Kinetics and Mechanism of Ligand Substitution in Binuclear Copper(II) Complexes as Model Compounds for Hemocyanin

Siegfried Schindler* and Horst Elias*

Anorganische Chemie III, Eduard-Zintl-Institut, Technische Hochschule Darmstadt, Hochschulstraße 10, D-6100 Darmstadt

Helmut Paulus
Institut für Physikalische Chemie, Abteilung Strukturforschung, Technische Hochschule Darmstadt, Petersenstraße 20, D-6100 Darmstadt

Dedicated to Prof. Dr. Dirk Reinen on the occasion of his 60th birthday

Z. Naturforsch. 45b, 607–618 (1990); received November 17, 1989

Binuclear Copper(II) Complexes, X-Ray, UV/VIS Spectra, Ligand Substitution, Kinetics and Mechanism

A series of binuclear, hydroxyl-bridged copper(II) complexes 1–10 with the general formula [Cu2(LR2D)(OH)](Cli04)2 was prepared and characterized. The mono basic, five- or seven-dentate ligands HLR2D were obtained either by reduction of the Schiff bases derived from the condensation of 2,6-diformyl-4-methylphenol and the amines H2N–D (complex 1: D = –(CH2)2–NH2; 2: –(CH2)2–NMe2; 3: –(CH2)3–NET2; 4: –(CH2)3–NET2; 5: –(CH–)2–4-imidazolyl; 6: –CH2–2-pyridyl; 7: –(CH2)2–2-pyridyl) or by reacting 2,6-di-(chloromethyl)-4-methylphenol with 2-[(2-methylamino)ethyl]pyridine (complex 8), N,N,N′,N′-tetraethyldiethylenetriamine (complex 9) or bis[2-(2-pyridyl)ethyl]amine (complex 10) under HCl elimination. The UV/VIS spectra of complexes 1–8 with two four-coordinate copper centers and of 9 and 10 with two five-coordinate copper centers are discussed. An X-ray structure analysis of complex 6 (= [Cu2(LR2D)(OH)](Cli04)2 with R = H and D = –CH2–2-pyridyl) was carried out. 6 crystallizes in the orthorhombic space group Pbca and the two copper centers have practically co-planar, doubly O-bridged cis-N-O, coordination geometries with one perchlorate anion being weakly coordinated to each copper. Stopped-flow spectrophotometry was used to study the kinetics of copper removal from [Cu2(LR2D)(OH)]2+ with EDTA in the pH range 8.5–6.6 at 20 °C and I = 0.2 M (NaClO4). The reaction is first-order in both complex and EDTA, the rate law being: rate = k|complex|LEDTAให้. At pH 8.0 second-order rate constants k range from 40.3 × 10−3 M−1 s−1 (complex 6) to 3.72 × 10−2 M−1 s−1 (complex 9). It follows from the pH dependence of k that both species H2EDTA− and HEDTA3− react independently with [Cu2(LR2D)(OH)]2+, the reactivity of H2EDTA− being considerably greater than that of HEDTA3−. The experimental facts support the operation of an associative (A) mechanism with the removal of the first copper being rate-controlling. The kinetic effects of the donor group D and substituent R are attributed to steric and/or electronic interactions.

Introduction

From the coordination chemistry point of view the oxy-state of the active site of the oxygen carrying metalloprotein hemocyanin (= Hc) [1–3] can be classified as a binuclear copper(II) complex with the two coppers exogenously bridged by peroxide according to (1) [4–6].

[\text{His}–\text{Cu}^2\text{II}–\text{O}–\text{O}–\text{His} \rightleftharpoons \text{His}–\text{Cu}^2\text{II}–\text{O}–\text{O}–\text{His}]

(1)

The presence of an additional endogenous bridge OR (water?) in oxy-Hc is discussed, although convincing experimental evidence for its existence is scarce [2, 7–13]. Kinetic studies on Hc include deoxygenation and oxygenation, redox, addition and demetalation reactions [2, 3, 14, 15].

* This contribution summarizes the main results of the Dr.-Ing. Dissertation submitted to Technische Hochschule Darmstadt, D17, by Siegfried Schindler in 1989.

* Reprint requests to Prof. Dr. H. Elias.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen 0932–0776/90/0500–0607/$01.00/0
The broad interest in He and its role in the metabolism of molluscs and arthropods has initiated extensive research on low molecular weight binuclear copper(II) complexes as potential models for oxy-Hc [16, 17], the preparation of systems with oxygen binding properties being a main goal [18].

Reaction (2) is a standard procedure to remove copper from Hc and thus prepare the apo-protein [2, 3, 14, 15]. From the kinetic point of view this reaction is a rather complex multi-step process for most hemocyanins [2, 15] and its interpretation is hampered by the fact that the specific state of aggregation (number and linkage of subunits) of any hemocyanin can produce cooperative effects [15].

Considering the rather limited information available in the literature on the kinetic behaviour of binuclear copper(II) model complexes [19–22] we decided to carry out a systematic study on the kinetics of ligand substitution according to reaction (3)

\[
[Cu_2(LR_2D_2)(OH)] + 2 \text{EDTA} \rightarrow 2 \text{Cu(EDTA)} + LR_2D_2
\]

(charges omitted for clarity). The symbol \([Cu_2(LR_2D_2)(OH)]^{2+}\) stands for a series of cationic binuclear copper(II) complexes 1–10 in which the two coppers are bridged by a phenolate and a hydroxyl oxygen, the other coordination sites being occupied by nitrogen.

