Research Article

pH-Potentiometric Investigation towards Chelating Tendencies of p-Hydroquinone and Phenol Iminodiacetate Copper(II) Complexes

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Copper ions in the active sites of several proteins/enzymes interact with phenols and quinones, and this interaction is associated to the reactivity of the enzymes. In this study the speciation of the Cu2+ with iminodiacetic phenolate/hydroquinonate ligands has been examined by pH-potentiometry. The results reveal that the iminodiacetic phenol ligand forms mononuclear complexes with Cu2+ at acidic and alkaline pHs, and a binuclear O phenolate-bridged complex at pH range from 7 to 8.5. The binucleating hydroquinone ligand forms only 2 : 1 metal to ligand complexes in solution. The pK values of the protonation of the phenolate oxygen of the two ligands are reduced about 2 units after complexation with the metal ion and are close to the pK values for the copper-interacting tyrosine phenol oxygen in copper enzymes.

1. Introduction

Copper ions in the active sites of proteins/enzymes mediate a broad scope of chemical processes including electron transfer, dioxygen uptake, storage, and transport and catalytic conversions [1]. When surveying the known copper enzymes and their functions, it is striking that their reactivity is typically linked to dioxygen or compounds directly synthesized from O2-like phenols and quinones [2–7].

For example, copper proteins are involved in reversible dioxygen binding in hemocyanin [8], two-electron reduction to peroxide coupled to oxidation of substrates in amine and galactose oxidases [9], biogenesis of novel metalloenzyme cofactors (e.g., topaquinone in amine oxidases) [10], activation of hydroxylation in tyrosinase [11], and proton pumping in cytochrome c oxidase [12].

Detailed study of the solid and solution chemistry of Cu2+ phenolate/hydroquinonate complexes is essential for better understanding of the coordination of the metal ion in the enzymes and the mechanisms of the enzymatic catalysis. Derivatives of phenol or hydroquinone containing nitrogen [13–22] as donor atoms are the vast majority of the ligands used to model the active site of the copper enzymes. Despite the importance of phenolate/hydroquinonate chelating ligands as models of copper enzymes, ligands with other than nitrogen donor atoms such as aminocarboxylate derivatives of phenols, have been much less studied. These ligands exhibit very attractive features for modelling metal enzymes, such as the highly solubility in aqueous solution, forming stable complexes with metal ions and the similarity of the donor groups to those in biological systems. In addition, the one-electron oxidized p-semiquinone radical of the ligand 2,5-bis[N,N-bis(carboxymethyl)aminomethyl] hydroquinone (H6bicah) has been stabilized in aqueous solution by ligation to metal ions [23] and thus serves as model for the enzymes that operate via a p-semiquinone radical, acting in one-electron transfer reactions, including cytochrome c and copper amine oxidases. In previous pH-potentiometric studies [24] of Cu2+ with the phen iminodiacetate ligand HBIDA (Scheme 1) the equilibrium calculations have been performed assuming that all the species of Cu2+ with HBIDA in solution at various pHs are mononuclear 1:1 and 1:2
metal to ligand complexes. A recent detailed crystallographic study [25] of the Cu2+-phenol iminodiacetate H4cacp, H4cah and H6bicah (Scheme 1) complexes isolated at a pH range 2.0–9.0 has shown that binuclear O phenolate-bridged Cu2+ complexes (Scheme 2) are also present in solution. It is apparent that previous pH-potentiometric studies of these systems should be repeated including also the dinuclear species in the calculations.

Herein, we describe the pH-potentiometric titration study of Cu2+ with the iminodiacetate phenolate tripod ligands H4cacp and H6bicah. In contrast to H4cacp, H6bicah exhibits two metal ion binding sites bridged through the hydroquinone moiety. The potentiometric study showed that only the H4cacp ligand forms in solution O phenolate-bridged binuclear complexes, which is in agreement with the previous crystallographic study [25]. The pK values of the protonation of the phenolate oxygen of the two ligands reduced about 2 units after complexation with the metal ion are close to the pK values for the copper-interacting tyrosine phenol oxygen in copper enzymes, such as glyoxal oxidase [26].

