Copper(II)–Histidine Stereochemistry. Structure of L-Histidinato-D-histidinatodiaquocopper(II) Tetrahydrate

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Abstract: Crystals of L-histidinato-D-histidinatodiaquocopper(II) tetrahydrate, grown at pH 8, are triclinic, with cell dimensions (subcell) $a = 6.894$, $b = 8.478$, $c = 9.553$ Å, $\alpha = 98.61$, $\beta = 116.13$, $\gamma = 95.88°$, space group $P\bar{1}$ with one formula unit of the complex per cell. Although some weak supercell reflections were present on films, only the subcell data were used to solve (Fourier methods) and refine (final $R = 0.076$) the structure. The copper atom is octahedrally coordinated by amino and imidazole nitrogens of an L-His and a D-His in a square planar arrangement, and by water molecule oxygens at the axial positions. The Cu···N distances are 2.03 (amino) and 2.00 Å (imidazole) and the Cu···OH_2 separation is 2.57 Å. The carboxyl groups of the histidines secondarily coordinate to the copper through hydrogen bonding to the axial waters (O···O distance is 3.01 Å). The structural results, along with consideration of potentiometric titrations and infrared data for the crystals, have been used to postulate a plausible structure for the physiologically important Cu(II)-(L-His)$_2$ complex in solution.

Introduction

Copper(II)-amino acid complexes have been isolated from normal human blood serum and have been shown to be in equilibrium with Cu(II) bound to albumin through an intermediary ternary complex, albumin-Cu(II)-amino acid.[2-5] These equilibrium complexes are considered to be the transport form of Cu(II) in blood. The predominant binary amino acid complex in blood serum is Cu(II)-(L-His)$_2$, and reversible transfer of Cu(II) between Cu(II)-(L-His)$_2$ and albumin and the existence of the albumin-Cu(II)-(L-histidine) ternary complex have been demonstrated. There have been extensive solution studies carried out on the Cu(II)-(L-His)$_2$ complex but several attempts to crystallize it at neutral pH have been unsuccessful. In this communication we report the production and x-ray structure determination of crystals of the mixed complex L-His-Cu(II)-D-His, obtained at approximately neutral pH, and possible implications concerning the structure of Cu(II)-(L-His)$_2$.

Preparation of the Crystals

$Cu(OH)_2$ gel, freshly prepared by treating anhydrous $CuCl_2$ with concentrated KOH, was washed until the effluent had a pH ~8.0. A solution 0.1 M in each of D-histidine and L-histidine was filtered slowly through the washed $Cu(OH)_2$ gel; the filtrate had the characteristic dark blue color of Cu(II)-histidine complexes. The pH of the blue filtrate was adjusted to 8.0, and the solution diluted with an equal volume of acetone; crystallization of hydrated L-His-Cu(II)-D-His started after 4 days at 4 °C.

Experimental Section

A. Crystallographic Studies. Unit cell parameters were obtained from least-squares refinement of diffractometer measurements of 2θ values for 17 general reflections.

Crystal data (λ Cu Kα = 1.541 78, λ Cu Kα_1 = 1.540 51, λ Cu Kα_2 = 1.544 33 Å) for L-histidinato-D-histidinatodiaquocopper(II) tetrahydrate: $(C_6H_8N_3O_2)_2Cu\cdot 6H_2O$, molar mass 479.94 g; triclinic, Delaunay reduced cell has $a = 6.894$ (1), $b = 8.478$ (1), $c = 9.553$ (1) Å, $\alpha = 98.61$ (1), $\beta = 116.13$ (1), $\gamma = 95.88$ (1)°, $U = 486.7$ (2) Å^3, $d_x = 1.64$ g/cm^3, $Z = 1$; μ(Cu Kα) = 22.2 cm^{-1}, space group $P\bar{1}$ (confirmed by structure analysis).

