Inorg. Chem. **2004**, *43*, 3338–3340

X-ray Structure of Physiological Copper(II)–Bis(L-histidinato) Complex

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Received December 9, 2003

The isolation and the X-ray crystal structure of physiological copper(II)–L-histidine complex are reported. The neutral five-coordinate complex shows distorted square pyramidal geometry with bidentate and tridentate L-histidine ligands. The basic character of the pendent imidazole group and H-bonding interactions of bidentate L-histidine ligand are important for copper transport. The unique structural features help explain the origin of its thermodynamic stability and kinetic reactivity in human blood along with the ternary copper(II)-amino acid complexes. The role of L-histidine in interaction with copper(II)–albumin, in cellular uptake of copper, and in treatment of Menkes disease can be studied using these results.

Copper is an essential trace element required by all living organisms.[1] L-Histidine plays a pivotal role in copper transport before its entry into cellular transport systems and incorporation into enzymes and proteins.[2] A small fraction of copper(II) bound to L-histidine maintains an exchangeable pool of copper in equilibrium with albumin in human blood.[3] The exchange of copper between L-histidine and albumin is a ligand metathesis reaction, and such reaction modulates the availability of copper to the cell. Albumin inhibits L-histidine-mediated enhanced copper(II) transport in placental and hepatic cell cultures. Menkes disease is a genetic neurodegenerative disorder causing death in children due to impaired copper metabolism.[4] The parenteral administration of copper(II)−L-histidine when initiated very early in life is most effective in preventing neurodegenerative problems in Menkes patients.[2,4] The role of L-histidine in interaction with copper−albumin, in cellular uptake of copper, and in treatment of Menkes disease is not clearly defined. This led to considerable interest in understanding physicochemical properties and structure of physiological copper(II)−L-histidine species. Nevertheless, the nature of this species remained inconclusive for the last four decades despite exhaustive characterization studies in aqueous solution.[5]

L-Histidine ligand has three potential sites for coordination: the amino nitrogen (N_{am}), the imidazole nitrogen (N_{im}), and the carboxylate oxygen ($O_{carboxyl}$). According to speciation studies, only one species exists at pH 7.4 (<99%) corresponding to the copper(II)−bis(L-histidinato) complex.[2] Despite efforts in many laboratories, the crystallization of this physiological complex was unsuccessful until now. In our quest to crystallize this compound, recently we isolated a novel copper(II) complex with a ligand derived from L-histidine.[6] Here, we report successful isolation, crystallization, and structure determination of copper(II)−bis(L-histidinato) complex.

An aqueous solution of copper(II)−L-histidine in 1:2 molar ratio was prepared using $CuSO_4 \cdot 5H_2O$ at pH 7.4. The UV−vis spectrum was characteristic of the formation of copper(II)-bis(L-histidinato) complex (**1**). An equal volume of dimethylformamide (DMF) was added to make 50/50 vol/vol water/DMF mixture. The crystallization induced by slow

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10.1021/ic035413q CCC: $27.50
Published on Web 05/04/2004

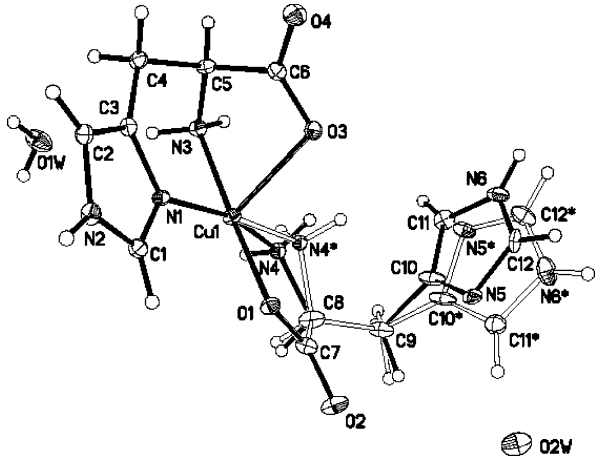

Figure 1. Structure of copper(II)−bis(L-histidinato) complex (**1**). Selected interatomic distances (Å): Cu(1)−O(1) 1.957(2), Cu(1)−N(4) 1.966(6), Cu(1)−N(4*) 2.034(6), Cu(1)−N(1) 1.984(3), Cu(1)−N(3) 2.003(3), Cu(1)−O(3) 2.277(2). Bond angles (deg): O(1)−Cu(1)−N(1) 92.08(10), N(1)−Cu(1)−N(3) 89.03(11), N(1)−Cu(1)−O(3) 92.85(11), N(3)−Cu(1)−O(1) 177.96(12).

