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Isolation and Characterization of the First Stable Bicarbonato Complexes of Bis(1,10-phenanthroline)copper(II). Identification of Lipscomb- and Lindskog-like Intermediates

Zong-Wan Mao,† Günter Liehr, and Rudi van Eldik*

Institute for Inorganic Chemistry
University of Erlangen-Nürnberg
Egerlandstrasse 1, 91058 Erlangen, Germany

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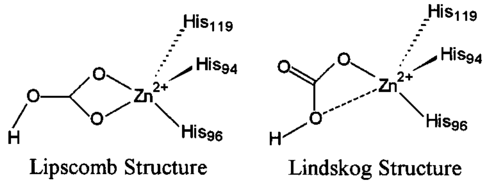
A number of experiments support the hypothesis that an intramolecular proton transfer is involved in the interconversion of CO_2 and HCO_3^- during the reversible hydration of CO_2 and dehydration of HCO_3^- catalyzed by human carbonic anhydrase II (HCAII).[1,2] Two representative mechanisms involving different bicarbonate binding modes were proposed by Lipscomb and Lindskog,[3,4] respectively, to account for the proton transfer process and have generated extensive interest over the past decade. In the Lipscomb mechanism, the intermediate involves a characteristic bidentate bicarbonate binding mode, i.e., both the carboxylate oxygen atoms are utilized as ligands for the zinc center. In the Lindskog mechanism, the intermediate involves a characteristic monodentate bicarbonate binding mode, i.e., one of the carboxylate oxygen atoms is bound to the zinc ion, whereas the hydroxyl oxygen is only weakly coordinated to the metal center. Therefore, the identification of the bicarbonate binding mode to HCAII is important in understanding the intramolecular proton transfer during the interconversion of CO_2 and HCO_3^- in the reversible catalytic cycle.



Lipscomb Structure   Lindskog Structure

Two X-ray structures involving monodentate bicarbonate binding to different carbonic anhydrases, i.e., a bicarbonate-HCAI intermediate and a mutant intermediate of bicarbonate-HCAII (Thr-200→His-200), have been determined and used to support the Lindskog structure.[5,6] However, theoretical calculations suggest that the bidentate coordination mode of bicarbonate contributes significantly toward the effective catalytic activity of the enzymes.[7] In recent progress in the engineering of carbonic anhydrase, both coordination modes were suggested to be part of the mechanism.[2b] In the study of model systems,[8] bicarbonato complexes of the type $L_nM^{II}(OCO_2H)$ (M = Zn, Cu, Co, Ni) were always considered to be unstable, and could so far not be structurally characterized. A chelated bicarbonate species was recently structurally identified for a Co(III) complex.[9]

