Gly-Gly-Gly)]·5H₂O with their standard deviations (in parentheses)

| No. | Atom | Fractional coordinates x 10⁴ | Thermal parameters x 10⁴ |
|-----|------|-----------------------------|--------------------------|
|      |      | \(x\) | \(y\) | \(z\) | \(B_{iso}\) | \(B_{iso}\) | \(B_{iso}\) | \(B_{iso}\) |
| 1    | Cu   | 2317 (09) | 4158 (01) | 4001 (01) | 194 (03) | 37 (01) | 41 (01) | 14 (01) | 17 (01) | 3 (01) |
| 2    | M(1) | 1771 (10) | 3511 (06) | 2336 (06) | 234 (10) | 41 (07) | 43 (07) | 10 (05) | 25 (05) | 0 (06) |
| 3    | H(1) | 1345 (15) | 4847 (05) | 1302 (02) | 301 (13) | 83 (12) | 37 (09) | 19 (16) | 9 (11) | 11 (08) |
| 4    | O(1) | 1354 (12) | 5772 (09) | 2726 (07) | 188 (21) | 84 (11) | 38 (06) | 24 (12) | 11 (10) | 12 (07) |
| 5    | O(2) | 0607 (10) | 6741 (06) | 1737 (06) | 352 (21) | 77 (08) | 68 (07) | 60 (10) | 38 (09) | 35 (06) |
| 6    | N(1) | 1754 (09) | 5560 (03) | 3365 (05) | 188 (17) | 43 (07) | 49 (06) | 19 (09) | 12 (06) | 6 (06) |
| 7    | O(3) | 1564 (12) | 6671 (07) | 4132 (07) | 245 (21) | 29 (09) | 34 (07) | 15 (10) | -7 (10) | -5 (06) |
| 8    | O(4) | 2104 (11) | 6193 (07) | 5444 (08) | 145 (19) | 43 (09) | 64 (09) | 1 (10) | 34 (10) | -75 (07) |
| 9    | Cu   | 1994 (09) | 6871 (07) | 5398 (07) | 269 (10) | 61 (06) | 60 (06) | 19 (09) | 21 (08) | -7 (05) |
| 10   | H(1) | 2667 (05) | 5578 (07) | 5432 (07) | 204 (17) | 43 (07) | 28 (06) | 32 (06) | 20 (08) | 8 (05) |
| 11   | O(5) | 3223 (12) | 4429 (07) | 3549 (07) | 202 (21) | 28 (08) | 36 (07) | 20 (10) | -6 (10) | 0 (06) |
| 12   | O(6) | 3270 (10) | 3996 (08) | 6105 (07) | 98 (16) | 56 (09) | 33 (07) | 7 (09) | 7 (09) | 12 (06) |
| 13   | O(7) | 4277 (09) | 4843 (03) | 3365 (05) | 232 (25) | 56 (06) | 44 (05) | 37 (07) | 7 (07) | 17 (05) |
| 14   | O(8) | 3699 (09) | 2854 (06) | 4995 (07) | 115 (15) | 39 (07) | 54 (07) | 4 (08) | 10 (08) | 12 (06) |
| 15   | O(9) | 3718 (11) | 1644 (07) | 4603 (07) | 189 (21) | 24 (06) | 50 (08) | 22 (10) | -5 (10) | -7 (06) |
| 16   | O(10)| 2628 (10) | 748 (07) | 3965 (07) | 239 (22) | 12 (08) | 26 (07) | 15 (10) | 7 (10) | 8 (06) |
| 17   | O(11)| 2360 (09) | -243 (03) | 3353 (05) | 230 (20) | 43 (06) | 77 (07) | 18 (08) | 15 (08) | 15 (05) |
| 18   | O(12)| 2668 (09) | 1048 (06) | 3875 (05) | 134 (15) | 45 (07) | 51 (06) | 10 (09) | 12 (06) | 2 (06) |
| 19   | O(13)| -1456 (12) | 242 (08) | 2378 (07) | 197 (21) | 59 (09) | 33 (07) | -23 (11) | -18 (10) | -5 (05) |
| 20   | O(14)| -2977 (12) | 509 (06) | 2204 (06) | 280 (24) | 41 (09) | 60 (09) | 33 (11) | 16 (12) | -5 (05) |
| 21   | O(15)| -962 (06) | 1129 (06) | 1518 (05) | 201 (15) | 91 (08) | 64 (06) | 1 (08) | 21 (08) | 24 (06) |
| 22   | O(16)| -3728 (06) | 33 (05) | 1484 (05) | 188 (14) | 66 (07) | 59 (06) | -2 (07) | -8 (07) | 8 (05) |
| 23   | O(17)| -6391 (05) | -1139 (05) | 2024 (07) | 263 (10) | 76 (04) | 50 (03) | 63 (10) | 17 (14) | 4 (06) |
| 24   | O(18)| -5609 (05) | 1448 (01) | 220 (03) | 271 (10) | 72 (04) | 58 (03) | 35 (05) | 23 (05) | 6 (03) |
| 25   | O(19)| -7238 (10) | 2138 (08) | 300 (07) | 267 (21) | 186 (12) | 110 (08) | 23 (12) | 24 (10) | 34 (08) |
| 26   | O(20)| 4567 (09) | 6968 (06) | 8241 (06) | 272 (28) | 94 (08) | 72 (06) | 47 (10) | 12 (10) | 26 (06) |
| 27   | O(21)| 2077 (10) | 2014 (07) | 6890 (06) | 340 (25) | 177 (05) | 77 (07) | 56 (11) | 31 (10) | 26 (06) |
| 28   | O(22)| -7974 (10) | 291 (08) | 891 (07) | 296 (21) | 166 (11) | 106 (08) | 65 (12) | 57 (10) | 42 (08) |