diffusion of ethanol in this mixture for one week at room temperature yielded small clusters of intense blue crystals. The complete structure of **1** was solved by the single-crystal X-ray diffraction method.[7] The formation of **1** is further confirmed by a combination of spectral and elemental analyses.[8]

Figure 1 shows a crystal structure of **1**, a neutral five-coordinate distorted square pyramidal complex. The structure of **1** is unique and different from all the structures suspected for copper(II)−L-histidine species at physiological pH.[5] One of the L-histidine ligands acts as the monoanionic bidentate form through $N_{(am)}$ and $O_{(carboxyl)}$ atoms, while the other binds as the monoanionic tridentate ligand toward copper(II) center through its $N_{(am)}$, $N_{(imidazole)}$, and $O_{(carboxyl)}$ atoms. The $O_{(carboxyl)}$ atom lies in an axial position (angle N(1)−Cu(1)−O(3) = 92.85(11)°). The bidentate L-histidine ligand shows a positional disorder. Overall, the structural parameters for **1** such as bond distances and angles are in the range of those for other copper(II)−amino acid complexes.[9]

In aqueous solution, copper(II)−L-histidine complexes assume various structures depending on the pH and the composition.[2] When an aqueous solution of copper(II) salt is mixed with 2 equiv of L-histidine, the resulting pH of the solution is approximately 3.7. The X-ray crystal structure for the resulting complex (**2**) reported by Evertsson[10] showed that $N_{(am)}$ and $O_{(carboxyl)}$ atoms are involved in copper coordination in a square planar geometry (Figure 2). The binding mode shows that the sequence in which the protons of L-histidine are removed by titration (pK_a: COOH, 1.8; imidazolium, 6.0; NH_3^+, 9.18) is not the sequence in which potential donor atoms are used in copper binding. This is due to thermodynamic stability of five-membered chelate ring formation in complex **2** over seven-membered ring formation involving imidazole group in coordination.[11] Our results show that only one imidazole group is bound to the copper center and the other imidazole is trapped in an unfavorable axial position where the $O_{(carboxyl)}$ group of the other L-histidine ligand is coordinated. This pendent imidazole group further fails to rotate in proximity of the remaining axial site in the coordination sphere, due to the *S*-stereochemistry of the L-histidine ligand. On the contrary, the previously reported structure for [Cu(II)(L-His)(D-His)$(H_2O)_2$]·$4H_2O$ shows the involvement of both imidazole groups due to the presence of racemic ligands.[12] Complex **1** can be formed reversibly from complex **2** by increasing pH, and the reorganization of coordination environment seems less feasible (Figure 2).

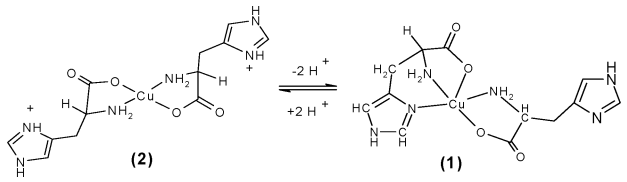

Figure 2. Proposed relation between **1** and **2**.