The above cell is a subcell; oscillation photographs about a and b showed that a(supercell) = $6a$ (subcell) and b (supercell) = $4b$ (subcell). In the a direction reflections of the form $(h,k,l) = (6n,k,l)$, where n = integer, were strong (subcell reflections), those having $(h,k,l) = (6n \pm 1,k,l)$ were weak, and all others were absent. In the b direction $(h,k,l) = (h,4n,l)$ were strong, $(h,k,l) = (h,4n \pm 1,l)$ were weak, and $(h,k,l) = (h,4n + 2,l)$ were absent. In view of the large number of possible reflections in the supercell it was decided to proceed with structure solution and refinement using only the subcell data. The final results of this analysis were sufficiently precise that no consideration of the supercell data was deemed necessary. In addition, Weissenberg photographs taken after data collection showed a considerable decrease in intensity of the weak supercell reflections compared to the subcell reflections.

The intensities of all reflections in the subcell having $2\theta_{CuK\alpha} \leq 132°$ (corresponding to a minimum interplanar spacing of 0.84 Å) were measured on an automated four-circle diffractometer with nickel-filtered Cu Kα radiation using the 2θ–θ scan technique. The intensities were corrected for background, and linearly corrected for fall-off in intensity (about 20%) and Lorentz and polarization factors applied. The crystal was small and no absorption corrections were applied. A total of 1707 unique reflections were measured of which 1537 (90%) had $I > 2\alpha(I)$ and were considered to be observed.

Structure Determination and Refinement. The histidine molecule and the three water oxygens in the asymmetric unit were located on a Fourier synthesis phased with the copper atom at the origin center of symmetry. After three cycles of full-matrix isotropic refinement with all reflections and using unit weights ($R = 0.21$) and one cycle of anisotropic refinement ($R = 0.12$), positions of the hydrogen atoms on the histidine were located on a difference map. To obtain a more satisfactory weighting scheme all reflections were divided into 20 ranges according to the magnitude of F_o and for each range the $\langle F_o \rangle$ was plotted against $\langle |F_o - F_c| \rangle$. A second-order polynomial fit to the curve gave $\sigma(F_o) = 0.0127^* F_o + 0.986 - 0.306/F_o$ (weight, $w = 1/\sigma^2(F_o)$). Subsequent cycles of anisotropic refinement, using the above weights, followed by difference Fourier maps, resulted in the location of the hydrogen atoms of the water molecules. Hydrogen atoms were included in the structure factor calculation (with isotropic temperature factors of the atoms to which they are attached) but not refined; the final residual indices are, for all data, $R = 0.091$, $R_w = 0.092$, and for observed data, $R = 0.076$, $R_w = 0.087$. The maximum shift/error in the final cycle was 0.05, the final $[\Sigma w(F_o - F_c)^2/(m - n)]^{1/2} = 0.97$ (m = number of observations, n = number of parameters.)

A final difference map showed some residual density (maximum of 0.4 e Å^{-3}) in the vicinity of the copper atom, the amino nitrogen atom, and water molecule 1. Scattering factors for copper (Cu$^+$), O, N, and C[6] and for H[7] were as cited. Table I lists the atomic fractional coordinates and thermal parameters; the observed and calculated structure factors are available.[8]

B. Potentiometric Studies. Potentiometric data were obtained utilizing procedures and equipment previously described.[5] Two samples were studied: Cu(II)-L-histidine (1:4 ratio) and Cu(II)-L-histi-

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Table I. Final Positional Parameters (Fractional) and Anisotropic Thermal Parameters ($\times 10^5$)