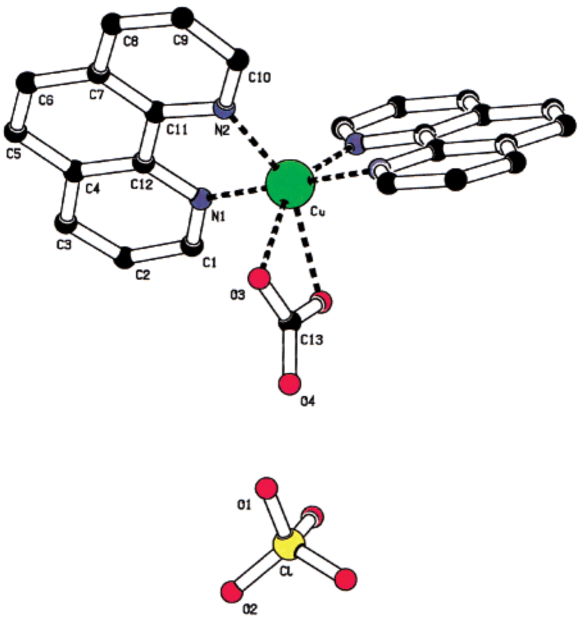


Figure 1. Molecular structure of **1**. Selected bond distances (Å) and angles (deg): Cu−N1 1.997(2), Cu−N2 2.119(2), Cu−O3 2.359(3), C13−O3 1.196(3), C13−O4 1.243(11), N1−Cu−N1* 180.00(12), N1−Cu−N2* 99.37(9), N1−Cu−N2 80.68(8), N1−Cu−O3* 90.04(9), N1−Cu−O3 90.04(9), N2−Cu−O3* 147.90(9), N2−Cu−O3 96.83(9), N2−Cu−N2* 114.93(9), O3−Cu−O3* 52.19(9), O3*−C13−O3 120.4(4), and O3*−C13−O4 119.8(2). The asterisk (*) refers to the symmetry equivalent positions as defined by the axis of symmetry through the Cu, C13, O4, and Cl atoms.

In our earlier work, we studied the catalytic activity of carbonic anhydrase and model Zn(II) complexes in an indirect way employing an indicator method to monitor the pH changes associated with the hydration/dehydration processes.[10] In efforts to use a UV−vis active metal center which will enable a direct monitoring of the catalytic process, we turned to model Cu(II) complexes and succeeded in isolating two bicarbonate complexes that closely mimic the Lipscomb- and Lindskog-type structures. In this work, $[Cu(phen)_2(H_2O)](ClO_4)_2$ was used as starting material,[11] which on reaction with bicarbonate produced two isomeric complexes of the type $[Cu(phen)_2(HCO_3)](ClO_4)$, in which bicarbonate is bound as a monodentate or as a bidentate ligand, respectively. These structures can serve as time-resolved pictures of the intramolecular proton-transfer process mentioned above.

Complexes **1** and **2** were synthesized as described in the Supporting Information. The isolated complexes were characterized by elemental analyses, IR spectroscopy, and single-crystal X-ray diffraction.[12−14] The molecular structures of complexes **1** and **2** are shown in Figures 1 and 2, respectively.

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* Address correspondence to this author.

† On leave from the School of Chemistry and Chemical Engineering, Zhongshan University, 510275 Guangzhou, P.R. China.

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(12) Anal. Calcd for $C_{25}H_{17}N_4ClO_7Cu$: C, 51.38; H, 2.93; N, 9.59. Found: C, 51.66; H, 2.92; N, 9.50 for **1** and C, 51.59; H, 2.75; N, 9.43 for **2**.

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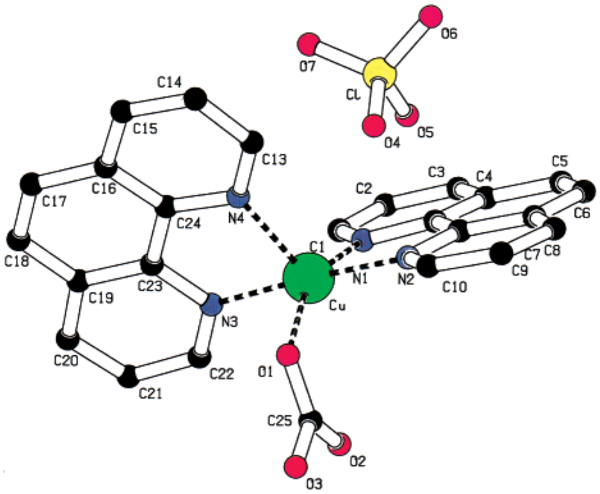

Figure 2. Molecular structure of **2**. Selected bond distances (Å) and angles (deg): Cu−N1 2.052(11), Cu−N2 1.987(11), Cu−N3 1.988(11), Cu−N4 2.173(11), Cu−O1 1.998(11), Cu−O2 3.045(9), C25−O1 1.264(9), C25−O2 1.197(9), C25−O3 1.364(9), N1−Cu−N2 82.2(3), N1−Cu−N3 97.6(3), N1−Cu−N4 113.7(3), N1−Cu−O1 152.8(3), N2−Cu−N3 177.8(2), N2−Cu−N4 101.2(3), N2−Cu−O1 90.2(3), N3−Cu−N4 80.9(3), N3−Cu−O1 89.0(3), and N4−Cu−O1 93.3(3).