In complexes 1–8 the two coppers are four-coordinate (set of donor atoms: N_3O_2), whereas in 9 and 10 they are five-coordinate (N_3O_2).

One of the main questions to be answered by the present study is the question of how the removal of the two equivalently bound coppers in complexes 1–10 occurs kinetically and how the kinetic pattern governing reaction (3) compares to that of native Hc.

**Experimental**

*Materials and reagents*

2,6-Diformyl-4-methylphenol was prepared according to Ullmann and Brittnner [23]. The amines H_3N–D needed for the preparation of the ligand systems in complexes 1–7 and the amines 2-[(2-methylamino)ethyl]pyridine and N,N,N',N'-tetraethylidithylenetriamine were commercially available (Aldrich and Merck; purity 95–99%). Bis[2-(2-pyridyl)ethyl]amine for complex 10 was obtained by reacting 2-vinyl pyridine with NH_4Cl [24] or with 2-(2-aminoethyl)-pyridine [25]. NaBH_4 (Merck), Na_2EDTA·2H_2O (Merck), NaClO_3·H_2O (Fluka), Cu(ClO_4)_2·6H_2O (Aldrich) and the buffers EPPS, MES and MOPS (Sigma) were used without further purification. Doubly
distilled water (quartz apparatus) was applied for the kinetic studies.

**Complexes**

Complexes 2–7 were prepared according to the following general procedure [26].

A solution of 5 mmol 2,6-diformyl-4-methylphenol in 50 ml EtOH was reacted with 10 mmol of the corresponding amine H,N–D to form the orange Schiff base. After addition of 1 g NaBH₄, dissolved in 20 ml water, and stirring for 1 h at room temperature the almost colorless solution was brought to pH 5 with dilute HClO₄ and filtered. Dropwise addition of 10 mmol Cu(CIO₄)₂, dissolved in 20 ml water, to the filtrate led to a green solution, which was stirred for 15 min and filtered. Standing of the filtrate for 1–3 days in an open beaker at room temperature led in most cases to green crystals of [Cu(H₂T.RD)(OH)](CIO₄)₂, which were recrystallized from hot water. Sometimes viscous oils instead of crystals were formed, which was a problem.

Complex 1 was prepared by the method described by Robson and Dickson [27], whereas complexes 8–10 were obtained according to Sorrell, Jameson and O'Connor [28].

**Caution!** The perchlorates thus prepared should be handled with great care to avoid explosions!

Results of elemental analysis (C, H, N) obtained for complexes 1–10 were in fair agreement with theory. The UV/VIS data (collected with a diode array spectrophotometer, Hewlett-Packard 8451 A) are compiled in Table I.

**X-ray structure determination**

Crystals of 6 were grown by slow evaporation of an aqueous solution of 6. Intensities were measured on a four-circle diffractometer (Stoe-Stadi-4) using graphite-monochromatized Mo-Kα radiation. Cell constants were determined by least-squares methods from the 2θ angles of 56 reflections (T = 25 °C) measured on the same instrument. LP and background corrections and a numerical absorption correction were applied.

The structure was solved by SHELXS-86 and refined to the values given in Table II. Hydrogen atoms were positioned geometrically (C–H distance 1.08 Å) and not refined. All crystallographic calculations were performed with the programs SHELX-76 and SHELXS-86 on an IBM 200D computer at Technische Hochschule Darmstadt. Scattering factors for C, H, N and O as stored in SHELX-76 [29]. Lists of atomic and thermal parameters as well as structure factor tables have been deposited*.

**Kinetic measurements**

Reaction (3) was followed at 350 nm with a modified [30] stopped-flow spectrophotometer (Durrum, D110) at constant ionic strength (I = 0.2 M; NaClO₄) and 20 °C under pseudo-first-order conditions ([EDTA]₀ ≈ 10[complex]₀). The absorbance/time data were computer-fitted to either

---

* Further information may be obtained from: Fachinformationszentrum Karlsruhe GmbH, D-7514 Eggenstein-Leopoldshafen 2, by quoting the depository number CSD-54410, the names of the authors and the journal citation.
Empirical formula  
Crystal system  
Cell constants (pm;\(\text{°}\))  
Space group  
Formula units/unit cell  
Calculated density (g \(\cdot\) cm\(^{-3}\))  
Number of reflections collected  
Number of symmetry-independent reflections  
Reflections with F > 2\(\sigma(F)\)  
\(\beta = \frac{\sum |F_0| - F_C|}{\sum |F_0|}\)  
Crystal form  
Linear absorption coefficient (cm\(^{-1}\))