**FIG. 1.** Stereoscopic view of one [Cu(Gly-Gly-Gly-Gly-Gly)]²⁻ complex. (This view is obtained if the complex at \(z,\bar{y},\bar{z}\) with respect to the coordinates in Table I is placed in a right-handed coordinate system.)
complex. The bond lengths and interbond angles are summarized in Fig. 2. The standard deviations are quoted with the reservation that they represent (characteristically for X-ray diffraction results) only the random errors of measurement and may therefore be underestimated.

In Table II are listed the deviations of individual atoms from planes fitted by least squares to groups of them. Planes 1 and 2 represent the coordination square, plane 3 the coordinated portion of the peptide molecule, planes 4 to 7 the four peptide groups (C-N(CO)-N-C'), plane 8 the carboxyl group, and planes 9 to 11 the three metal-binding peptide groups minus the Cu atoms (C-N(CO)-N-C'). The four donor nitrogen atoms are displaced from their plane of best fit in the sense of a very flattened tetrahedron, but their displacements are only of the order of 1 S.D. of their atomic positional coordinates. The distance of the copper atom from the plane of the donor atoms is negligible. The coordinated portion of the ligand molecule is, however, far from planar so that the chelate rings must be appreciably puckered. Among the three metal-binding peptide groups, only the second is unequivocally planar. For the first peptide group, the deviations of the atoms are of the order of 2 S.D. of the atomic positional coordinates, and the third peptide group is clearly not represented by its plane of best fit. If the Cu atoms adjacent to the N(peptide) atoms are omitted from the least squares calculations, then the three C-N-C bonds (29) is of only limited applicability with respect to the coordinated portion of the peptide in this complex. If we ignore the N-Cu bonds and use the least squares planes fitted to the peptide and carboxyl groups (planes 4 to 8 in Table II), then the torsion angles are: $\omega_1 = 178^\circ$, $\psi_1 = 4^\circ$, $\chi_1 = 188^\circ$, $\psi_2 = 355^\circ$, $\psi_3 = 183^\circ$, $\psi_4 = 248^\circ$, $\psi_5 = 185^\circ$, $\psi_6 = 91^\circ$, $\psi_7 = 144^\circ$. The values for $\omega_1$ and $\psi_1$ are represented by points on the "steric map" which are forbidden for a pair of linked peptide units (30), but which become allowed when adjacent N(peptide) atoms are coordinated to a metal. The values of $\omega_1$ and $\psi_1$ lie close to permitted portions of the steric map. They are associated with the free peptide group and terminal carboxyl group atoms.
Table II

Distances (in Angstrom units) from planes fitted by least squares to groups of atoms in Na₄Cu(Gly-Gly-Gly-Gly-Gly)-4.5H₂O