Complex **1** is made up of discrete $[Cu(phen)_2]^{2+}$, O_2COH^-, and ClO_4^- units. The complex has a 2-fold axis passing through the Cu, C13, O4, and Cl atoms. Two identical and significantly elongated Cu−O3 and Cu−O3* distances of 2.359(3) Å indicate that the HCO_3^- group is weakly coordinated to the Cu center in the bidentate mode, in which the Cu−C13 distance is 2.694 Å. If the HCO_3^- ligand is looked upon as one coordinated group, the stereochemistry is best described as distorted trigonal bipyramidal ($\alpha_1 = \alpha_2 = 122.5°$ and $\alpha_3 = 114.9°$). A comparison of the bond lengths of C13−O3 (1.196 Å) and C13−O4 (1.243 Å) suggests that the hydrogen atom of HCO_3^- is bound to the O4 atom, although it could not be located by X-ray analysis. Other evidence for this assignment is the distance of 2.575 Å between O4 of HCO_3^- and O1/O1* of the ClO_4^- anion, which indicates that there are two hydrogen bonds, O4−H···O1 and O4−H···O1*, between coordinated HCO_3^- and the ClO_4^- ions. An approximate square plane is formed by N2, N2*, Cu, O3, O3*, O4, O1, O1*, and Cl, from which the Cu, C13, and Cl atoms deviate by +0.0887, −0.0058, and −0.1577 Å, respectively. The molecules form a linear structure along the *b* axis through weakly interacting hydrogen bonds.

Complex **2** is made up of discrete $[Cu(phen)_2OCO_2H]^+$ and ClO_4^- units. The bond distances for Cu−O1 (1.998 Å) and Cu−O2 (3.045 Å) clearly indicate that HCO_3^- coordinates to the Cu center in the monodentate mode, which differs completely from that in complex **1**. The geometry around the Cu(II) ion shows a strong distortion from a trigonal bipyramidal to a tetragonal pyramidal arrangement once coordinated H_2O is substituted by HCO_3^-. Similarly, the difference between the bond lengths of C25−O2 (1.197 Å) and C25−O3 (1.364 Å) suggests that the C25−O2 bond has double bond character, and the proton is bound to the O3 atom. In this structure the perchlorate ion is further away from the bicarbonate anion than in complex **1**.

Table 1. Structural Information on Available Monomeric Complexes of HCO_3^-

complex	M−O$_1$/Å	M−O$_2$/Å	HCO_3^- binding mode	ref
$[RhH_2(P(i\text{-}Pr)_3)_2O_2COH]$	2.306(3)	2.279(2)	bidentate	17
$[PPN][W(CO)_5OCO_2H]$	2.19	3.4	monodentate	18
$[Co(tepa)O_2COH](ClO_4)_2$[a]	1.926(4)	1.932(3)	bidentate	9
$[Cu(phen)_2O_2COH]ClO_4$	2.359(3)	2.359(3)	bidentate	*c*
$[Cu(phen)_2OCO_2H]ClO_4$	1.998(9)	3.045(9)	monodentate	*c*
HCO_3^-−HCAII[b]	2.23	2.54	monodentate	5
HCO_3^-−HCAI	1.8	3.5	monodentate	6

[a] tepa = tris[2-(2-pyridyl)ethyl]amine, phen = 1,10-phenanthroline. [b] A mutant HCAII (Thr-200→His-200). [c] This work.