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>(\text{C}<em>{21}\text{H}</em>{24}\text{Cl}<em>{2}\text{Cu}</em>{2}\text{N}<em>{4}\text{O}</em>{10})</td>
</tr>
<tr>
<td>Crystal system</td>
<td>orthorombic</td>
</tr>
<tr>
<td>Cell constants ((\text{pm};\text{°}))</td>
<td>(a = 2006.7(6)) (\text{pm}) (b = 2828.0(8)) (\text{pm}) (c = 934.0(3)) (\text{pm})</td>
</tr>
<tr>
<td>Space group</td>
<td>(\text{Pbca})</td>
</tr>
<tr>
<td>Formula units/unit cell</td>
<td>8</td>
</tr>
<tr>
<td>Calculated density ((\text{g} \cdot \text{cm}^{-3}))</td>
<td>1.73</td>
</tr>
<tr>
<td>Number of reflections collected</td>
<td>4695</td>
</tr>
<tr>
<td>Number of symmetry-independent reflections</td>
<td>4681</td>
</tr>
<tr>
<td>Reflections with F &gt; 2(\sigma(F))</td>
<td>4002</td>
</tr>
<tr>
<td>(\beta = \frac{\sum</td>
<td>F_0</td>
</tr>
<tr>
<td>Crystal form</td>
<td>prism, 0.37 (\times) 0.42 (\times) 0.98 mm</td>
</tr>
<tr>
<td>Linear absorption coefficient ((\text{cm}^{-1}))</td>
<td>18.74</td>
</tr>
</tbody>
</table>

Table II. Crystal structure data for complex 6.

Results and Discussion

Preparation and properties of the complexes

Most of the complexes \([\text{Cu}_2(\text{LR}_2\text{D}_2)(\text{OH})](\text{ClO}_4)_2\) with \(\text{R} = \text{H}\) were obtained in a direct procedure, \textit{i.e.}, without isolation of the ligand. After reduction of the double Schiff base (obtained by condensation of 2,6-diformyl-4-methylphenol with the corresponding amine \(\text{H}_2\text{N}-\text{D}\)) with \(\text{NaBH}_4\), the addition of copper(II) perchlorate led to the products 2–7. For complexes 8–10 with \(\text{R} \neq \text{H}\) a different synthetic route with the precursor 2,6-di(chloromethyl)-4-methylphenol [28, 31] had to be followed. Complexes 2–4, 6, and 9 have not been described in the literature so far.

The isolation and purification of the green perchlorates 1–10 was in some cases difficult due to the formation of viscous oils instead of crystals upon cooling. Therefore, the solutions were left at room temperature for days to allow slow crystallization. For recrystallization very slow cooling of the aqueous solution (heated to approx. 80 °C before) was found to be the best way to avoid oil formation. Isolation of crystalline tetrafluoroborate complexes instead of the hazardous perchlorate complexes turned out to be even more difficult.

The perchlorate complexes dissolve easily in methanol, ethanol and acetone and sufficiently in water.

\(\text{UV/VIS spectra}\)

The \(\text{UV/VIS}\) spectra of the “four-coordinate” complexes 1–8 are characterized by a weak d–d absorption (\(\varepsilon = 125–220 \text{ M}^{-1}\text{ cm}^{-1}\)) in the range 606–624 nm and a rather strong absorption (\(\varepsilon = 1460–2980 \text{ M}^{-1}\text{ cm}^{-1}\)) in the range 328–346 nm. The latter one can be assigned to charge transfer processes of the type \(\text{HO}^- \rightarrow \text{Cu}^{2+}\) and \(\text{PhO}^- \rightarrow \text{Cu}^{2+}\), respectively [32], which are energetically not very different. For some complexes a weak shoulder in the range 350–390 nm can be observed.

A somewhat different behaviour is found for the “five-coordinate” complex 10, which shows two similarly strong CT bands at 348 and 394 nm, respectively [32]. Complex 9, which is also “five-coordinate”, differs from 10 in the sense that only one CT band is observed. Both the CT band (372 nm) and the d–d band (831 nm) of complex 9 are significantly red-shifted, however, which makes the spectrum of 9 differ from that of 10 as well as from those of complexes 1–8.

A more detailed analysis of the CT band observed for the “four-coordinate” complexes 1–8 leads to some interesting results: (i) methylation and ethylation of the primary \(-\text{NH}_2\) groups in 1, as demonstrated by the series 1 (\(-\text{NH}_2\) \(\rightarrow\) 2 (\(-\text{NMe}_2\) \(\rightarrow\) 3 (\(-\text{NET}_3\)), is accompanied by a
stepwise increase in $\lambda_{\text{max}}$ and $\varepsilon_{\text{max}}$ (see Table I), (ii) the change from a chelate five ring (as formed by the donor group - D in complex 3) to a chelate six ring (as present in the corresponding complex 4) reduces the intensity of the CT band considerably, and (iii) the spectroscopic effect produced by introducing a methyl group on the "remote" nitrogen (compare 8 with $R = \text{Me}$ and 7 with $R = \text{H}$) is negligibly small.

As shown in Fig. 2, the most obvious spectroscopic change associated with ligand substitution according to reaction (3) is the loss of the CT band of the binuclear complex $[\text{Cu}_2(\text{LR}_2\text{D}_2)(\text{OH})]^2^+$.  

**X-ray structure**  
In the case of complex 6 it was possible to grow single crystals suitable for an X-ray structure analysis. The complex crystallizes in the orthorhombic space group Pbca (see Table I). Fig. 1 a shows that the two coppers in complex 6, as a first approximation, can be considered to have co-planar cis-N$_2$O$_2$ coordination geometry. It follows from Fig. 1 b, however, that there are substantial deviations from planarity as far as the arrangement of the benzene and pyridine rings is concerned.

