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XAFS Study of Coordination Structure of Cu(L-His)$_2$ in Solution

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Multiple coordination modes are present in the Cu^{II}-histidine complex in solution and the copper coordination environment varies with pH. In this work, we have investigated the coordination geometry of $Cu(His)_2$ complex using X-ray absorption fine structure (XAFS) analysis. Copper K-edge XAFS spectra were acquired on aqueous Cu^{2+} samples with histidine at different pH values. The coordination environments were further confirmed by chemically modified histidine. Results show that the caboxylate groups coordinate at acidic condition, while amino and imidazole nitrogens get coordinated at higher pH. For the coordination geometry of $Cu(His)_2$ in solution at physiological pH, the sixfold coordination is preferentially formed, while the fivefold coordination can co-exist in equilibrium.

Key words: X-ray absorption fine structure, Coordination structure, Copper, Histidine, Chemical modification, pH

I. INTRODUCTION

Copper is the third most ubiquitous transition-metal element in living organisms, following iron and zinc [1]. Its oxidation-reduction characteristics and the presence as an integral part of the active sites of many enzymes play important roles in many biologic processes [2]. Histidine residue is the most favorite binding site of Cu^{II} in copper enzymes and proteins in the copper transporter systems. Histidine shows high binding affinity to Cu^{II} with imidazole coordination, giving the $\lg\beta_2$=18.3 [1]. A small fraction of Cu^{II} bound to L-histidine maintains an exchangeable pool of copper in equilibrium with albumin in human blood, and the Cu^{II} exchange between L-histidine and albumin modulates the availability of this biometal to the cell [3]. Additionally, parenteral administration of Cu^{II} (bis-L-histidine) has been proved effective in cures for Menkes disease [4].

L-histidine has four potential metal coordianting sites, the carboxylate oxygen (O_C), the amino nitrogen (N_{am}) and two imidazole nitrogen (N_{im}) [3]. The coordination affinities of these binding sites are highly influenced by their protonation status, thus depending on the pH-value. The pK_a values for carboxylate group, amino nitrogen and imidazole are 1.82, 9.17, and 6.04, respectively [5]. Therefore, the O_C coordination can occur at more acidic condition, N_{am} can only coordinate at high pH and the amino group is deprotonated. The coordination property of imidazole group, which has a pK_a near the physiological pH and often plays important roles in protein functions, can be sensitive to subtle environmental variations. In addition to the variation of coordination sites in histidine, different coordination numbers of Cu^{II} (2,- 3-, 4-, 5- and 6-coordination) add more diversity of the coordination mode of Cu^{II}-bis-L-histidine complex. Many different techniques have been applied to elucidate the coordination of $Cu(His)_2$, including circular dichroism (CD), UV-Vis, infrared spectroscopy, Raman, NMR, ESR, potentiometric, calorimetric, and crystallography [5, 6]. Polarographic experiments revealed that the [$Cu(His)_2$] is the dominant species at physiological conditions [3]. In a recent study, the coordination between imidazole ring and copper ion was emphasized and illustrated using diethyl pyrocarbonate (DEPC) modified histidine and mass spectroscopy [7]. However, despite of all these studies, disagreement on the mode of histidine binding to copper and the structure of $Cu(His)_2$ in solution still exists, especially the coordination mode of $Cu(His)_2$ at physiological pH due to its important biological implication. Disputes arose from coordination geometry and the number of imidazole nitrogen involved in the coordination. Additionally, controversies exist at the pH value of the imidazole nitrogen coordination. Compared with other spectroscopic methods such as IR, Raman, UV-Vis, and crystallography, X-ray absorption spectroscopy (XAS) is particularly well suited for the investigation of the local solvent structure of ions, due to its atomic discrimination and its acuteness to dilute solutions [8]. Moreover, the X-ray absorption near-edge structure (XANES) spectra at the metal K-edge of transition metal complexes in aqueous solution have been the object to attain coordination geometry around

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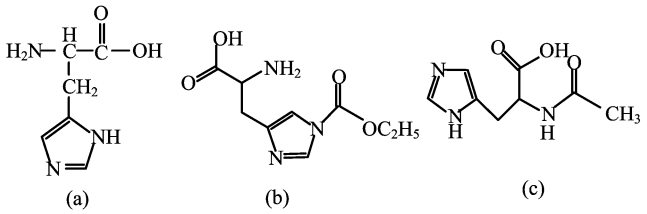

FIG. 1 Chemical formula of (a) histidine, (b) DEPC-histidine, and (c) acetyl-histidine.

the metal ion [9].