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Cu	0.0	0.0	0.0	2811	1790	1113	−317	591	348
N1	−0.1929	−0.0403	−0.2402	4499	3493	973	−2174	−159	841
C2	−0.1798	0.0639	−0.3462	2772	1231	735	10	475	184
C1	−0.3280	0.1883	−0.3751	2331	1440	2469	−595	660	−442
O1	−0.3553	0.2523	−0.4880	3276	1834	4405	601	779	1326
O2	−0.4110	0.2148	−0.2836	3219	3753	4270	−1067	2399	−2340
C3	0.0608	0.1469	−0.2803	2449	1524	959	304	817	492
C4	0.1394	0.2663	−0.1284	1575	1410	1180	38	638	360
N2	0.1321	0.2220	0.0029	1936	1478	876	−275	526	125
C5	0.2095	0.3577	0.1155	2053	2051	1154	−145	567	33
N3	0.2661	0.4823	0.0643	2233	1332	1722	−246	696	−142
C6	0.2225	0.4274	−0.0875	2145	1411	1816	21	890	627
OW1	−0.3210	0.1095	0.0236	3671	5505	1901	168	1459	1431
OW2	0.2608	0.4042	0.4919	5262	2407	2826	1401	1374	910
OW3	−0.1589	0.2739	0.3230	8109	3289	3183	564	2203	885
Standard deviations									
Cu	0	0	0	3	3	3	2	2	2
N,C,O	0.0009	0.0008	0.0008	20	20	20	10	10	10

Atom	x	y	z	Atom	x	y	z
HN1a	−0.275	0.025	−0.210	HC6	0.240	0.475	−0.145
HN1b	−0.320	−0.105	−0.300	HW1a	−0.355	0.125	−0.060
HC2	−0.225	0.0	−0.440	HW1b	−0.290	0.180	0.120
HC3a	0.145	0.060	−0.265	HW2a	0.305	0.495	0.525
HC3b	0.070	0.195	−0.355	HW2b	0.385	0.390	0.570
HC5	0.235	0.365	0.215	HW3a	−0.195	0.330	0.380
HN3	0.310	0.565	0.120	HW3b	−0.005	0.315	0.385

dine-D-histidine (1:2:2 ratio), with Cu(II) concentration = 10×10^{-3} M in both cases. Titrations were performed in the range pH 2–11. The resulting titration curves were very similar.

C. Infrared Spectrum. Crystals of L-His-Cu(II)-D-His hydrate were powdered and a KBr pellet of the compound was made. A Beckman IR-20 double beam infrared spectrophotometer was used to obtain the percent transmission spectrum in the range 2000–1000 cm^{-1}.

Results and Discussion

A. Description of the Structure. The structure and conformation of L-histidine-D-histidinediaquocopper(II) tetrahydrate are shown by a stereoscopic plot in Figure 1. The copper atom is coordinated by an L-histidine and a D-histidine molecule, each in bidentate fashion through N(amino) and N(imidazole) atoms, to form a square planar arrangement. Two water molecules also bind to the copper, in axial positions with respect to the square plane, resulting in octahedral coordination for the Cu(II) (maximum deviation from octahedral geometry of the angles involving these six ligands is 4°). The apical water molecules, in addition to directly coordinating the copper, also serve as hydrogen-bonding bridges between the copper atom and an oxygen atom of the histidine carboxyl group in each case, thereby increasing the stability of the coordination complex. A similar copper-water-O(carboxyl) bridge has previously been noted[9] (in $K_2Cu(H_{-1}GlyGly)_2 \cdot 6H_2O$, though it appears to be of a weaker nature in that compound as the O-H···O(carboxyl) distance is 2.78 Å there vs. 2.26 Å in the present structure (values are for H···O distances; see Figure 2 for O···O distances).

Figure 2 presents the interatomic distances and angles in the coordination complex. The Cu-amino nitrogen distance (2.033 Å) is somewhat longer than the Cu-imidazole nitrogen separation (1.996 Å), in accordance with what has been found in a number of similar compounds; the values of these distances also agree with previously reported cases.[10] The bond lengths and angles in the histidine moiety are typical of values found in structure determinations of other histidine-containing compounds and need little comment. The similar values of the two C5-N distances in the imidazole ring indicate electron delocalization over these bonds. The water molecules occupying the axial octahedral coordination positions are 2.570 Å from the copper; copper-water (axial) distances of 2.46–2.78 Å have been observed in other compounds.[9-13] Torsion angles in the copper-histidine complex are given in Table II.

