Metal Ion Binding by Amino Acids

Potassium Hydrogen L-Glutamate Monohydrate K(L-GluH)·H_2O

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The title compound is obtained by neutralization of aqueous solutions of L-glutamic acid with potassium hydroxide, and crystallization from aqueous methanol. The compound shows a pH of 7 in water at ambient temperature and an optical rotation $[\alpha]_D^{20}$ of −4.30 (c = 3.0). In the crystal (orthorhombic, space group $P2_12_12_1$) the potassium ion is in a distorted trigonal-prismatic environment of six oxygen atoms of four different amino acids and of the water molecule. No nitrogen coordination is observed. Through the bridging function of some of the α-carboxylate oxygen atoms (O1, O2) and through complexation of the metal through the γ-carboxylate groups (O3), a layer-coordination polymer, with strings of potassium ions running parallel, is formed. The layers are cross-linked by hydrogen bonds involving the ammonium and γ-carboxylate functions as well as the water molecule.

Complexation of the alkali metal ions by amino acids and proteins plays an important role in many biological processes[1-3]. Evidence suggests that aspartate and glutamate units are among the principal mediators of metal ion binding. Though stability constants for the alkali aspartate and glutamate complexes are low[4,5], this weak coordination at several sites of a protein can be highly significant, especially for its tertiary structure. Many reactions at or in peptides therefore show marked alkali ion catalysis, and some of the metal ions act as antagonists[6,7].

As part of a current program on metal ion binding by amino acids, with special attention directed towards complexes of aspartic and glutamic acid, we also have investigated the structure of the title compound, which is present in and may be obtained from solutions containing potassium and L-hydrogen glutamate ions at pH = 7, i.e. near-physiological conditions. In previous studies we have determined the structure of the corresponding L-hydrogen aspartates of lithium and potassium[8]. A preliminary report on the structure of Na(L-GluH)·H_2O has appeared, but no details are available[9].

It should be noted that potassium L-hydrogen aspartate and L-hydrogen glutamate are components of drugs for a broad spectrum of applications, including the therapy of electrolyte deficiency, certain heart conditions, and other indications[6,7].

Results and Discussion

The potassium ion complex of the L-hydrogen glutamate anion is formed through neutralization of L-glutamic acid with one equivalent of potassium hydroxide. It crystallizes from concentrated aqueous solutions on addition of methanol as a monohydrate K(L-GluH)·H_2O which shows a melting point of 113.5°C. The aqueous solutions exhibit a pH of 7.5 at ambient temperature and a specific optical rotation $[\alpha]_D^{20}$ of −4.3 (c = 3.0 g). In the ^{13}C-NMR spectrum of these solutions the five non-equivalent carbon atoms give rise to five individual signals with the expected ^{13}C-^{1}H couplings (see Experimental). The data are very similar to those of other alkali or earth alkali salts of glutamic acid indicating at least partial dissociation in solution and relatively weak cation/anion contacts.

The crystal structure of the potassium hydrogen L-glutamate hydrate thus obtained has been determined by single-crystal X-ray diffraction at 23°C. K(L-GluH)·H_2O forms orthorhombic crystals, space group $P2_12_12_1$, with four molecules in the unit cell (Table 1). The compound forms a coordination polymer in the form of a layer structure as shown in Figure 1. Strings of potassium ions running parallel to the crystallographic *b* axis (horizontally in Figure 1) are bridged by the two oxygen atoms of the α-carboxylate groups of the L-glutamate anions, with each of the two oxygen atoms being engaged in a different string of metal ions (O1 and O2, respectively). The γ-carboxylate group is attached to a potassium ion through only one of its oxygen atoms (O3), while the other (O4) is engaged in hydrogen bonding with one of the hydrogen atoms of the $NH_3^{\oplus}$ function.

In addition to the double bridging by the α-carboxylate oxygen atoms O1 and O2 (or their symmetry equivalents O1′, O2′, O1″ and O2″, etc.) and the monodentate contact of the γ-carboxylate oxygen atom O3, the coordination sphere of each potassium center also contains one water molecule with its oxygen atom O5. The total coordination number of $K^{\oplus}$ is therefore 6, and the geometry of the array of the nearest neighbours can be described as trigonal-prismatic (Figure 2). The low coordination number 6 is unex-