In this work, we studied $Cu(His)_2$ at different pH values using XAFS in order to clarify the coordination environment. It was performed in solution using fluorescent mode. To further confirm the coordination sites in histidine, the N-acetyl histidine was used to verify the coordination amino group and DEPC modified histidine was used to verify the coordination of imidazole group [10].

II. EXPERIMENTS

A. Sample preparation

L-histidine was purchased from Sangon Biotech (Shanghai) Co., Ltd. Other chemicals were purchased from Sinopharm chemical reagent Co., Ltd. All reagents were in analytical grade and were used without further purification. Copper complexes were prepared by dissolving 1.9166 g (0.2 mol) of L-histidine or acetyl-histidine in 50 mL distilled water, and 10 mL 0.5 mol/L of $CuCl_2{\cdot}2H_2O$ were added to this solution under continuous stirring. The pH-value was adjusted to 2.8, 4.5, 7.4, and 9.8 with diluted NaOH solution. Histidine modification was carried out by two equivalents of DEPC. The DEPC modified histidine was checked in prior to the XAFS experiment by means of UV. The DEPC modification on histidine shows a characteristic absorption peak at 240 nm, which obviously decreases with copper binding [7]. The chemical formula of histidine and modified histidines are shown in Fig.1.

B. XAFS data collection

XAFS spectra of the copper K-edge (8.979 keV) were performed on the National Laboratory of Synchrotron Radiation NLSR (USTC, China) at beamline U7C. Ring energy was 0.8 GeV, and ring current was 250 mA. Energy calibration was carried out with a copper foil. The liquid sample spectra were recorded at room temperature in fluorescent mode using a Si(111) double crystal monochromator and high purity 7-element Ge array detectors with 45° incidence angle, with hanning windows. Scanning time was set as 90 min for each scan, and each sample is scanned for once, since multiple scans didn't improve the signal-to-noise ratio significantly.

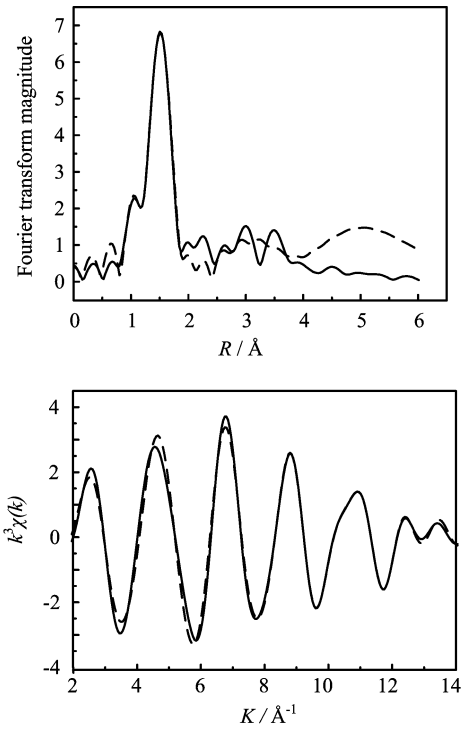

FIG. 2 (a) Non-phase shift corrected Fourier transforms of the experimental data of $Cu(His)_2$ at pH=7.4 (solid line) and the data fitting using IFEFFIT [16] (dashed line). (b) $k^3\chi(k)$ for the first- and second-nearest shells for the $Cu(His)_2$ complexe at pH=7.4. Solid lines indicate the experimental data and dashed lines indicate the simulation result.