The structural analyses clearly indicate that bicarbonate can substitute the water molecule in $[Cu(phen)_2(H_2O)]^{2+}$ and coordinate not only in the bidentate but also in the monodentate mode. The bicarbonate binding modes in **1** and **2** are very close to the extreme limits for bidentate and monodentate binding, respectively, so that they could be regarded as models for the idealized intermediates suggested by Lipscomb and Lindskog, respectively. Furthermore, they form part of the continuum of binding states predicted in theoretical studies.[7] Complex **1** closely resembles the structure of bicarbonate bound to Co(II)-substituted carbonic anhydrase for which two bicarbonate oxygen atoms were found between 2.3 and 2.5 Å from the cobalt ion.[15] Complex **2** most closely resembles the structure reported for the bicarbonate-HCAII (Thr-199→Ala) mutant in which only one bicarbonate oxygen was found to be close to the zinc ion.[16]

So far only a few structures for monomeric heavy metal or inert metal complexes of HCO_3^- have been reported, and are summarized in Table 1. In complex **1**, the Cu−O3 and Cu−O3* bond lengths of 2.359 Å are significantly longer than the Co−O bond lengths of 1.926 and 1.932 Å reported for the more stable bicarbonate chelate in $[Co^{III}(tepa)O_2COH]^{2+}$, tepa = tris(2-(2-pyridyl)ethyl)amine,[9] and also differ from the usual coordination of carbonate, via a monodentate mode. This implies that complex **1** is a very labile species in solution and presumably only exists in the solid state through self-assembly. By way of comparison, the monodentate structure **2** seems to have higher stability due to a normal Cu−O bond length of 1.998 Å.

Following numerous attempts to prepare bicarbonate metal complexes, both monodentate and bidentate complexes of the type $[Cu(phen)_2(HCO_3)](ClO_4)$ were successfully isolated and structurally characterized before they could react further in subsequent reactions. The isolated complexes reveal insight into the continuum of structures proposed to be involved in the reversible intramolecular conversion of coordinated hydroxo-carbon dioxide to bicarbonate. In this sence their identification should advance our understanding of the reversible catalytic cycle of HCAII, as well as of other reaction mechanisms involving bicarbonate.

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Supporting Information Available: Synthesis of complexes **1** and **2** and tables with structural information on complexes **1** and **2**, including details on structure determination, final coordinates of all atoms, bond distances, bond angles, torsion angles, contact distances, and hydrogen bonds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) IR data (in cm^{-1}) for coordinated bicarbonate (KBr pressed pellet): 1592 s (ν_1), 1365 m, 1308 s (ν_2), 824 w (π) for **1** and 1654 s (ν_1), 1371 s, 1259 s (ν_2), 823 m (π) for **2**.

(14) Crystal data for **1**: $C_{25}H_{17}N_4ClO_7Cu$, SG: $C2/c$, $a = 16.757(5)$ Å, $b = 11.585(5)$ Å, $c = 12.565(5)$ Å, $\beta = 111.320(5)°$, $V = 2272(2)$ Å^3, $Z = 4$, $d_c = 1.72$ g cm^3, $F(000) = 1124$, crystal size 0.55 × 0.15 × 0.05, $R = 0.0661$ ($I > 2\sigma(I)$), $wR2 = 0.2007$. Crystal data for **2**: $C_{25}H_{17}N_4ClO_7Cu$, SG: $P\bar{1}$, $a = 10.24(5)$ Å, $b = 10.34(5)$ Å, $c = 12.54(5)$ Å, $\alpha = 71.80(2)°$, $\beta = 70.90(2)°$, $\gamma = 71.89(2)°$, $V = 1159(9)$ Å^3, $Z = 2$, $d_c = 1.672$ g cm^3, $F(000) = 592$, crystal size 0.18 × 0.35 × 0.40, reflections collected = 7716, Independent reflections = 4016, GOF on $F^2 = 1.36$, $R = 0.059$ ($I > 2\sigma(I)$), $wR2 = 0.1872$. Intensity data were collected on a Philips PW1100/0 diffractometer with graphite monochromated Mo Kα radiation in the $\omega/2\theta$-scan mode at 213 K. The structures were solved by direct methods using the SIR-92 computer program and refined by full-matrix least-squares on F^2 using SHELXL93.

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