The distance Cu(1)-O(5) is 259.8 pm (see Table III), which suggests a weak coordination of the perchlorate anion (for the sake of clarity the second perchlorate, coordinated to Cu(2), was omitted in Fig. 1 b). As to be expected, the distance copper-nitrogen (aliphatic) (as characterized by the distances Cu(1)-N(2) and Cu(2)-N(3)) amounts to 197.65 pm (mean) and is thus by 5.5 pm longer than the Cu-N distance in the corresponding non-hydrogenated Schiff base complex with a carbon-nitrogen double bond [33]. The Cu-O (phenolate) distances in 6 are slightly longer than the Cu-O (hydroxyl) distances (see Table III) and the Cu-Cu distance (292.4 pm) is very close to the Cu-Cu distance in the corresponding Schiff base complex (294.7 pm) [33].

### Table III. Bond lengths (pm) and bond angles (Deg) characterizing the inner coordination sphere of the metal in complex 6.

<table>
<thead>
<tr>
<th>Angles$^a$</th>
<th>Distances$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(2)-Cu(1)-O(1)</td>
<td>80.21</td>
</tr>
<tr>
<td>O(2)-Cu(1)-N(1)</td>
<td>102.28</td>
</tr>
<tr>
<td>O(2)-Cu(1)-N(2)</td>
<td>170.04</td>
</tr>
<tr>
<td>O(1)-Cu(1)-N(1)</td>
<td>177.27</td>
</tr>
<tr>
<td>O(1)-Cu(1)-N(2)</td>
<td>92.91</td>
</tr>
<tr>
<td>N(1)-Cu(1)-N(2)</td>
<td>84.77</td>
</tr>
<tr>
<td>O(2)-Cu(2)-O(1)</td>
<td>80.10</td>
</tr>
<tr>
<td>O(2)-Cu(2)-N(4)</td>
<td>100.92</td>
</tr>
<tr>
<td>O(2)-Cu(2)-N(3)</td>
<td>171.33</td>
</tr>
<tr>
<td>O(1)-Cu(2)-N(4)</td>
<td>171.47</td>
</tr>
<tr>
<td>O(1)-Cu(2)-N(3)</td>
<td>93.89</td>
</tr>
<tr>
<td>N(4)-Cu(2)-N(3)</td>
<td>84.11</td>
</tr>
<tr>
<td>Cu(1)-O(1)-Cu(2)</td>
<td>98.48</td>
</tr>
<tr>
<td>Cu(1)-O(2)-Cu(2)</td>
<td>100.48</td>
</tr>
</tbody>
</table>

$^a$ Upper limits of standard deviation for distances and angles are 4 pm and 0.4 °, respectively.

Fig. 1. View of the coordination geometry of complex 6 (1a: view perpendicular to the plane Cu(1)-O(1)-Cu(2), perchlorate anions omitted; 1b: view along the axis (O(1)-O(2), only one perchlorate anion included).
The overall results are, (i) the structural details found for complex 6 are very similar to those described in the literature for complex 5 [34] and related complexes [18, 28], (ii) Fig. 1a demonstrates that, from the steric point of view, both copper centers appear to be easily accessible, which is of significance for the ligand substitution reaction studied.

Kinetic studies at pH 8.0

Ligand substitution according to reaction (3) is accompanied by a considerable decrease in absorbance at approx. 350 nm and a small increase in the range 700–800 nm (see Fig. 2). The stopped-flow experiments carried out at 350 nm (and in some cases also at 600 nm) led to absorbance/time data which could be fitted to a single exponential according to equation (4). Fig. 3 gives an example for the quality of the fitting procedure, which leads to the experimental rate constant $k_{obs}$ (error: ±1–2%). As shown in Fig. 4 the data obtained for $k_{obs}$ at different concentrations of EDTA increase linearly with [EDTA]. These findings imply that reaction (3) is first-order in complex and follows rate law (6).

rate = $-d[$complex$]/dt = k_{obs}[$complex$] = k[$EDTA$]_{total}[$complex$]$  

(6)
A ligand-independent rate contribution, which would appear as intercept in Fig. 4, is not found. This means that there is no proton or water induced reaction channel. The fact that the observed change in absorbance (as shown in Fig. 3 for complex 5) covers the full range expected for the complete conversion of $[\text{Cu}_2(\text{LR}_2\text{D}_2)(\text{OH})]^2^-$ to the product, leads to the conclusion that removal of the first copper ion is rate-controlling and slow as compared to the removal of the second copper ion.

The second-order rate constants $k$ thus derived from the dependency $k_{\text{obs}} = f([\text{EDTA}]_{\text{total}})$ for the various complexes are compiled in Table IV. Analyzing the data one recognizes that the differences in rate are quite dramatic. Complex 6, for instance, reacts by a factor of approximately $10^6$ faster than complex 9. Another important result is that monitoring of the reaction at 600 nm instead of 350 nm leads to data for rate constant $k$, which are identical within the limits of error (see results obtained for complexes 2 and 3).