C. XAFS data analysis

The data analysis was performed on the NSRLXAFS2.0 software package [11]. The theoretical backscattering amplitude and phase shift functions were calculated by the FEFF7 code [12]. At the initial step of simulation, the coordination number N_c was set as 4. Bond length R, coordination number and coordinated atoms (nitrogen and oxygen in this work) were then optimized in order to fit the experimental curves. The first-shell contributions have been fitted using the theoretical references. The input file based on $Cu(His)_2$ crystal at pH=7.4 for FEFF was created by the ATOMS software [13]. In the first coordination sphere fits, only single scattering paths of the first neighbors are taken into account.

III. RESULTS

The Cu K-edge XAFS spectra of the complex Cu-histidine have been measured at four pH values (2.8, 4.5,

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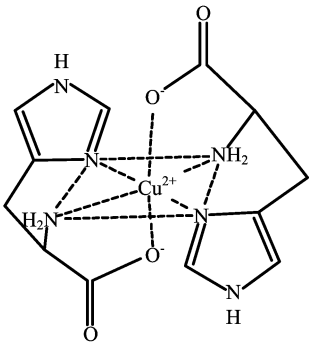

FIG. 3 Proposed molecular structure of Cu(His)$_2$ complex at pH=7.4.

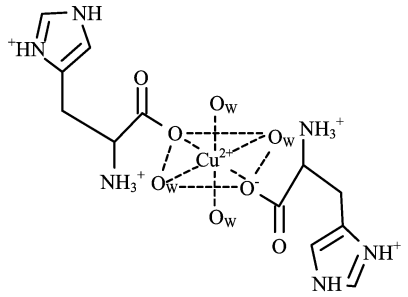

FIG. 4 Proposed molecular structure of Cu(His)$_2$ complex at pH=2.8.

7.4, and 9.8) since the main compositions reach their maximum at those pH values [13, 14]. The XANES spectra of Cu-histidine complexes exhibited the pre-edge at 8.98 keV, showing a typical Cu^{II} complex absorption [15]. A representative XAFS spectrum and fitting curve are shown in Fig.2. Figure 2(a) Shows the Fourier transformat into R-space. Figure 2(b) shows the convert scale from energy E into wavenumber k. The fitting was acquired using IFEFFIT [16]. The coordination numbers were estimated by the pattern of XANES signal from Cu(His)$_2$ and then calculated by fitting EXAFS data [15].

A. pH=7.4

The coordination of carboxylate group can be expected due to its deprotonation at neutral pH. Although the imidazole group and amino group are partially protonated at the physiological pH (the protonation ratios are 4.18% and 98.33%, respectively), both N_{am} and N_{im} still coordinated to copper, suggesting the strong binding affinity of these nitrogen atoms, as shown in Fig.3. Analyses of EXAFS data gave the interatomic distances of 1.96 Å for the four nitrogen atoms in the equatorial plane, and 2.28 Å for the two axial oxygen atoms. These distances are typical bond length for $Cu^{II}-N$ and $Cu^{II}-O$ [1]. This coordination structure was confirmed using chemically modified histidine. The EXAFS fitting results of the Cu(His)$_2$ and modifications at pH=7.4 are shown in Table I. As expected, the acetylation disabled the coordination of amino group, and the DEPC modification incapacitated the coordination of imidazole group. Either modification inactivated one nitrogen, and alters the histidine from tridentate to bidentate ligand, resulting in a 4-coordination Cu(His)$_2$ complex. However, the histidine modification by DEPC was prevented by Cu^{II} coordination. Once coordinated with copper ion, the N_{im} became inactive to DEPC, so it could be inferred that the coordination between copper and N_{im} was rather firm. We also noticed when O_C was at axial position, the bond length is larger than that at equatorial position.

TABLE I EXAFS fit results of the Cu(His)$_2$ and modifications at pH=7.4.