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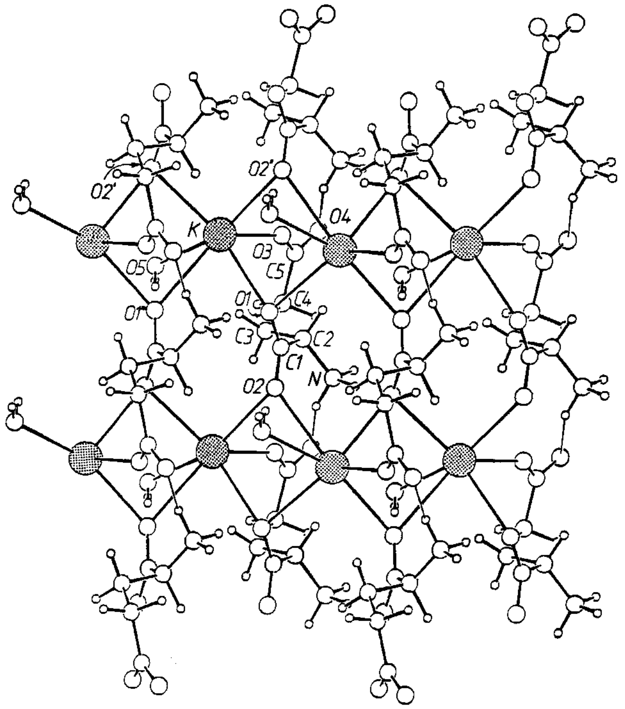

Figure 1. Layer structure of K(L-GluH)·H_2O with atomic numbering (arbitrary radii). α-Carboxylate oxygen atoms (O1 and O2) of each L-GluH anion are bridging the potassium ion in adjacent strings (horizontal). One of the γ-carboxylate oxygen atoms (O3) has a monodentate contact with only one potassium ion, alternately from above and below the layer. Oxygen atoms O4 and the primary ammonium groups $NH_3^{\oplus}$ are engaged in hydrogen bonding. The water molecule (with oxygen atom O5) has only one short contact to a potassium ion but can be described as semibridging

pected for potassium, since in most related complexes a larger number of inner-sphere contacts has been observed[8]. There is no obvious reason for this low connectivity in K(L-GluH)·H_2O, and the results thus indicate a high flexibility and non-directionality in potassium complexation and solvation. Each L-GluH$^{\ominus}$ ion is chelating one potassium ion with formation of an eight-membered ring and is connected to three other potassium ions as shown in Figure 3. As pointed out in connection with the hydrogen bonding involving O4, the amino group of the L-glutamic acid in K(L-GluH)·H_2O is protonated and converted into an $NH_3^{\oplus}$ function. Mainly from these primary ammonium groups, there originates a system of hydrogen bonds which is not only operative within each layer, but also interconnects the layers to give a three-dimensional network. The prominent hydrogen bonds (<3.0 Å) are listed in Table 4.

The configuration of the water molecule is noteworthy: Both the refinement of the hydrogen positions and the difference Fourier syntheses of the crystal structure determination led consistently to one rather small angle [K–O5–H51 79(2)°] and one rather large angle [K–O5–H52 138(2)°] with the [H51–O5–H52] angle being 102(2)°. Though a crystallographic artefact cannot be excluded, it seems more likely that cooperative effects including two hydrogen bonds (Table 4) and a weak potassium coordination [O5–K′ 3.538(2) Å] are responsible for the severe distortion of the hydrate water molecule.

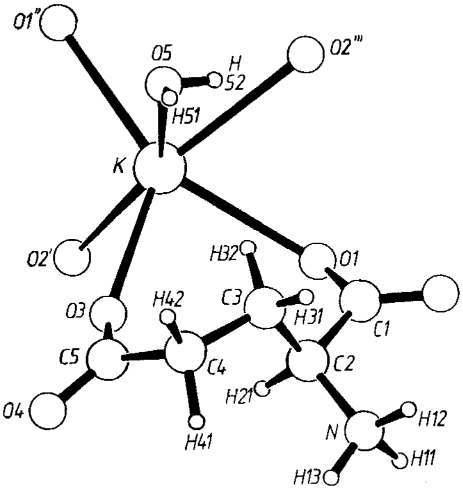

Figure 2. Inner coordination sphere of the potassium ion in K(L-GluH)·H_2O with atomic numbering (arbitrary radii)