2. Experimental Section

2.1. Materials. Copper(II) acetate monohydrate, p-hydroquinone, 4-hydroxybenzoic acid, iminodiacetic acid, paraformaldehyde, potassium chloride, and potassium hydrogen phthalate were obtained from Aldrich. Sodium hydroxide and hydrogen chloride were purchased from Merck. All chemicals were reagent grade and used without further purification.

2.2. Ligand Preparation. The ligands referred to this study 2,5-bis[N,N′-bis(carboxymethyl)aminomethyl]-hydroquinone (H6bicah) and 2-[N,N′-bis(carboxymethyl)aminomethyl]-4-carboxyphenol (H4cacp) were synthesized based on the Mannich type reaction reported in the literature [27, 28]. The synthesis of the organic ligands (Scheme 1) was performed under inert nitrogen atmosphere and their purity was checked and confirmed by means of 1H-NMR spectroscopy. 1H-NMR spectra were recorded on a 300.13 MHz Avance Brucker spectrometer.

2.3. Potentiometric Studies and Computational Data Analysis. The potentiometric equilibrium measurements of H4cacp and H6bicah ligands in the absence and in the presence of metal ions were carried out with a JENWAY 3020 pH meter fitted with an Ag-AgCl reference electrode in saturated KCl solution. A glass electrode was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO2-free NaOH solution, and the equivalence point was determined by Gran’s method which yields the standard potential E◦ of the electrode, using the GLEE computational program [29]. The actual concentration of NaOH (0.157 mol dm−3) was standardized by titration with potassium hydrogen phthalate, and the HCl solution (0.111 mol dm−3) was standardized by titration of the standard NaOH solution. The temperature was maintained at 298 K and the ionic strength of each experimental sample was adjusted to 0.100 mol dm−3 with the addition of KCl supporting electrolyte. Typical concentrations of experimental solutions were 5.00 mmol dm−3 in ligand with molar concentration of copper (II) ion half, equivalent, and twice to that of the ligand. Degassed distilled water was used for the preparation of the solutions and the oxygen and carbon dioxide contamination of the reaction mixtures from the atmosphere was avoided by continuous passing of purified nitrogen gas in the reaction cell.

The proton association constants of H4cacp and H6bicah ligands and the formation constants of 1:1 (H4cacp: Cu2+) and 1:2 (H6bicah: 2Cu2+) metal-ligand systems were obtained using the program TIRMET which is a computational program based on mass-balance and charge-balance equations, written in our laboratory according to the basic principles first reported by Martell and Motekaitis [30, 31]. In this program the input consists of the components and their concentrations, the initial values of the equilibrium constants for each species considered to be present, the potentiometric equilibrium data determined experimentally, and conditions of the potentiometric experimental procedure (E◦, pKw = 13.78 at 298 K, y = 0.78). The program sets up simultaneous mass-balance equations for all components at each neutralization value involving the concentration of acid added to the assay and solves for each species present in the pH region 2.00–10.0. Then, equilibrium constants are varied in order to minimize the differences between the calculated and observed values, resulting in the fitting of the calculated results to the experimental curves. The concentration stability constants, βpqr = [MqLr身上]/[M]q[L]r[身上], were considered to be estimated according to the model proposed by the computational program PSEQUAD [32]. The species considered present in the assays are those expected to be formed according to established principles of coordination chemistry including the formation of deprotonated and protonated metal chelates, respectively [24, 33–35]. All potentiometric titrations were performed three times for each system (about 100 data points each) in the pH range 2.00–10.0 without significant variation.

3. Results and Discussion

3.1. Ligands. Potentiometric titrations of phenol (H4cacp) and p-hydroquinone (H6bicah) iminodiacetate derivatives indicate stepwise protonation steps arising from their characteristic functional groups, amine, carboxylates, and phenolate, in the measurable pH range. The protonation constants (overall stability protonation constants log β) are listed in Tables 1 and 2, respectively, and their distribution speciation diagrams are illustrated in Figure 1.