	Shell	R/Å	N_c	σ^{2a}/Å^2
Cu(His)$_2$	Cu-N_{im}	1.96	2	0.005
	Cu-N_{am}	1.96	2	0.005
	Cu-O_C	2.28	2	0.005
Cu(AcHis)$_2$	Cu-O_C	2.00	2	0.0005
	Cu-N_{im}	1.96	2	0.001
Cu(DEPC-His)$_2^b$	Cu-N_{am}	1.98	2	0.008
	Cu-O_C	2.02	2	0.001
Cu(His)$_2$-DEPC^c	Cu-N_{im}	1.96	2	0.001
	Cu-N_{am}	1.99	2	0.004
	Cu-O_C	2.28	2	0.001

[a] σ^2: Debye-Waller factor.
[b] DEPC-His: DEPC modified histidine.
[c] Cu(His)$_2$-DEPC: product from reaction of Cu(His)$_2$ with DEPC.

B. pH=2.8

At pH=2.8, both imidazole group and amino group are strongly protonated and their coordination can be prohibited. The proposed molecular structure of Cu(His)$_2$ complex at pH=2.8 is given in Fig.4. The EXAFS fitting results of Cu(His)$_2$ and modifications at pH=2.8 are shown in Table II. EXAFS data showed that neither the modification of carboxyl group nor the DEPC-modified imidazole group of histidine changed the coordination mode in the histidine-modified copper complexes. The carboxylate group was deprotonated due to its low pK_a value (1.82). Furthermore, Casella and Gullotti reported that the $\nu_{C=O}$ at 1736 cm^{-1} in IR spectra decreased intensively when pH value was above 2, which reflected the deprotonation of the carboxyl group with increasing pH [18]. These indicated that only carboxylate oxygen O_C and water oxygen (O_W) were involved in the coordination to the copper ion. This observation is consistent with the previous results from circular dichroism and electron spin resonance measurements that histidine is in mono-dentate coordination below pH=3 and the imidazole ring does

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TABLE II EXAFS fitting results of the copper complexes at pH=2.8.

	Shell	R/Å	N_c	σ^2/Å^2
$Cu(His)_2$	Cu-O_W	2.04	2	0.004
	Cu-O_C	1.92	2	0.004
	Cu-O_W	1.92	2	0.004
$Cu(AcHis)_2$	Cu-O_W	1.99	2	0.007
	Cu-O_C	1.92	2	0.007
	Cu-O_W	1.92	2	0.007
$Cu(DEPC\text{-}His)_2$	Cu-O_W	2.08	2	0.007
	Cu-O_C	1.94	2	0.007
	Cu-O_W	1.94	2	0.007
$Cu(His)_2$-DEPC	Cu-O_W	2.07	2	0.005
	Cu-O_C	1.93	2	0.005
	Cu-O_W	1.93	2	0.005

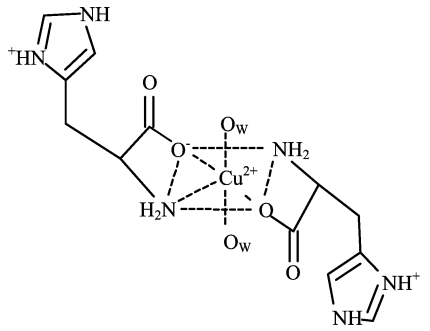

FIG. 5 Proposed molecular structure of $Cu(His)_2$ complex at pH=4.5.

not participate in the coordination [5, 18].

C. pH=4.5

While two distinct coordination modes have been identified at acidic and neutral conditions (O_6 and N_4O_2), the $Cu(His)_2$ coordination at an intermediate pH was also analyzed in order to further verify the coordination of two different nitrogen atoms. EXAFS data on the $Cu(His)_2$ complex showed a N_2O_4 coordination mode at pH=4.5, as shown in Fig.5, indicating that one nitrogen from each histidine were released from copper coordination at such pH condition. The EXAFS fitting results of the $Cu(His)_2$ and modifications at pH=4.5 are shown in Table III. EXAFS data from histidine modification in this work further supported this result. The imidazole modification by DEPC did not alter the copper coordination, whereas the acylated histidine gave an O_6 coordination mode. These results suggest that, while acidifying solution to pH=4.5, the N_{am} remains coordinated while N_{im} coordination was replaced by O_C. Different from the copper coordination at neutral pH, in which the depletion of imidazole resulted in a four-coordination structure, water coordination appeared in the DEPC modified histidine at pH=4.5.

TABLE III EXAFS fitting results of the $Cu(His)_2$ and modifications at pH=4.5.