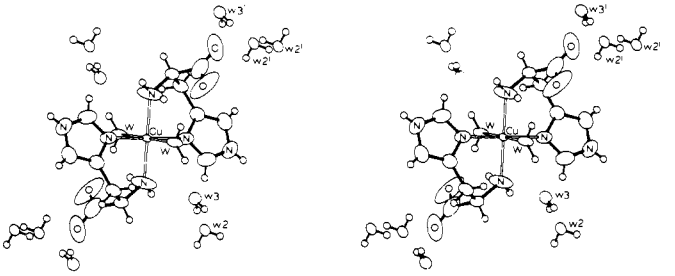

Figure 1. Stereoscopic drawing of L-histidinato-D-histidinatodiaquocopper(II) tetrahydrate.

The imidazole ring is planar but not coplanar with the central square plane. The angle between normals to the planes is 17°, similar to the 18 and 20° angles between similar groups in Cu-GlyGly-L-His methylamide.[11]

The intra- and intermolecular hydrogen bonding scheme in the crystal structure is extensive and involves all possible hydrogen-bondable atoms. Carboxyl O2, in addition to accepting an H bond from the axially coordinated water molecule (W1) (forming the previously mentioned intramolecular bridge to the copper), also interacts with a hydrogen on the amino nitrogen in the same molecule; this interaction undoubtedly serves to further stabilize the observed conformation. In addition, each O2 also accepts intermolecular H bonds from an imidazole N3 hydrogen and from a water molecule (W2). Each carboxyl O1 atom is also involved in four hydrogen bonding contacts, with an amino nitrogen and W2 and W3 water molecules. Finally, the water molecules themselves are linked together by H bonding (Figure 2).

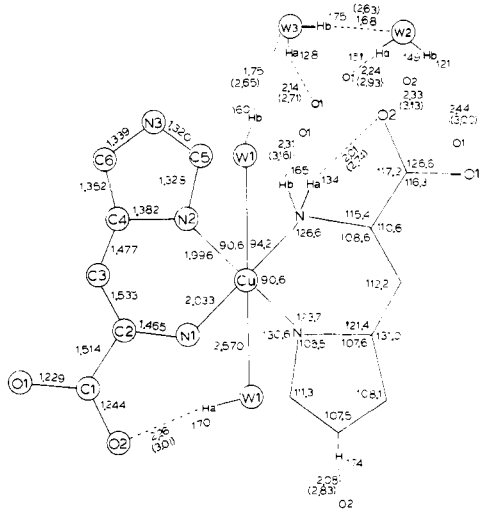

Figure 2. Interatomic distances (Å) and angles (deg) in the molecule and details of the hydrogen-bonding network. The values in brackets beside hydrogen bonds are distances between "heavy" atom centers. Standard deviations are 0.005 Å and 0.2° for distances and angles around the copper, and 0.008 Å and 0.6° for those involving only C, N, and O atoms.

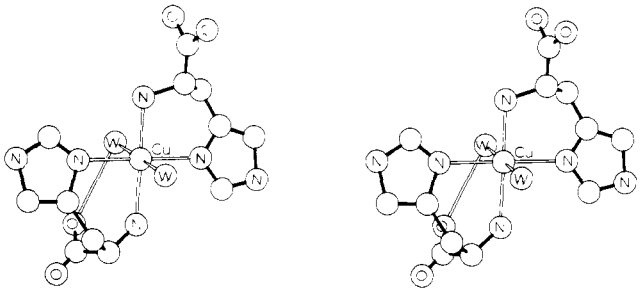

Figure 3. Stereoscopic drawing of postulated structure for Cu(II)-(L-His)$_2$ in solution.

The individual bond lengths and angles involving hydrogen atoms are not particularly accurate and, except for hydrogen atoms shown to illustrate the H-bonding scheme, are not given here; the average value of "heavy" atom-hydrogen distances is 0.86 Å.