It has to be pointed out that for complexes 3 and 8 fitting of the absorbance/time data to equation (4) was slightly less satisfying than for all the other complexes. Fitting to equation (5) with two exponentials improved the quality of the fit. Table V presents the data thus obtained for $k_{\text{obs}}(1)$, $k_{\text{obs}}(2)$, $A_1$ and $A_2$. One recognizes that the data obtained for rate constant $k_2$ (i.e., for the slower step) are very close to those of rate constant $k$ (see Table IV), which results from fitting of the data to only one exponential according to equation (4). In addition, the relative contribution of the fast reaction, as characterized by the amplitude $A_1$ (see Table V) is very minor as compared to that of the slow reaction, as characterized by $A_2$.

**Kinetic studies in the pH range 6.6–8.5***

According to rate law (6), which was derived on the basis of kinetic data obtained at pH 8, reaction (3) is first-order in complex and first-order in the total concentration of the attacking ligand EDTA. Since EDTA carries a total of four carboxyl groups, which differ in their $pK_a$ values, one has to consider the question of which of the species $H_2\text{EDTA}^{2-}$, $\text{HEDTA}^{3-}$ or $\text{EDTA}^{4-}$ is most domi-

---

**Table IV. Second-order rate constant $k^{a,b}$ for the reaction of complexes 1–10 with EDTA according to (3) at pH 8.0 and I = 0.2 M.**

<table>
<thead>
<tr>
<th>Complex</th>
<th>$k^{(20°)}$ M$^{-1}$s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$9.690 \pm 0.5$</td>
</tr>
<tr>
<td>2</td>
<td>$372 \pm 8$</td>
</tr>
<tr>
<td>2</td>
<td>$390 \pm 20^a$</td>
</tr>
<tr>
<td>3</td>
<td>$212 \pm 5$</td>
</tr>
<tr>
<td>3</td>
<td>$209 \pm 4^a$</td>
</tr>
<tr>
<td>4</td>
<td>$19.3 \pm 0.5$</td>
</tr>
<tr>
<td>5</td>
<td>$152 \pm 4$</td>
</tr>
<tr>
<td>6</td>
<td>$40,300 \pm 700^a$</td>
</tr>
<tr>
<td>7</td>
<td>$440 \pm 10$</td>
</tr>
<tr>
<td>8</td>
<td>$16.6 \pm 0.5$</td>
</tr>
<tr>
<td>9</td>
<td>$0.0372 \pm 0.0004^a$</td>
</tr>
<tr>
<td>10</td>
<td>$= 0.04^b$</td>
</tr>
</tbody>
</table>

*a Rate constant $k$ calculated from the slope of the straight lines obtained by plotting $k_{\text{obs}}$ versus $[\text{EDTA}]_{\text{total}}$; data for $k_{\text{obs}}$ determined at six different concentrations of $[\text{EDTA}]_{\text{total}}$ in the range 0.001–0.01 M; b conditions: $[\text{complex}]_0 = 1 \times 10^{-5} \text{M}$; $[\text{EDTA}]_{\text{total}} \geq 10[\text{complex}]_0$; c $pH$ adjusted with the buffer EPPS (0.05 M); d ionic strength adjusted with NaClO$_4$; e from absorbance/time data collected at 600 nm instead of 350 nm; f this reaction was measured under stoichiometric conditions ($[\text{complex}]_0 = 0.5 [\text{EDTA}]_0 = 1 \times 10^{-4} \text{M}$); the absorbance/time data were fitted to $A = [(A_n - A_m)/1 + k \cdot t \cdot [\text{complex}]_0 + A_m]$; g this slow reaction was followed by normal spectrophotometry; h estimated.

**Table V. Rate constants and amplitudes for the biphasic reactions of complexes 3 and 8 with EDTA.**

<table>
<thead>
<tr>
<th>Complex</th>
<th>$k_i^{b}$ M$^{-1}$s$^{-1}$</th>
<th>$A_i^c$</th>
<th>$k_b^{b}$ M$^{-1}$s$^{-1}$</th>
<th>$A_2^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>$1,440 \pm 50$</td>
<td>0.065</td>
<td>$203 \pm 3$</td>
<td>0.47</td>
</tr>
<tr>
<td>8</td>
<td>$77 \pm 4$</td>
<td>0.079</td>
<td>$15.0 \pm 0.3$</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*a Conditions: pH 8.0 (buffer EPPS); T = 20 °C; I = 0.2 M (NaClO$_4$); b calculated from the slope of the straight lines obtained by plotting $k_{\text{obs}}(1)$ and $k_{\text{obs}}(2)$, respectively, versus $[\text{EDTA}]_{\text{total}}$; c obtained from the fit of the absorbance/time data to equation (5).

---

* Spectrophotometric titration studies showed that the complexes decompose at $5.5 \pm pH \geq 9$.
again to straight lines without intercept. So, rate law (6) is obeyed in the full pH range studied.