	Shell	R/Å	N_c	σ^2/Å^2
$Cu(His)_2$	Cu-O_W	2.05	2	0.009
	Cu-O_C	2.00	2	0.002
	Cu-N_{am}	1.98	2	0.007
$Cu(AcHis)_2$	Cu-O_W	2.02	2	0.006
	Cu-O_C	1.92	2	0.006
$Cu(DEPC\text{-}His)_2$	Cu-O_W	1.92	2	0.006
	Cu-N_{am}	1.98	2	0.003
	Cu-O_C	2.01	2	0.003
	Cu-O_W	2.12	2	0.005
$Cu(His)_2$-DEPC	Cu-N_{am}	1.95	2	0.004
	Cu-O_C	1.99	2	0.001
	Cu-O_W	2.05	2	0.005

TABLE IV EXAFS fit results of the $Cu(His)_2$ and modifications at pH=9.8.

	Shell	R/Å	N_c	σ^2/Å^2
$Cu(His)_2$	Cu-N_{im}	1.96	2	0.003
	Cu-N_{am}	1.98	2	0.005
	Cu-O_C	2.02	2	0.001
$Cu(AcHis)_2$	Cu-N_{im}	1.96	2	0.003
	Cu-O_C	2.01	2	0.001
$Cu(DEPC\text{-}His)_2$	Cu-N_{am}	1.98	2	0.007
	Cu-O_C	2.02	2	0.002
$Cu(His)_2$-DEPC	Cu-N_{im}	1.96	2	0.005
	Cu-N_{am}	1.99	2	0.008
	Cu-O_C	2.00	2	0.006

D. pH=9.8

All three potential copper binding sites, carboxylate, imidazole, and amino groups are deprotonated at such pH, so that the natural histidine demonstrates a N_4O_2 coordination mode. Thus the coordination mode in this condition is the same as that at neutral pH, in which the histidine shows a tridentate ligand and all three groups get involved in the coordination, although minor bond length differences can be observed at pH=9.8 (Table IV). The DEPC modification and N-acetylation released the coordination of imidazole and amino, respectively. Consequently, the depletions of these nitrogen coordination resulted in four-coordination structures, which was the same as that at neutral pH.

IV. DISCUSSION

Two coordination modes have been proposed on bis-L-histidine copper(II) complexes [1]. One is the "lycine-

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type", in which one histidine offers an oxygen atom and an amine nitrogen while the other histidine offers two nitrogen atoms which come from the amine group and the imidazole group respectively. The other is the "histamine-histamine type", in which each histidine donates an imidazole nitrogen and an amine nitrogen. The glycine-type coordination exists under acidic environment, due to the pK_a of carboxylate is much smaller than that of N in histidine. As pH increasing, the imidazole and amino groups become deprotonated and get involved in Cu^{II} coordination. An equilibrium mixture of "glycine-histamine" has been proposed at pH=8.1 [1].

Deprotonation of ligands is usually required for metal coordination, so that the protonation status can directly influence the coordination affinity. Compared with the other two binding sites, the carboxylate group coordinates to copper at more acidic condition due to the low pK_a (1.8). The protonation of imidazole and amino groups prevents their coordination at pH=2.8 since their pK_a are much higher (6.0 and 9.18 for imidazole and amino groups, respectively) [6]. These groups get coordinated when pH increases. It is worth noting that the coordination sequence may differ from the deprotonation order. The amino group can coordinate at lower pH than imidazole group, although the pK_a of imidazole is smaller. This observation can be explained by the inductive effect on the deprotonations of N_{am} and N_{im} by O_C coordination, and the electrostatic interaction between the Cu^{2+} ion and the π system of the histidine [5, 19], and hence results in the more favored coordination with N_{am} despite of the higher pK_a.