The pH-metric titration curve of H4cacp indicates three major protonation steps due to the phenolate or the benzoic-carboxylate oxygen group, the carboxylate oxygen group, and the amino group with pKs values 8.47, 4.84, and 2.42, respectively (Table 1). The low pKs (2.42) value attributed to the amine nitrogen atom demonstrates intramolecular hydrogen bonding between the deprotonated amino group
is similar to that found for an analogue ligand [N-(o-hydroxybenzyl)]iminodiacetic acid] [24]([HBIDA, Scheme 1) (2.34) while for the nonphenolic, iminodiacetic acid (ida) the corresponded value is 2.94 [33]. The pH-metric titration of the symmetric bis-substituted iminodiacetate p-hydroquinone derivative H4bicah gave two steps each one corresponding to two successive protonation of the two phenolate oxygens and the two carboxylate groups with pK\textsubscript{a} values 8.47 and 7.26, respectively (Table 2). It was not possible to determine the pK\textsubscript{a} value for the amine nitrogen group because this value was very low.

3.2. Cu(II)-H4cacp. The Cu(II)-H4cacp titration curves were evaluated on the assumption of the formation of various 1:1, 1:2 and 2:1 metal to ligand species with different protonation steps. The extensive crystallographic study of the isolated complexes from solutions of Cu(II)-H4cacp at various pHs reported by Stylianou et al. [25] was also used for the better suggestion of the species in solution (Scheme 2). The best fit with the experimental data (Figure 2(a)) was obtained with the speciation model listed in Table 1. Species distribution curves for the complexes formed in the Cu(II)-H4cacp system as a function of pH are depicted in Figure 3.

Cu(II) ion forms with H4cacp three major mononuclear species, the protonated [Cu(H2cacp)(H2O)] at pH below 5.0, the deprotonated [Cu(Hcacp)(H2O)]\(^{−}\) at pH between 5.0 and 6.5 the mono-hydroxo species [Cu(Hcacp)(OH)]\(^{2−}\) at pH above 9.0 and a minor 1:2 metal to ligand [Cu(H2cacp)]\(^{2−}\) species at pH 5.

The process from the deprotonated mononuclear species to the protonated one, which corresponds to the consumption of one H\(^{+}\) per molecule of complex equation (1), is accompanied by a color change from green to blue attributed to the protonation of the phenolic oxygen. The protonation of the phenolic oxygen will result in weakening or non-bonding of the Cu−OH(phenol) bond which is in agreement with the color change (the mononuclear nonphenolic amino acetate complexes of Cu\(^{2+}\) at acidic pHs exhibit blue color). The crystallographic data of the complex isolated at pH 3.2 [25] confirm the weak interaction between the protonated phenol oxygen atom and the metal ion [Cu–OH(phenol), 2.529(2) Å]:

\[
\text{OH} \quad \text{Cu} \quad \text{O} \quad +\text{H}^+ \quad \text{Cu}
\]

(1)

The estimated pK\textsubscript{a} involved in this protonation step is 5.22 ± 0.02 and is comparable to that calculated by UV-vis spectroscopic studies and was found to be 5.91 ± 0.05 [25]. The overall stability formation constants of complexes [Cu(Hcacp)]\(^{−}\) and [Cu(H2cacp)(H2O)] are greater than those of the iminodiacetate copper (II) complexes [Cu(ida)]\(^{−}\) (log \(β\) 10.42) and [Cu(H(ida))]\(^{−}\) (log \(β\) 12.35) [33]. The higher stability is ascribable to the coordination of the phenolate oxygen atom. This is also supported by the X-ray crystallographic studies which show that the deprotonated form, even at low pHs, strongly interacts with the metal ion. In addition, the planar configuration of the phenyl ring fixes
the orientation of the flexible carboxylate groups in positions favorable to chelating, especially in the case of the copper(II) ion which forms stable complexes in an octahedral/or square pyramidal coordination geometry pattern [36].