On the basis of the pK values 10.17 (HEDTA³⁻), 6.11 (H₂EDTA²⁻) and 2.68 (H₃EDTA¹⁻) (for an ionic strength of 0.1 M [35]) one can easily calculate that in the pH range 8.5–6.6 the species HEDTA³⁻ is the most dominant one, with a few percent of EDTA⁴⁻ being present at pH 8.5 and approximately 30% of H₂EDTA²⁻ at pH 6.6. Neglecting the species EDTA⁴⁻ one can use the mass balance [EDTA]total = [HEDTA³⁻] + [H₂EDTA²⁻] to derive relationship (7) \(10^{-6.11} = K_a \) of the species H₂EDTA²⁻.

\[
k_{\text{obs}} = k[\text{EDTA}]_{\text{total}} = k(1 + [H^+]/10^{-6.11})[\text{HEDTA}^3-] \tag{7}
\]

This equation would imply that HEDTA³⁻ is the only reacting species and that the observed pH dependence of rate constant \(k\) is due to the pH effect on [HEDTA³⁻]. Calculation of the corresponding rate constant \(k(\text{HEDTA}^3-)\) from the \(k\) values listed in Table VI according to (8) shows,

\[
k(\text{HEDTA}^3-) = k(1 + [H^+]/10^{-6.11}) \tag{8}
\]

however, that the parameter \(k(\text{HEDTA}^3-)\) thus defined is still pH dependent. This means very clearly that there has to be another EDTA species additionally involved in reaction (3). As a matter of fact, a satisfying and consistent interpretation of the observed pH dependence of reaction (3) is finally found with the two-term rate law (9).

\[
\text{rate} = k(H_2\text{EDTA}^2-)[\text{H}_2\text{EDTA}^2-] + k(\text{HEDTA}^3-)[\text{HEDTA}^3-][\text{complex}] \tag{9}
\]

Since both concentrations \([H_2\text{EDTA}^2-]\) and \([\text{HEDTA}^3-]\) can be easily calculated for any concentration \([\text{EDTA}]_{\text{total}}\) and any pH value, computer-fitting of the \(k_{\text{obs}}\) data to equation (9) allows the determination of the specific rate constants \(k(H_2\text{EDTA}^2-)\) and \(k(\text{HEDTA}^3-)\) (see Table VI).

It turns out that the reactivity of the species \(H_2\text{EDTA}^2\) is substantially greater than that of the species HEDTA³⁻ (see last column in Table VI). This means that at low pH (where considerable amounts of the species \(H_2\text{EDTA}^2\) are present) reaction (3) is accomplished mainly through the first term in rate law (9), whereas at high pH the second term controls the reaction.

**Further kinetic results**

In addition to the study of reaction (3) with EDTA as attacking ligand further experiments

<table>
<thead>
<tr>
<th>Complex</th>
<th>pH 8.5d</th>
<th>pH 8.0d</th>
<th>k(20°C)b</th>
<th>M⁻¹s⁻¹</th>
<th>pH 7.6c</th>
<th>pH 7.0e</th>
<th>pH 6.6f</th>
<th>k(H₂EDTA²⁻)·10⁻²g</th>
<th>M⁻¹s⁻¹</th>
<th>k(HEDTA¹⁻)·10⁻¹h</th>
<th>M⁻¹s⁻¹</th>
<th>k(H₂EDTA²⁻)</th>
<th>k(EDTA³⁻)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>277 ± 6</td>
<td>372 ± 8</td>
<td>950 ± 60</td>
<td>2,230 ± 70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>151 ± 5</td>
<td>22 ± 3</td>
<td>68.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>157 ± 2</td>
<td>212 ± 5</td>
<td>411 ± 9</td>
<td>875 ± 3</td>
<td>7,100 ± 100</td>
<td>-</td>
<td>-</td>
<td>56 ± 1</td>
<td>14.1 ± 0.8</td>
<td>39.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>15.3 ± 0.9</td>
<td>16.6 ± 0.5</td>
<td>24.1 ± 0.8</td>
<td>32 ± 1</td>
<td>65 ± 2</td>
<td>1.51 ± 0.06</td>
<td>1.53 ± 0.09</td>
<td>9.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Conditions: [complex]₀ = 1·10⁻² M; [EDTA]total ≥ 10 [complex]; I = 0.2 M (NaClO₄); b rate constant \(k\) calculated from the slope of the straight lines obtained by plotting \(k_{\text{obs}}\) versus [EDTA]total data for \(k_{\text{obs}}\) determined at six different concentrations of [EDTA]total in the range 0.001–0.01 M; c determined by computer-fitting of the \(k_{\text{obs}}\) data to equation (9); d buffer: EPPS, 0.05 M; e buffer: MOPS, 0.05 M; f buffer: MES, 0.05 M.
were carried out with NTA (= nitrilotriacetic acid) and CDTA (= trans-1,2-diaminocyclohexanetetraacetic acid). As compared to EDTA the tetradentate ligand NTA turned out to be equally suitable for removal of the copper according to (3). The reaction between complex 3 and NTA at pH 8.1 also follows rate law (6) and is also biphasic with \( k_1 = 3.900 \pm 100 \text{ M}^{-1} \text{s}^{-1} \) and \( k_2 = 393 \pm 4 \text{ M}^{-1} \text{s}^{-1} \). NTA is thus more reactive than EDTA by a factor of about 2 (see Table V). The ligand CDTA, like EDTA and NTA, reacts with \([Cu_2(LR_2D_2)(OH)]^{2+}\) but the kinetics appear to be more complicated.