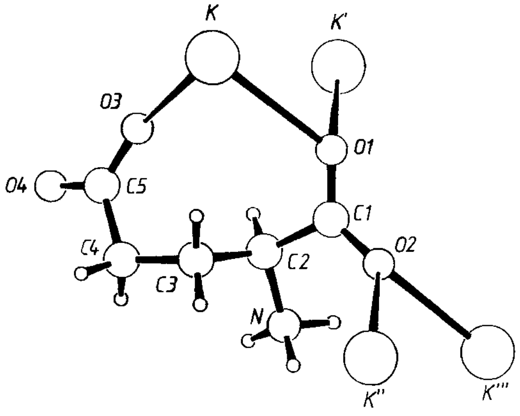

Figure 3. Environment of the hydrogen L-glutamate ion, L-GluH$^{\ominus}$, in the crystal of K(L-GluH)·H_2O with atomic numbering (arbitrary radii)

The structure of K(L-GluH)·H_2O shows no relation to that of K(L-AspH)·$2H_2O$, where the potassium atom is seven-coordinate[8]. It is probably similar, however, to the structure of the analogous *sodium* complex, Na(L-GluH)·H_2O, (orthorhombic space group $P2_12_12_1$), where the sodium ion also has coordination number 6[9]. On the other hand, structural details are at variance regarding both the connectivity pattern and the bond distances and angles, mainly as expected on the ground of differences in ionic radii.

In summary, the structural features of the crystalline state of K(L-GluH)·H_2O show a preference of potassium for carboxylate coordination with large ring chelation and metal bridging through oxygen coordination only. This is in agreement with findings for other aspartate and glutamate complexes of the larger (heavier) alkali and earth alkali metals. Potassium clearly has little selectivity regarding its coordination number, since small changes in stoichiometry (like aspartate/glutamate) are sufficient to induce a reduction from 7 to 6. With respect to their coordination number, both lithium and magnesium appear to be more directional: No other than the coordination numbers 4 (Li) or 6 (Mg) have been found.

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Experimental

All experiments were carried out in bidistilled, degassed water. – NMR: Jeol CX 270 and 400.

Potassium L-Hydrogen Glutamate Monohydrate [K(L-GluH)·H_2O]: L-Glutamic acid (14.7 g, 100 mmol) and potassium hydroxide (5.60 g, 100 mmol) are dissolved in 100 ml water at 23°C. Slow evaporation of the solution to one third of the initial volume and careful addition of an upper layer of methanol (30 ml) onto its surface induces crystallisation. The crystals, m.p. 113.5°C, are soluble in water to give solutions of pH = 7.5 and an optical rotation of $[\alpha]_D^{20} = -4.3$ (c = 3.0). – ^{13}C NMR (D_2O): δ = 32.45 [t, J(CH) = 129 Hz, C-β], 38.84 [t, J(CH) = 126 Hz, C-γ], 60.01 [d, J(CH) = 145 Hz, C-α], 180.11 and 186.72 (2 s, α-, γ-CO_2).

$C_5H_{10}KNO_5$ (203.24) Calcd. C 29.42 H 4.93 N 6.86
Found C 29.48 H 5.03 N 6.78

Determination of the Crystal Structure

Suitable crystals of K(L-GluH)·H_2O from aqueous methanol were sealed into a glass capillary and investigated directly on a diffractometer. Table 1 gives the crystal data and details of the structure solution procedure. The data were corrected for Lp effects, but correction for absorption and decay (−0.3%) appeared to be unnecessary. The structure was solved by direct methods (SHELXS-86)[10] and completed by Fourier syntheses. All H atoms could be located in difference syntheses and were included in the final refinement cycles using isotropic displacement parameters (SHELX-76)[11]. The absolute configuration of the amino acid was confirmed by refinement of the inverse data set, which gave significantly higher R values [R (wR) = 0.029 (0.031)].

In the refinement of the hydrogen positions as well as in the difference syntheses one of the angles K–O–H of the potassium-coordinated water molecule showed consistently a small value of 79°, compensated by a value of 138° for the other K–O–H angle. Artefacts cannot be ruled out completely for these results, but it is plausible that hydrogen bonding (Table 4) and/or partial bridge functionality between two potassium ions (Figure 1) are the origin of this phenomenon.

Table 2 shows the atomic coordinates, Table 3 gives important distances and angles, and Table 4 a list of hydrogen bonds in the crystal. Complete lists of coordinates have been deposited[12].