B. Implications for the Structure of Cu(II)-(L-His)$_2$. Because of the physiological importance of copper-L-histidine complexes in the transport of Cu(II) in the blood, many studies have been carried out to try to determine the modes of histidine chelation of copper. Interpretations of various spectral data have led to proposals that in solution of near neutral pH the structure of Cu(II)-(L-His)$_2$ consists of (1) two bidentate histidines bound to copper through amino N and carboxyl O atoms;[14] (2) two bidentate histidines, bound to copper through amino N and carboxyl O of one His and through amino and imidazole N's of the other;[15] (3) a bidentate (amino and imidazole N's) and a tridentate (N's in the square plane, carboxyl O chelated axially) histidine;[16] and (4) an equilibrium mixture of the species in (3) above with another tridentate-bidentate species in which the bidentate His binds copper through amino N and carboxyl O.[17]

Attempted preparations of crystals of Cu(II)-(L-His)$_2$ for structural study by x-ray diffraction have been unsuccessful. The crystal structure of [Cu(II)-(L-HisH)$_2$(H$_2$O)$_2$](NO$_3$)$_2$ has been reported;[13] the copper in that complex is chelated by two -histidines through amino nitrogens and carboxyl oxygens in a square plane, with water molecules at the apical positions. However, that complex was prepared at pH 3.7; above pH 5 much evidence indicates that the imidazole group replaces the carboxyl group as a primary copper chelator. The crystal

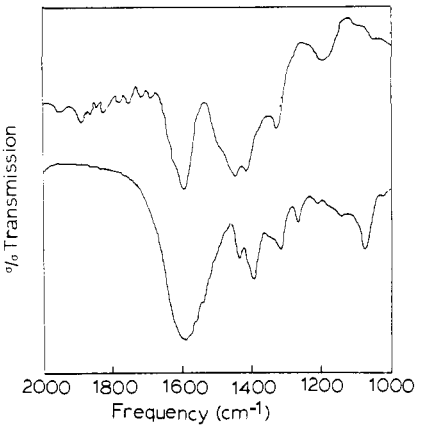

Figure 4. Infrared spectra of (top) 1:2 Cu(II)-L-histidine in D$_2$O at pD 9.0 (ref 19), and of (bottom) L-histidine-Cu(II)-D-histidine hexahydrate crystals grown at approximately pH 8.0.

Table II. Conformational Angles for L-His-Cu(II)-D-His

Torsion angle	Value, deg	Torsion angle	Value, deg
N1-C2-C1-O1	−165	C3-C2-C1-O2	−109
N1-C2-C1-O2	15	C3-C2-C1-O1	71
N1-C2-C3-C4	−68	Cu-N1-C2-C1	−93
C1-C2-C3-C4	59	Cu-N1-C2-C3	32

structure of a complex consisting of Cu(II) bound to L-histidine and L-threonine, at approximately physiological pH, has been determined;[18] the copper is bound by a tridentate L-His (amino and imidazole N's in the square plane, carboxyl O at an approximately axial position) and a bidentate L-Thr (amino N, carboxyl O in square plane) with a water molecule completing a distorted octahedron, the distortion being caused by the L-His carboxyl O (O···Cu = 2.58 Å, N(amino)-Cu-O(carboxyl) angle = 68°). It was concluded[18,19] from this crystal structure determination and spectral and potentiometric studies[19] that the structure of Cu(II)-(L-His)$_2$ in physiological solution probably consists of either a bidentate (amino and imidazole N's) histidine and a tridentate (N's in square plane with carboxyl O in distorted apical position) histidine binding to copper, or two tridentate histidines.

The results of the present structure determination of hydrated Cu(II)-L-His-D-His have led us to propose a somewhat different structure as a possibility for Cu-(L-His)$_2$ in solution. The proposed arrangement is shown stereoscopically in Figure 3; it consists of Cu(II) chelated by two bidentate L-His molecules through amino and imidazole nitrogens, trans to each other in the two histidines, in a square planar arrangement, with water molecules coordinating copper at the axial octahedral positions. In addition, one of the L-His moieties is bridged to the Cu(II) through hydrogen bonding to an axial water, as is the case in the Cu(II)-L-His-D-His structure. The evidence upon which this structure is based, in addition to the Cu(II)-(L-His)-(D-His) crystal structure results, consists of the following.