One very significant result of this potentiometric titration study is the detection of the dimeric species $[\text{Cu}_2(\text{Hcacp})_2]^{2-}$. Previous potentiometric studies have postulated that the dimeric complexes are not favored in solution because of steric effects and electrostatic destabilization which do not allow a dimerization process [35]. Harris et
Scheme 1: Iminodiacetic derivatives of phenol/p-hydroquinone ligands with their abbreviations. The ligands referred to the potentiometric/stability studies are denoted in parentheses.

Scheme 2: Molecular drawings of the structures of the phenol and p-hydroquinone iminodiacetate copper(II) complexes, isolated at a pH range 2.0–9.0 according to a recent detailed crystallographic study [25].
Cu²⁺−di- and mono-hydroxo complexes \([Cu_2(Hcacp)2]^{2−}\) and \([Cu_2(bicah)(OH)(H_2O)]^{3−}\) complex of Cu²⁺ and the phenol iminodiacetate ligand al. had suggested the formation of a mononuclear phenolate \((log β_1)\), and acidity constants (pKₐ) for the species formed in H₆bicah and Cu(II)-H₆bicah system, over the pH range 2.00−10.0 thus obtained from the potentiometric study (25°C, I = 0.10 mol dm⁻³ KCl, pKₐ = 13.78, and γ = 0.78).

\[
\begin{array}{ccc}
(p, q, r) & \text{Species} & log β & \text{pK}_a \\
(0, 1, 0) & [H_2bicah]^4− & 8.41 ± 0.02 & 8.47^a \\
(0, 1, 2) & [H_4cacp]^{2−} & 13.40 ± 0.01 & 7.26^b \\
(2, 1, −2) & [Cu_2(bicah)(OH)H)]^{3−} & 11.57 ± 0.15 & \\
(2, 1, −1) & [Cu_2(bicah)(H_2O)(OH)]^{3−} & 15.72 ± 0.11 & \\
(2, 1, 0) & [Cu_2(bicah)(H_2O)]^{2−} & 32.90 ± 0.16 & \\
(2, 1, 1) & [Cu_2(bicah)(H_2O)]^{2−} & 39.33 ± 0.16 & \\
(2, 1, 2) & [Cu_2(bicah)(H_2O)]^{2−} & 45.52 ± 0.12 & \\
\end{array}
\]

\(^4\) Phenolate oxygen group, \(^b\) carboxylate oxygen group.

al. had suggested the formation of a mononuclear phenolate complex of Cu²⁺ and the phenol iminodiacetate ligand HBIDA at pH above 6.0 (Scheme 1), but they have not mentioned the possibility of dimeric binuclear species in solution [24]. However, recently Stylianou et al. [25] have isolated and crystallographically characterized the dimeric species \([Cu_2(Hcacp)2]^{2−}\) from aqueous solution at alkaline pHs 8.0−9.0, indicating that such species are present in solution. In this complex the two Cu²⁺ are bridged through the deprotonated phenolate oxygen (Scheme 2). The speciation diagram of Cu(II)-H₆bicah system in Figure 3 shows that \([Cu_2(Hbicah)(H_2O)]^{2−}\) is the major complex at pH range 7.0−8.5 reaching a maximum of 20% of the total metal ion concentration at pH 8.0 and an overall stability formation constant 11.26 ± 0.02 equation (2).

\[
\begin{align*}
2 + 2H^+ & \rightarrow \text{Cu}^{2+} + \text{Cu}^{2+} + 2H^+ + 2\text{OH}^− \\
\end{align*}
\]