Table 1. Crystal structure data for K(L-GluH)·H_2O

$C_5H_{10}KNO_5$; M_r = 203.241; orthorhombic, $P2_12_12_1$ (No. 19); a = 6.289(1), b = 7.240(1), c = 18.153(2) Å; V = 826.5 $Å^3$; Z = 4; $d_{calcd.}$ = 1.633 g/cm^3; μ(Mo-K_α) = 6.2 cm^{-1}; F(000) = 424 e; T = 23°C; diffractometer: Enraf-Nonius CAD4; radiation: Mo-K_α, λ = 0.71069 Å, monochromator: graphite; scan: Θ/2Θ; scan width (in ω): 0.8 + 0.35 tanΘ; $(\sin\Theta/\lambda)_{max}$: 0.637; hkl range: +8, +9, ±23; measured reflections: 2118; unique reflections: 1794: R_{int} = 0.012; refined parameters: 149; observed reflections: 1709 [$F_o > 4.0\cdot\sigma(F_o)$]; R[a] = 0.024, R_w[b] = 0.026; $\Delta\varrho_{fin}$ (max/min) = 0.40/−0.52 e/$Å^3$.

[a] $R = \Sigma(||F_o| - |F_c||)/\Sigma F_o$. – [b] $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2]^{1/2}$, $w = 1/\sigma^2(F_o)$; function minimized: $\Sigma w(|F_o| - |F_c|)^2$.

Table 2. Fractional atomic coordinates and equivalent isotropic displacement parameters (hydrogen atoms: isotropic displacement parameters) for K(L-GluH)·H_2O; $U_{eq} = \Sigma_i\Sigma_j U_{ij} a_i^* a_j^* a_i a_j$

ATOM	X/A	Y/B	Z/C	U(eq.)
K	-0.02488(6)	0.77389(5)	0.28876(2)	0.140
O1	0.3177(2)	0.5539(2)	0.23987(7)	0.092
O2	0.6687(2)	0.5216(2)	0.25375(6)	0.083
O3	-0.0266(2)	0.5385(2)	0.40120(7)	0.112
O4	-0.0463(2)	0.4101(2)	0.51149(7)	0.113
O5	0.1340(3)	1.0462(2)	0.37947(9)	0.123
N	0.6189(2)	0.3110(2)	0.37699(8)	0.069
C1	0.4806(3)	0.5077(2)	0.27550(8)	0.063
C2	0.4408(3)	0.4342(2)	0.35350(8)	0.064
C3	0.4130(3)	0.5944(2)	0.4082(1)	0.084
C4	0.2874(3)	0.5403(3)	0.47638(9)	0.077
C5	0.0539(3)	0.4933(2)	0.46158(9)	0.088
ATOM	**X/A**	**Y/B**	**Z/C**	**U(iso)**
H11	0.654(3)	0.216(3)	0.338(1)	0.037(5)
H12	0.754(3)	0.375(3)	0.384(1)	0.040(6)
H13	0.582(3)	0.238(3)	0.420(1)	0.042(6)
H21	0.306(3)	0.358(3)	0.350(1)	0.025(5)
H31	0.547(3)	0.641(2)	0.4225(9)	0.023(5)
H32	0.337(3)	0.700(3)	0.3814(9)	0.025(5)
H41	0.345(4)	0.411(3)	0.503(1)	0.040(6)
H42	0.297(3)	0.648(3)	0.512(1)	0.042(6)
H51	0.204(4)	1.053(4)	0.418(1)	0.070(9)
H52	0.227(4)	1.040(4)	0.349(1)	0.061(8)

Table 3. Selected distances [Å] and bond angles [°] in the structure of K(L-GluH)·H_2O with standard deviations in units of the last significant digit in parentheses

Distances			
K -O1	2.822(1)	K -O2	2.730(1)
K -O1'	2.788(2)	K -O2'	2.971(2)
K -O3	2.659(1)	K -O5	2.756(2)
C1 -O1	1.257(2)	C1 -O2	1.251(2)
C1 -C2	1.533(2)	C2 -N	1.494(2)
C2 -C3	1.536(2)	C3 -C4	1.520(2)
C4 -C5	1.532(2)	C5 -O3	1.251(2)
C5 -O4	1.257(2)		
Angles			
O1 -K -O2	95.1(1)	O1 -K -O3	83.3(1)
O2 -K -O3	75.3(1)	O1 -K -O5	108.3(1)
O2 -K -O5	150.9(1)	O3 -K -O5	90.1(1)
O2'-K -O1	90.6(1)	O2'-K -O5	56.6(1)
O2'-K -O2	151.1(1)	O2'-K -O1	71.5(1)
O1'-K -O5	80.3(1)	O1'-K -O3	127.3(1)
O1'-K -O2	88.7(1)	O1'-K -O1	148.9(1)
K -O1-C1	127.6(1)	K -O2-C1	130.4(1)
K -O3-C5	147.0(1)	O1 -C1-O2	125.9(1)
O1 -C1-C2	115.8(1)	O2 -C1-C2	118.3(1)
N -C2-C1	110.4(1)	N -C2-C3	110.6(1)
C1 -C2-C3	110.7(1)	C2 -C3-C4	113.0(1)
C3 -C4-C5	114.4(1)	O3 -C5-O4	123.6(2)
O3 -C5-C4	118.9(2)	O4 -C5-C4	117.4(2)