A distance in parentheses indicates that the atom was not included in the calculation of that plane.

| Atom      | Plane | | | | | | | | | |
|-----------|-------|---|---|---|---|---|---|---|---|---|
|           | 1     | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |
| Cu        | (-0.005) | -0.004 | 0.056 | (-0.239) | (0.281) | (0.027) |
| N(1)      | -0.005 | -0.004 | 0.186 | (-0.082) | (0.193) | (-0.110) |
| C(1)      | (0.111) | 0.080 | 0.013 |      |      |      |
| C(2)      | (0.168) | -0.059 | -0.017 |      |      |      |
| O(1)      |     | -0.206 | 0.004 |      |      |      |
| N(2)      | 0.006 | 0.007 | 0.033 | -0.011 |      |      |
| C(3)      | (-0.029) | -0.024 | 0.012 | 0.003 |      |      |
| C(4)      | (-0.139) | 0.026 | 0.002 |      |      |      |
| O(2)      |     | 0.135 | -0.002 |      |      |      |
| N(3)      | -0.006 | -0.005 | -0.067 | -0.009 |      |      |
| C(5)      | (-0.091) | -0.029 | 0.006 | -0.05 |      |      |
| C(6)      | -0.039 | -0.007 | 0.022 |      |      |      |
| O(3)      |     | -0.055 | 0.006 |      |      |      |
| N(4)      | 0.005 | 0.006 | 0.028 | 0.052 |      |      |
| C(7)      |     | (0.210) | 0.106 | -0.044 |      |      |
| C(8)      |     |      |      | 0.001 |      |      |
| O(4)      |     |      |      | 0.000 |      |      |
| N(5)      |     |      |      | 0.000 |      |      |
| C(9)      |     |      |      |      | -0.001 | 0.004 |
| C(10)     |     |      |      |      | -0.012 |      |
| O(5)      |     |      |      |      | 0.005 |      |
| O(6)      |     |      |      |      | 0.004 |      |

[FIG. 3. Stereoscopic view of a dimeric complex in Na₄Cu(Gly-Gly-Gly-Gly-Gly)-4.5H₂O. Hydrogen bonds are represented by hollow bonds.]

The nearest neighbor of the copper atom in an axial direction is an O(peptide) atom, O(2'), at 3.17 Å. This contact is clearly nonbonded. The atom O(2') belongs to a complex which is related to the original complex through the center of symmetry at 0, 0.5, 0.5. The two complexes, linked by a pair of hydrogen bonds, N(5)—H···O(2') and N(5')—H···O(2) (2.93 Å), form dimers in the crystal (Fig. 3). The protons in these hydrogen bonds cannot be supplied by the O(peptide) atoms, so that we...
have crystallographic evidence that the protons have not disso-
ciated from the fourth N(peptide) atoms of the pentapeptide
molecules. This is as expected, since the atoms N(5) do not
participate in metal binding.

The metal-peptide complex anions are stacked on top of one
another parallel to the crystallographic z-axis. The N(amine),
O(peptide), and O(carboxyl) atoms form a profusion of hydrogen-
bonded and electrostatic interactions with water molecules and
sodium ions, respectively. These contacts are listed in Table
III. The assignment of hydrogen donors and acceptors in the
hydrogen bond scheme is unequivocal and is included in Table
III.

In view of the current interest in interactions between alkali
metal ions and biological molecules, we now examine the coor-
dination geometry of the sodium ions. There are two crystallo-
graphically independent types of sodium ion, Na(1) and Na(2).
Each of them has contacts in the range 2.3 to 2.9 A with six
oxygen atoms (Table IV). The atoms around Na(1) are O(peptide)
atoms of three different complexes, an O(carboxyl) atoms of
three complexes, and two O(water) atoms. The nearest neighbors
of Na(2) are O(carboxyl) atoms of two complexes and four
O(water) atoms. The sodium ions thus provide altogether 23
crystallographically nonequivalent links between peptide com-
plexes and their environment. Of these,

three are C=O(peptide) . . . Na+ . . . O(peptide) . . . C
three are C=O(peptide) . . . Na+ . . . O(carboxyl) . . . C
one is C=O(carboxyl) . . . Na+ . . . O(carboxyl) . . . C
six are C=O(peptide) . . . Na+ . . . OH-
ten are C=O(carboxyl) . . . Na+ . . . OH-

The environments of both sodium ions are octahedral, that of
Na(2) being the more regular (Table IV). The sodium ions are
arranged in blocks of four, in which each Na(1) shares two octa-
hedron edges, and each Na(2) three, with adjacent sodium ions.