It was also attempted to remove the copper from the binuclear complexes 2 and 3 with monodentate ligands such as cyanide and thiocyanate ion, the use of CN\(^-\) ion being of special interest with regard to the corresponding reaction of oxy-He according to (2). The result was that the SCN\(^-\) ion does not react, whereas the CN\(^-\) ion is too fast for the stopped-flow time scale (the reaction was complete to more than 60% within the mixing time of the apparatus).

**Discussion of the kinetic results**

Considering the binuclear character of the complexes \([Cu_3(LR_2D_2)(OH)]^{3+}\) and the fact, that reaction (3) takes place in aqueous medium, one would expect to observe two separate steps (reflecting the removal of the two copper ions) and the participation of water molecules or protons as species that are able to initiate ligand-independent reaction pathways. These expectations are only partly fulfilled.

Rate laws (6) and (9) clearly prove that there is no water or proton induced reaction channel, which is in some way surprising. The second-order reaction of the binuclear complexes with either \(H_2EDTA^{2-}\) ion or \(HEDTA^{3-}\) ion is obviously much faster than that with \(H_2O\) or \(H^+\), at least at \(pH > 6.6\). Only for two of the ten complexes studied biphasic kinetics are observed, the contribution of the fast step (rate constant \(k_1\)) to the overall rate being very small (see amplitudes \(A_1\) and \(A_2\) listed in Table V). Both rate constants \(k_1\) and \(k_2\) obtained for complexes 3 and 8, which react with EDTA in a biphasic fashion, describe second-order reactions following rate law (6). Since, in addition, the data obtained for \(k_2\) (rate constant for the slow step; see Table V) and \(k\) (second-order rate constant resulting from the fit of the absorbance/time data to one exponential; see Table IV) are very similar, it is most plausible to assign \(k_1\) and \(k_2\) according to reactions (10) and (11), respectively

\[
[Cu_2(LR_2D_2)(OH)] + EDTA \xrightarrow{k_2} \text{slow}
\]

\[
[Cu(LR_2D_2)(H_2O)] + Cu(EDTA) \quad (10)
\]

\[
[Cu(LR_2D_2)(H_2O)] + EDTA \xrightarrow{k_1} \text{fast}
\]

\[
\text{Cu(EDTA)} + LR_2D_2 \quad (11)
\]

(charges omitted). The intermediate mononuclear species \([Cu(LR_2D_2)(H_2O)]\) is expected to be the following cation:

\[
\begin{align*}
\text{[Cu(LR_2D_2)(H_2O)]}^+ \\
\begin{array}{c}
\text{[Cu(LR_2D_2)(H_2O)]}^+ \text{[Cu(LR_2D_2)(H_2O)]}^+ \\
\end{array}
\end{align*}
\]

The coordinated water molecule in this intermediate represents a very labile position and it is very understandable, therefore, that the \(k_1\) step, as compared to the slower \(k_2\) step, is a fast consecutive reaction which cannot be observed in most cases (one should point out that complexes 3 and 8, for which biphasic kinetics are observable, represent relatively "slow" complexes).

The overall result then is that copper removal according to (3) is controlled by the second-order reaction between the binuclear, OH-bridged complex \([Cu_3(LR_2D_2)(OH)]^{3+}\) and EDTA, as characterized by rate constant \(k\) (see Table IV). It follows from the pH dependence of \(k\) that both species \(H_2EDTA^{2-}\) and \(HEDTA^{3-}\) attack the complex according to (12) and (13). From the electrostatic point of view (cation/anion interaction) one would expect reaction (13) to be faster than (12). The fact that \(k(H_2EDTA^{2-})/k(HEDTA^{3-}) \gg 1\) (see
Table VI could indicate, however, that the availability of protons to be transferred from the attacking EDTA species to the hydroxyl bridge of the binuclear complex is more important than the charge effect.

At pH 8.0, where most of the kinetic data were collected, the relative concentration of the species $\text{H}_2\text{EDTA}^{3-}$ is very small and the kinetics are therefore simply described by reaction (13) and rate law (6). Rate constant $k (= k(\text{HEDTA}^{3-})$, as compiled in Table IV, is the adequate parameter at pH 8.0 to reflect the reactivity of complexes $1-10$ which differ in the nature of the groups D and R and in coordination number.

The discussion of rate constant $k$ as a characteristic measure for the reactivity of complexes $1-10$ necessitates a mechanistic interpretation of reaction (3) and rate law (6), respectively. Taking into account the tendency of planar four-coordinate copper(II) complexes to add another donor group and become thus five-coordinate [36], it is most plausible to postulate, according to Scheme I, a fast pre-equilibrium (14), which is followed by rate-controlling cleavage of the Cu–O(hydroxyl) bond and simultaneous proton transfer from the coordinated species HEDTA$^{3-}$ to the hydroxyl bridge (see (15)). All subsequent steps (such as removal of the first copper to form the mononuclear intermediate, attack of a second HEDTA$^{3-}$ ion at the second copper as well as its removal) appear to be fast consecutive steps which are not observed experimentally in most cases*.