Table 4. Hydrogen bonds [Å] in the crystal structure of K(L-GluH)·H_2O

A–B···C	A–B	B···C	A···C	symmetry operation[a]
O5–H51···O4	0.83(3)	2.04(3)	2.84(1)	a
O5–H52···O2	0.81(3)	1.98(3)	2.72(1)	b
N–H11···O1	1.01(2)	1.84(2)	2.85(1)	c
N–H12···O3	0.97(2)	1.85(2)	2.81(1)	d
N–H13···O4	0.97(2)	1.83(2)	2.783(2)	

[a] a: 0.5 + x, 1.5 − y, 1 − z; b: −x − 1, 0.5 + y, 0.5 − z; c: 1 − x, y − 0.5, 0.5 − z, d: 1 + x, y, z.

CAS Registry Numbers

K(L-GluH): 19473-49-5 / K(L-GluH) · H_2O: 6382-01-0 / L-Glutamic acid: 56-86-0

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[1] V. Klingmüller, *Biochemie, Physiologie und Klinik der Glutaminsäure,* Cantor KG, Aulendorf 1955.

[2] M. Schenk, E. Kolb, *Grundriß der Physiologischen Chemie,* Gustav Fischer Verlag, Jena 1982.

[3] C. A. Evans, R. Guerremont, D. L. Rabenstein in *Metal Ions in Biological Systems* (H. Sigel, Ed.), vol. 9, p. 41ff., Marcel Dekker Inc., New York 1979.

[4] H. C. Freeman in *Inorganic Biochemistry* (J. Eichhorn, Ed.), vol. 1, p. 129ff., Elsevier, Amsterdam 1973.

[5] N. J. Birch in *Metal Ions in Biological Systems* (H. Sigel, Ed.), vol. 14, p. 257ff., Marcel Dekker, New York 1984.

[6] B. Helwig, *Moderne Arzneimittel,* 5th ed., p. 802ff., Wissenschaftliche Verlagsgesellschaft, Stuttgart 1980.

[7] *Pharmazeutische Stoffliste,* 4th ed., Arzneibüro der Bundesvereinigung Deutscher Apothekerverbände, Frankfurt 1984.

[8] H. Schmidbaur, I. Bach, D. L. Wilkinson, G. Müller, *Chem. Ber.* **122** (1989) 1427, 1433, 1439, and 1445; **121** (1988) 1441; H. Schmidbaur, I. Bach, J. Riede, G. Müller, J. Helbig, G. Hopf, *ibid.* **121** (1988) 795; H. Schmidbaur, I. Bach, G. Müller, J. Helbig, G. Hopf, *ibid.* **120** (1987) 867; H. Schmidbaur, G. Müller, J. Riede, G. Manninger, J. Helbig, *Angew. Chem.* **98** (1986) 742; *Angew. Chem. Int. Ed. Engl.* **25** (1986) 1013.

[9] S. T. Rao, M. Mallikarjunan, *Acta Crystallogr., Sect. A,* **31** (1975) 48 (Suppl. Abstr. 03.5–5).

[10] G. M. Sheldrick in *Crystallographic Computing 3* (G. M. Sheldrick, C. Krüger, R. Goddard, Eds.), p. 175, Oxford Univ. Press 1985.

[11] G. M. Sheldrick, *SHELX-76, Program for Crystal Structure Determination,* Univ. of Cambridge, Cambridge, England 1976.

[12] Further details of the crystal structure investigation are available on request from the Fachinformationszentrum Karlsruhe, Gesellschaft für wissenschaftlich-technische Information mbH, D-7514 Eggenstein-Leopoldshafen 2, on quoting the depository number CSD-54287, the names of the authors, and the journal citation.

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