The sharing is as follows:

O(6) . . . O(10) between Na(1) and Na(2)
O(6) . . . O(7) between Na(1) and Na(2)
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TABLE IV

Environments of sodium ions in Na<sub>4</sub>[Cu(Gly-Gly-Gly-Gly-Gly)]·4.5H<sub>2</sub>O

The labels and superscripts of the oxygen atoms are explained in the legend to Table III.

| Atoms | g | Atoms | Angle | Atoms | Angle |
|-------|---|-------|-------|-------|-------|
| Na<sub>1</sub>…O(1') | 2.86 | O(1')…Na<sub>1</sub>…O(3ix) | 88° | O(6')…Na<sub>2</sub>…O(6') | 93° |
| Na<sub>1</sub>…O(3ix) | 2.30 | O(4w') | 94 | O(7') | 90 |
| Na<sub>1</sub>…O(4w') | 2.36 | O(6) | 143 | O(8w') | 88 |
| Na<sub>1</sub>…O(6) | 2.51 | O(7) | 87 | O(9w') | 173 |
| Na<sub>1</sub>…O(7) | 2.52 | O(10) | 100 | 0 | 79 |
| Na<sub>1</sub>…O(10) | 2.47 | O(3ix)…Na<sub>1</sub>…O(4w') | 109 | O(6')…Na<sub>2</sub>…O(7') | 89 |
| Na<sub>2</sub>…O(6) | 2.49 | O(4w')…Na<sub>1</sub>…O(6) | 121 | O(7')…Na<sub>2</sub>…O(8w') | 82 |
| Na<sub>2</sub>…O(8w') | 2.49 | O(7) | 97 | O(9w') | 84 |
| Na<sub>2</sub>…O(10) | 2.43 | O(10) | 164 | O(10) | 89 |
| Na<sub>2</sub>…O(9w') | 2.43 | O(8w')…Na<sub>1</sub>…O(7) | 87 | O(8w')…Na<sub>2</sub>…O(9w') | 94 |
| Na<sub>2</sub>…O(9w') | 2.38 | O(10) | 76 | O(10) | 100 |
| Na<sub>2</sub>…O(10) | 2.34 | O(7)…Na<sub>1</sub>…O(10) | 76 | O(9w')…Na<sub>2</sub>…O(10) | 107 |

Fig. 5. Left, detail from Fig. 4, showing the labels of the oxygen atoms surrounding 4 sodium ions. Center, idealized version of left, emphasizing the sharing of octahedron edges. The sodium environments are drawn as regular octahedra and viewed along the normal to a plane containing the four sodium ions. Right repetition of center without the outlines of the octahedra. The oxygen atoms are seen as parts of two hexagonally close packed layers, between which the sodium ions occupy octahedral holes.

Scheme 1

$$O(\text{peptide})--Na^+--O(\text{carboxy1})$$

$$O(\text{carboxy1})--Na^+--O(\text{peptide})$$

Difficult to trace these connections in Fig. 4, and a block of four sodium octahedra with all atoms labeled is shown again in Fig. 5, left. If the octahedra are idealized and viewed from a direction perpendicular to a plane containing the four sodium ions, then it becomes clear that the oxygen atoms form part of two adjacent hexagonally close packed layers with the sodium ions in octahedral holes (Fig. 5, center and right).

The blocks of sodium octahedra are arranged along the edges of the unit cells (Fig. 4), providing columns of positive charge between the regions of negative copper-peptide complexes. In addition to the links which each sodium ion makes between the oxygen atoms in its own coordination octahedron, the sharing of octahedron elements creates a three-dimensional network of more complicated bridges. For instance, there are six nonequivalent sequences (Scheme 1). Conversely, the peptides act as bridges between the blocks of octahedra which are anchored to them at their O(\text{peptide}) and O(\text{carboxy1}) atoms. In the y-direction the columns of octahedra are also joined by hydrogen bonds via the disordered water molecules (e.g. Na(2')…O(8)—H…O(11)…H—O(9)…Na(2vii)).