The formal kinetic treatment of this mechanism (see Scheme I) which can be classified as an associative (A) mechanism, leads to rate law (16), if $[\text{HEDTA}^{3-}]_0 \gg [\text{complex}]_0$. If, in addition, $K \leq 10$ (only weak interaction between HEDTA$^{3-}$ and the complex) the term $K \cdot [\text{HEDTA}^{3-}]_0[\text{complex}]$ is small under the given conditions ($\leq 0.1$) and can be neglected, therefore. This means that rate law (16) is reduced to (17) and the experimental second-order rate constant $k (= k(\text{HEDTA}^{3-})$ for pH 8) corresponds to $k_p \cdot K \cdot [\text{HEDTA}^{3-}]_0[\text{complex}]$ (17)

The experimentally observed variation in rate constant $k$ (see Table IV) is therefore caused by either changes in equilibrium constant $K$ or rate constant $k_p$. In other words: it is either the ability of complexes $1-10$ to form adducts with the attacking ligand EDTA or the strength of the Cu–O(hydroxyl) bond (plus the availability of protons on the attacking EDTA species) that determines the size of $k$ and, hence, the rate of copper removal according to (3), (12) or (13), respectively.

The “five-coordinate” complexes 9 and 10 are clearly those, where internal blocking of the axial sites prevents adduct formation according to (14) and causes extreme low reactivity, therefore. The decrease in $k$ observed for the sequence $1(D = -(\text{CH}_2)_2-\text{NH}_2) \rightarrow 2(D = -(\text{CH}_2)_2-\text{NMMe}_2) \rightarrow 3(D = -(\text{CH}_2)_2-\text{NET}_2)$ could also be due to increased steric crowding near the copper which hinders adduct formation with EDTA. On the other hand, the basicity of the nitrogen in the donor group D is surely affected by the alkyl groups, which consequently could have an effect on the strength of the Cu–O(hydroxyl) bond and, hence, on rate constant $k_p$. The more than tenfold reduced reactivity of complex 4 as compared to 3 signals an interesting chelate ring size effect, which one would prefer to classify as an effect on $k_p$. With the donor function D being an $\alpha$-pyridyl group such as in 6 and 7, the chelate ring size effect just mentioned is of the same order and direction. The fact that $k(7) \gg k(8)$ is most remarkable in the sense that even a “remote” methyl group (R=Me instead of R=H) is able to produce a strong rate-reducing effect, which is not predicted by Scheme I.

* Experimental support for this interpretation could possibly come from the study of mononuclear complexes such as 11 reacting with EDTA under identical conditions.

[Chemical structure diagram]
The mechanistic interpretation according to Scheme 1 makes the experimental rate constant $k$ a composite parameter ($k = k_p \cdot K$) and therefore a somewhat “ambiguous” number, as far as its interpretation is concerned. Considering rate law (16) one can think of experiments at very high concentrations of EDTA, which would modify (16) to have the form rate $= k_p$ [complex]$_0$ (since $K[HEDTA]$ $\ll 1$). It is to be expected, however, that such measurements in the “saturation range”, leading to rate constant $k_p$, cause experimental problems concerning buffering and adjustment of the ionic strength. Another possible experimental approach is the study of the Lewis acid properties of complexes 1–10 according to (17) ($Nu$ = nucleophile).

$$\textit{[Cu}_2\textit{(LR}_2\textit{D}_2\textit{)(OH)}]^{2+} + x\textit{Nu} \rightleftharpoons \textit{[Cu}_2\textit{(LR}_2\textit{D}_2\textit{)(OH)]}^{2+} \cdot \textit{(Nu)}_x \cdot K_x$$

(17)

The knowledge of equilibrium constant $K_x$ could shed some light on the size of rate constant $k$ as being controlled either thermodynamically ($K$) or kinetically ($k_p$).

Conclusions

The binuclear copper(II) complexes $[\textit{Cu}_2\textit{(LR}_2\textit{D}_2\textit{)(OH)}]^{2+}$ are more or less planar systems with four-coordinate (1–8) or five-coordinate (9, 10) copper centres. Addition of EDTA leads to removal of the copper ions, the rate of copper removal being first-order in complex and EDTA, respectively. The pH dependence suggests that both species $\text{HEDTA}^{3-}$ and $\text{H}_2\text{EDTA}^{2-}$ are able to react with the complexes in a second-order reaction, the species $\text{H}_2\text{EDTA}^{2-}$ being considerably more reactive. The kinetic results clearly show that the removal of the first copper is rate-controlling and that the mononuclear intermediate thus formed is much more reactive towards EDTA than the binuclear complex. The sum of the kinetic findings supports the operation of an associative (A) mechanism. The rate of copper removal is strongly affected by the nature of the donor group D and substituent R.

The model character of complexes $[\textit{Cu}_2\textit{(LR}_2\textit{D}_2\textit{)(OH)}]^{2+}$ for the active site of oxyhemocyanin is limited since they react much faster and follow single-step kinetics.

The authors are grateful for support from the Deutsche Forschungsgemeinschaft and the Verband der Chemischen Industrie e.V. Cooperation with Prof. Dr. T. N. Sorell (University of North Carolina, Chapel Hill), in whose laboratory S. S. prepared some of the complexes, is also acknowledged.
(13) B. Linzen, N. M. Soeter, A. F. Riggs, H.-J. Schnei­