3.3. Cu(II)-H₆bicah. The Cu(II)-H₆bicah titration curves were evaluated on the assumption of the formation of various 1:1 and 2:1 metal chelates with different protonation steps. The best fit between the simulated curves and the experimental data (Figure 2(b)) was obtained by the speciation model listed in Table 2. Species distribution curves for the complexes formed in the Cu(II)-H₆bicah system as a function of pH are depicted in Figure 4. In contrast to H₄cacp, H₆bicah exhibits two metal binding sites, thus, the ligand may ligate up to two metal ions. The potentiometric study shows that the 1:1 species are unstable and the equilibrium is favoured only to the formation of 2:1 metal to ligand complexes. In addition, the binucleating ligand, H₆bicah, exhibits larger steric hindrance than H₄cacp and thus does not form Ophenolate-bridged complexes with Cu²⁺ in solution or in solid state. At pH above 9.5 the di- and mono-hydroxo complexes \([Cu_2(bicah)(OH)]^{3−}\) and \([Cu_2(bicah)(OH)(H_2O)]^{3−}\) are the major species with stability formation constants 11.57 ± 0.15 and 15.72 ± 0.11, respectively. The brown \([Cu_2(bicah)(H_2O)]^{2−}\) is the major species between pH 7.0 and 9.5 and the green monoprotonated \([Cu_2(Hbicah)(H_2O)]^{2−}\) at pH range 5.0 to 7.0. The second phenol is protonated at pH below 5.0 resulting in the formation of the blue neutral \([Cu_2(Hbicah)(H_2O)]^{2−}\) which has been previously characterized by single crystal X-ray crystallography (Scheme 2) [25]. The two pKₐ values for the two equilibriums of the stepwise protonation of the two phenolate oxygen atoms equation (3) have been calculated as 5.89 ± 0.10 and 6.43 ± 0.10 for pKₐ1 and pKₐ2, respectively. These values are close to the values 6.25 ± 0.08 and 7.19 ± 0.08 for pKₐ1 and pKₐ2, respectively, found by spectrophotometric studies [25]. These differences are observed because the model used for the calculations in the spectrophotometric studies was incomplete (only the equilibriums in (3) were taken into account):

\[
\begin{align*}
\text{Cu}^{2+} & \text{H}_{\text{bicah}}^− + \text{H}^{+} \rightarrow \text{Cu}^{3+} + \text{H}_{\text{bicah}}^2− \\
\text{Cu}^{2+} & \text{H}_{\text{bicah}}^2− \rightarrow \text{Cu}^{3+} + \gamma \text{H}_{\text{bicah}}^− + \text{H}^{+} \\
\end{align*}
\]

The fact that there is almost 0.5 pK unit difference between the two deprotonation steps indicates that the electronic interaction between the two metal centres through the hydroquinone bridge is significant. A comparison between the overall stability constants of the two ligands in this study shows that the bifunctional ligand H₆bicah forms more stable complexes than H₄cacp in solution. This extra stabilization is attributed to the larger increase of entropy expected for the formation of the binuclear Cu²⁺-H₂bicah complexes compared to the mononuclear Cu²⁺-H₂cacp.

4. Conclusions

The speciation of Cu²⁺ with the iminodiacetate phenol/hydroquinone ligands H₄cacp/H₆bicah in aqueous solution was investigated by pH-potentiometry. Ligand H₄cacp, at pH below 5.0 forms with Cu²⁺ the mononuclear 1:1 and 1:2 complexes. At higher pH the phenol proton is deprotonated and at pH range 5.0−7.0 the major species is the mononuclear 1:1 complex. However at pH 7.0−8.0 the formation of a binuclear complex takes place and it is attributed to a Ophenolate-bridged complex. The binucleating ligand H₆bicah forms only 2:1 metal to ligand complexes in the pH range 2.0 to 9.0. The major species are the complete phenol protonated complex at pH below 4.5, the monoprotonated at pH range 4.5 to 7.0, and the complete phenol deprotonated species between pHs 7.0 and 9.0. The H₆bicah did not form binuclear Ophenolate-bridged complex in solution probably due to steric hindrance originated from the binucleating nature of the ligand. On the other hand, this solution study shows that binuclear Ophenolate-bridged species...
must also be considered in speciation studies of Cu^{2+} ions with mononucleating phenolate ligands such as H_{4}cacp.

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