It has been reported that histidine is protonated at very acidic condition (pH=1) and copper exhibits a hexa-aqua complex [5]. Histidine gets involved in copper coordination when the pH increased above 2.0 while the carboxylate group starts deprotonation. Two O_C replace the water molecules in copper binding while histidine gets coordinated, and the Cu−O_6 coordination mode remained at such condition. Our data from XAFS measurements are in agreement with the previous reports. For the Cu(His)$_2$ complex at acidic condition, the earlier EXAFS data gave the average Cu−O bond distances at 1.95 Å [5], whereas the X-ray crystallography revealed that in solid state, the Cu−O_W distances are 2.46 and 2.78 Å for the water above and below copper-N_{am} planar, respectively [20]. The data fitted from the EXAFS spectra in this work differentiated the two types of oxygen coordination, showing that the four O_W from water were at the planar geometry with Cu−O_W distances ranging from 2.0 Å to 2.05 Å, while the two O_C from carboxylate groups were at the axial position with Cu−O_C distances 1.98 Å. This result suggests that at this acidic condition, the coordination mode of Cu(His)$_2$ complex is similar to Cu(Gly)$_2$ complex.

Controversy exists on the coordination mode of Cu(His)$_2$ complex at weak acidic condition [5]. The UV-Vis/NIR data showed a four-coordination mode at pH=4.5 with $N_{am}O_CN_{am}N_{im}$ atoms in the equatorial plane [5]. While ESR studies suggested that $N_{am}O_CN_{am}O_C$ coordination remains the primary composition until pH=8 [21]. The fitting EXAFS data in this work show a Cu^{II}-N_2O_4 coordination mode. Compared with that in more acidic condition, the increase of pH from 2.8 to 4.5 causes the substitution of two water molecules by two nitrogen atoms in copper binding. The coordination of N_{am} is supported by the data from the acetylated histidine, which disables the nitrogen coordination and results in a Cu^{II}-O_6 complex at pH=4.5. In contrast, the imidazole modification by DEPC does not alter the coordination mode, which confirms the uncoordination of N_{im} atom at this pH.

The coordination of Cu(His)$_2$ at the physiological pH=7.4 has been mostly studied. The crystal structure indicates that the structure of Cu(His)$_2$ is a five-coordinate complex in a distorted square pyramid geometry [6]. Mass spectrometry study suggested that the structure of five-coordinate Cu(His)$_2$ is also present in solution [7]. However, major studies on solution samples indicated that the five-coordinate Cu(His)$_2$ in solid state converts to a six-coordinate structure with a N_4O_2 mode [5]. The discrepancy is very likely due to the different techniques used in the studies, as each technique only allows the determination of certain structural features. The EXAFS data in this work support the six-coordinate mode of Cu-N_4O_2, in this case both amino and imidazole get involved in the copper coordination. We also tried to fit the EXAFS data with five-coordination mode, but it gave higher Debye-Waller factor than that from six-coordination mode. The data from modified histidine further confirmed this coordination mode. Either amino acetylation or imidazole modification can release two coordination nitrogen atoms and results in the four-coordinated Cu-N_2O_2 structure. Although this result can not exclude the small fraction of five-coordinate Cu(His)$_2$ in equilibration, our data suggested that the six-coordinate mode of Cu-N_4O_2 was the dominant species in solution. Indeed, the presence of five-coordinate Cu(His)$_2$ in the equilibration can well explain the half protection of histidine from DEPC modification.

The coordination mode at pH=9.8 remains the same as that at pH=7.4. This result is in agreement with previously reported results using UV-Vis/NIR methods, in which the d-d transition band remained constant at pH>7 [5].

V. CONCLUSION

We have studied the coordination modes of Cu^{II}-L-histidine at different pH using XAFS in this work. The coordinations of amino nitrogen and imidazole nitrogen have been further confirmed by using chemically modified histidines. The results show that, while pH increases from acidic to basic condition, the complex changes from glycine-like to histamine-like coordination

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mode. Although this observation in general agrees with the literature results, this work clarifies the controversial coordination mode of $Cu(His)_2$ at physiological pH. Our results show that six-fold coordination geometry with bond lengths from 1.96 Å to 2.02 Å is plausible, while the five-fold condition could co-exist in equilibration. Additionally, the bond length in $Cu(His)_2$ complex at pH=4.5, on which the controversy exists in the literatures, was verified in this work.

VI. ACKNOWLEDGMENTS

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