DISCUSSION

The crystal structure of the complex confirms the correctness of the view based on the physicochemical evidence discussed earlier that the metal-binding loci are the terminal N(amino) and three deprotonated N(\text{peptide}) atoms. The complex which crystallizes
from a solution is merely the least soluble under the given conditions. Clearly, the present result alone does not disprove the existence in solution of species in which the terminal NH2 group does not participate in metal binding.

Within the limits of precision implied by the standard deviations (see legend to Fig. 2), the bond lengths and angles in the metal-binding section of the peptide (N(1) to C(7)) are in agreement with the mean values found in other copper-peptide complexes (31). In the “free” peptide and carboxyl groups (C(7) to O(6)), there are significant deviations from the average dimensions in peptides (32). These deviations all involve atom (C(7): N(4)−C(7) = 1.50(l) Å (mean = 1.46 Å), N(4)−C(7)−C(8) = 114.1(7)° (111°), C(7)−C(8)−O(4) = 117.1(7)° (120.5°), C(7)−C(8)−N(5) = 119.6(7)° (116°). We avoid the temptation to seek a physical cause for these exceptions, such as might reside in the fact that C(7) is the atom in which the chelated and free parts of the peptide meet. The reservations attached to our criteria of precision have already been stated, and our comparisons are made with average values from which deviations occur even among the individual peptide dimensions upon which they are based.

While these observations show that the [Cu(Gly-Gly-Gly)]**− ion fits into the general pattern of known copper-peptide and peptide structures, it is instructive to make a more detailed comparison between this complex and Na[Cu(Gly-Gly-Gly)]•H2O (32). The modes of chelation in the two complexes are chemically identical, and the corresponding bond lengths and angles involving the copper and donor atoms are equal within the limits of precision. As expected (31), the orders of bond lengths and angles are, respectively,

\[
\text{Cu−N(amino)} > \text{Cu−N(peptide)}
\]

\[
\text{Cu−N(amino)} − \text{C}_\text{α} < \text{Cu−N(peptide)} − \text{C}_\text{α}
\]

A closer examination shows that we have to distinguish between N(peptide) atoms like N(2) and N(3), which are shared by two chelate rings, and atoms such as N(4), which belong to only one chelate ring. A difference between the Cu−N bond lengths involving the two types of N(peptide) atoms was already noted tentatively in the paper describing the tetrapeptide complex (5), and systematic angular differences were observed in a tabulation of other copper-peptide complexes (Section V,B,2 of Reference 31). These differences are real properties of copper-peptide binding and not subtle effects of the interactions between the metal-peptide anions and their environments, because there is little similarity between the “crystal packing,” hydrogen bonding, and electrostatic interactions in the structures of the tetrapeptide and pentapeptide complexes. The average dimensions in the [Cu(Gly-Gly-Gly-Gly)]**− and [Cu(Gly-Gly-Gly-Gly-Gly)]**− ions are:

\[
N(\text{amino}) \quad N(\text{peptide})
\]

\[
\begin{array}{ccc}
\text{Two chelate rings} & \text{One chelate ring} \\
\text{C}_\text{α} & \text{C}_\text{α} & \text{C}_\text{α} \\
\text{C′} & \text{C′} & \text{C′} \\
2.03 & 1.92 & 1.95 \\
125° & 116° & 117° \\
119° & 126° & 125° \\
\end{array}
\]

The close agreement between the dimensions of the penta- and tetrapeptide complexes does not extend to the conformations. For instance, the displacements of the donor atoms from their plane of best fit are 10 times greater in [Cu(Gly-Gly-Gly)]**− (0.01 to 0.06 Å) than in [Cu(Gly-Gly-Gly-Gly-Gly)]**− (0.005 to 0.006 Å). On the other hand, the sum of the squares of the deviations from a plane fitted to the chelated part of the pentapeptide ligand (plane 3 in Table II) is twice (0.132) the corresponding sum for the tetrapeptide (0.066). These are undoubtedly crude criteria, but even they suggest that the conformations—especially the degrees of puckering of the chelate rings—are sensitive to those environmental factors from which the linear and angular parameters are largely independent.

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