The imidazole side chain of L-histidine is imperative for copper chelation in many metalloproteins[13] and is also an important factor in thermodynamic stability of ternary copper(II)−amino acid complexes.[11] The structural arrangement in complex **1** is the result of a combination of steric effects induced by the tridentate L-histidine ligand and the tendency of the copper(II) ion toward square planar geometry. The tridentate coordination of L-histidine ligand in complex **1** provides additional stability over binary copper(II)−amino acid complexes. The stability of **1** is comparable with that of ternary complexes, viz. [Cu(II)(L-His)(L-Ser)] and [Cu(II)(L-His)(L-Gln)] in solution.[14]

The H-bonding interactions of the pendent imidazole group play an important role in the isolation and crystallization of complex **1**. The addition of DMF reduces H-bonding interactions involving the pendent imidazole group and anions in aqueous solution, facilitating the crystallization process preceded by the salt precipitation. This is apparent

(7) To resolve the disorder in bidentate L-histidine ligand, the complete structure of **1** was solved three times independently. The structure obtained after each refinement was similar to that reported in the manuscript. Diffraction data (3183 independent reflections) were collected on a Bruker Nonius-Kappa CCD system using Mo Kα radiation. Crystallographic data: $C_{12}H_{19}CuN_6O_{5.5}$, fw 398.87 g mol^{-1}, monoclinic, space group $P2_1$, a = 6.9600(2) Å, b = 11.6650(5) Å, c = 9.6300(3) Å, β = 94.273(2)°, V = 779.67(5) Å^3, T = 150(1) K, Z = 2, ρ_{calcd} = 1.699 Mg/m^3, μ = 1.443 mm^{-1}, R1 = 0, 0359, wR2 = 0.0740 for $I > 2\sigma$.

(8) Anal. Calcd for $C_{12}H_{19}CuN_6O_{5.5}$: C, 36.10; H, 4.76; N, 21.06. Found: C, 36.08; H, 4.78; N, 20.88. UV−vis data: λ_{max} = 642 nm, $\epsilon_{\lambda max}$ = 88 M^{-1} cm^{-1}. CD data (λ_{max}, nm): 693, 323, 221. IR (KBr, cm^{-1}): $\nu_{as}(CO_2^-)$ centered around 1600, $\nu_s(CO_2^-)$ = 1393, ν(imidazole) = 1111. EPR (frozen solution): $g_{||}$ = 2.23, $g_\perp$ = 2.06, $A_{||}$ = 170 G.

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by the fact that crystallization using copper(II) sulfate is easier than that for copper(II) chloride. The process of crystallization can also be optimized using different salts with various DMF contents. However, the water molecule (O2WB) was only half occupied in the crystal structure of **1**, and it appears that it caused the reorientation of the unbound imidazole when it was present due to a H-bonding to N(5). The observed disorder is due to the presence of two conformations of the side chain of the pendent L-histidine ligand. The H-bonding interactions involving lattice water molecules are shown to induce different structures for copper(II)−amino acid systems but have little effect on the first shell coordination around copper.[15]

The structural similarities between complex **1** and the ternary copper(II)−amino acid complexes involving L-histidine ligand can explain their comparable concentrations in human blood.[16] The concurrence of the high stability associated with tridentate ligation and the kinetic reactivity of the bidentate binding of L-histidine ligand highlights the contribution of complex **1** in the copper transport. The bidentate ligation in the structure of complex **1** may play a crucial role during the exchange reaction with albumin. The imidazole group of L-histidine is known to act as a base, accepting a proton during enzyme catalysis.[17] A similar basic character was demonstrated by catalyzing the aldol condensation of acetone to form an asymmetric Schiff's base compound during our previous crystallization attempts for complex **1**.[6]

In summary, we have reported the isolation and the X-ray crystal structure of a physiological copper(II)−L-histidine complex. The structural features help explain the origin of its stability and kinetic reactivity in human blood along with the ternary copper(II)−amino acid complexes. The basic character of the imidazole group and H-bonding interactions of the bidentate L-histidine ligand in this complex can be used for the effective delivery of radio imaging agents across cell membranes and for the improvement of the treatment of Menkes disease.

Acknowledgment. We thank Fondation Électricité de France for financial support to P.D. We thank also Professor R. Morris for fruitful discussions and Drs. A. Lough and J. Britten for the use of the single crystal X-ray diffraction facility. Research was supported by Grant FRN-1800 from the Canadian Institutes of Health Research.

Supporting Information Available: X-ray structural data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

IC035413Q

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