(1) The potentiometric titration curves for solutions of Cu-(L-His)$_2$ and Cu-(DL)His$_2$ are similar; this indicates that in both systems the involvement of amino and imidazole nitrogens in copper binding is likely the same, suggesting similar bonding patterns. This agrees with the observed lack of stereoselectivity in the formation of Cu(II)-His$_2$ complexes in solutions where both D- and L-His are present.[20]

(2) Part of the evidence supporting a tridentate mode of binding of L-His to Cu(II) has been the interpretation of a shift in the infrared antisymmetric CO_2^- band in Cu(II)-(L-His)$_2$ solution at neutral pH, ~30 cm^{-1} from its position in the absence of copper, as indicative of Cu(II)-O (carboxyl) bond-

ing.[19] We have recorded the infrared spectrum of powdered crystals of L-His-Cu(II)-D-His hydrate, and it is shown in Figure 4 along with the Cu(II)-$(L\text{-His})_2$ solution spectrum.[19] The positions of the antisymmetric CO_2^- bands in the two spectra are identical. Although we cannot rule out the possibility that the position of the band in the solid is strongly affected by intermolecular interactions that do not exist in solution, neither can we positively ascribe the CO_2^- band shifts to different phenomena in the two media; thus the interaction of the histidine carboxyl group with copper through a water bridge, as occurs in the crystal structure of Cu(II)-$(DL)His_2$ hydrate, is at least as plausible an interpretation for the shift of the CO_2^- band as is the previous postulation.

(3) As reported by Freeman,[10] there is evidence "that all bis (α-amino acid)-Cu(II) complexes exist in solution as dihydrated distorted octahedral species, and that the differences in coordination of the Cu atoms in the crystalline complexes occur in the axial directions". The crystal structure results reported here agree with this suggestion of solution structure; in addition, the octahedral geometry of the Cu(II)-$(DL)His_2$ hydrate complex is much less distorted (virtually distortion free) than the octahedral geometry in the crystal structure of Cu(II)-L-His-L-Thr hydrate[18] in which the L-His carboxyl oxygen is chelated directly to Cu(II) in an axial position.

(4) In all of the structures so far elucidated of Cu(II) coordinated to histidine-containing species at neutral pH, the square planar coordination has been by amino, peptide, and imidazole nitrogen atoms, where this has been geometrically possible,[21] rather than by carboxyl oxygens. Model building indicates that placing two L-histidine molecules trans to each other with their amino and imidazole N's coordinating copper in a square plane is sterically more favorable than a cis placement, in which the imidazole rings approach each other too closely. Thus the model (Figure 3) incorporates these structural features.

In summary, the results of the crystal structure determination of L-histidinato-D-histidinatodiaquocopper(II) tetrahydrate, along with the above outlined potentiometric, spectral, and steric data, have been used to propose a plausible structure for Cu(II)-$(L\text{-His})_2$. In solution, the free unbridged carboxyl group of the model may coordinate another Cu(II) complex at an axial position, either directly or through a water bridge, or may interact solely with water molecules. Depending on concentration, a dynamic equilibrium between these modes might be expected.

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Supplementary Material Available: A listing of observed and calculated structure factors (9 pages). Ordering information is given on any current masthead page.

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(21) A referee has pointed out that carboxyl oxygen atoms occupy some square plane coordination positions in crystals of two forms of Cu(Gly-L-His-Gly) obtained from neutral pH solutions (R. Österberg, B. Sjöberg, and R. Söderquist, *Acta Chem. Scand.*, **26**, 4184 (1972); *J. Chem. Soc., Chem. Commun.*, 983 (1972)). In those structures three nitrogen atoms of each tripeptide occupy square planar positions around each copper; however, it is geometrically not possible for the fourth nitrogen to also coordinate to the copper, and the fourth square plane position is occupied by an oxygen from the free carboxyl end of a different tripeptide. Since there is no possibility of further nitrogen binding, this does not alter the conclusion that nitrogen square plane binding to Cu(II) is favored at neutral pH, and therefore is incorporated into the postulated model for Cu(II)-$(L\text{-His})_2$.