Towards Copper(II) Hemocyanin Models: The Synthesis and Molecular Structure of a Binuclear Copper(II) Complex of a Heptadentate Ligand

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Received December 18, 1984

The bioinorganic approach to the study of metalloproteins in general involves the synthesis of relatively low molecular weight model compounds designed to mimic the enzymatic site both in structure and chemistry [1]. One such enzyme system which has been the subject of recent study by this approach is the dicopper enzyme hemocyanin [2–7]. The function of this 'type 3' copper enzyme involves the binding and transport of molecular oxygen in the hemolymph of several molluscs and arthropods. The nature of the copper site is not unambiguously known, however recent EXAFS [8], resonance Raman [9] and other physicochemical studies [10, 11] have suggested that each copper in the oxygen bound form of the protein is pentacoordinate. Two or three imidazole (histidine) ligands, a bridging phenolate from a tyrosine residue and an equatorially bound O_2^{2-} make up the coordination spheres of the copper atoms. In this oxy form of the protein the geometry results in an antiferromagnetic coupling between the copper atoms making the copper site EPR nondetectable. In the absence of a bridging substrate molecule an EPR detectable Met dimer (Cu(II)–Cu(II)) has been recognized [10]. Early inorganic model systems for this binuclear Cu(II) enzyme employed Schiff base and other unsaturated ligands [11]. Although these studies addressed some of the features of the binuclear copper sites, the constraints imposed by the ligand geometry precluded accurate models of the Cu(II) centers in hemocyanin. It has only been recently that investigations of complexes containing more biologically relevant ligands have been reported [2–6]. In this communication we report the synthesis and molecular structure of a binuclear Cu(II) complex of a heptadentate ligand which incorporates two features pertinent to the Cu(II) site in hemocyanin, namely imidazole ligation and a bridging phenolate moiety.

The ligand (3) was prepared by the route outlined in Fig. 1. Preparation of the starting material (1) was achieved by reaction of 2,6 bis(hydroxymethyl)*p*-cresol with HCl gas in diethyl ether [4]. We have recently described the preparation of the other starting material (2) [13]. To a methanol solution (50 ml) of 2.7 g of (2) at −10 °C, 0.99 g of NEt_3 were added followed by 1.0 g of (1). The solution was allowed to warm to room temperature and then refluxed for 5 min. Cooling and stirring at room temperature for 24 h resulted in the precipitation of a fine white powder. ^1H NMR and IR spectroscopy as well as elemental analysis of this product was consistent with the formulation (3)·HCl·H_2O‡. During the course of our work other authors have described a similar preparation of (3) [5].

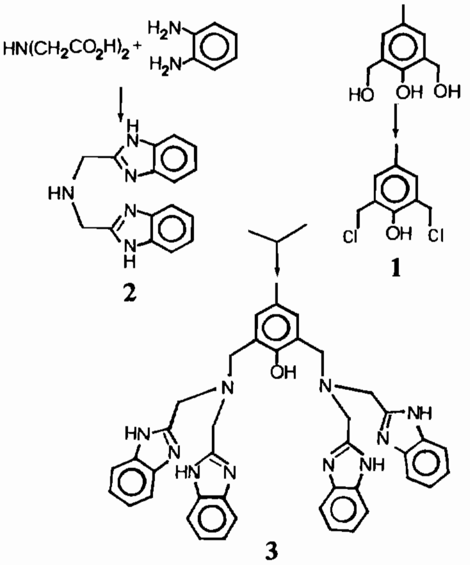

Fig. 1. The synthetic route to ligand (3).

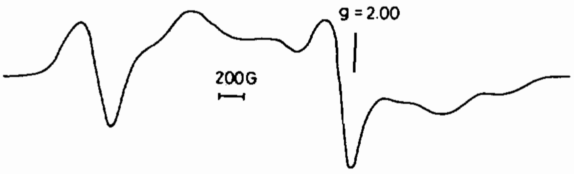

Fig. 2. The solid state EPR spectrum of (4) at 25 °C.

The copper complex of (3) was prepared by reaction of 90 mg of (3) with 90 mg of $Cu(H_2O)_6(ClO_4)_2$ in methanol. On standing green-brown crystals of (4) were deposited from the solution. The visible spectrum of this compound showed absorptions at 720 nm ($\epsilon = 115\ M^{-1}$) and 468 nm ($\epsilon = 447\ M^{-1}$). The EPR spectrum is shown in Fig. 2. Broad signals occur at g values of 2.07 and 4.69. The observation of the forbidden half field transition suggests that (4) contains two copper atoms in close proximity to each

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‡*Anal.* Calcd. for $C_{41}H_{41}N_{10}O_2Cl$ C: 66.43%, H: 5.57%, N: 18.89%; found C: 67.30%, H: 5.79%, N: 18.79%.

other [10]. These spectroscopic data as well as the analytical data were consistent with the formulation of (4) as a dimeric Cu(II) complex of formula $LCu_2(H_2O)_5(ClO_4)_3$, where L is the phenolate of (3)*.

Further investigation of the geometry of the complex (4) was undertaken by X-ray crystallographic methods. Brown-green crystals of (4) were obtained by slow evaporation from a methanol solution of (4). A crystal was mounted in a capillary and diffraction experiments were performed on a four-circle Syntex $P2_1$ diffractometer with graphite monochromatized MoKα radiation. The compound (4) crystallizes in space group $P2_1/c$ with $a = 12.143(4)$ Å, $b = 21.356(5)$ Å, $c = 21.328(3)$ Å, $\beta = 107.95(2)°$, $V = 5262(2)$ Å^3 and $Z = 4$. A total of 2671 reflections with $F^2 > 3\sigma F^2$ were collected. Solution of the structure was obtained by the heavy atom (Patterson) method in combination with successive Fourier map calculations. Refinement of the structure to the current residual values of $R = 0.1093$ and $R_w = 0.1219$ was performed employing block diagonal least-squares techniques on F. Disordering of the solvent molecules and the perchlorate anions is apparent and is presumably primarily responsible for the relatively high R values. Despite this problem the structure of the trication is clear (Fig. 3). Each of the copper atoms have two benzimidazoles, a tertiary amine nitrogen and a water molecule coordinated to them. The two copper atoms are bridged by the phenolate oxygen of (3). The coordination spheres of both of the copper atoms are distorted square pyramidal, however the two environments are not equivalent. The bridging oxygen is in the square plane of the Cu2 coordination sphere while it is apical to Cu1. Similarly the tertiary amine nitrogen atom coordinated to Cu2 (*i.e.* N6) is in the apical position, while the other tertiary amine nitrogen atom (*i.e.* N1) is in the square plane of Cu1. Cu–N bond distances are typical, the Cu2–N6 bond being significantly longer

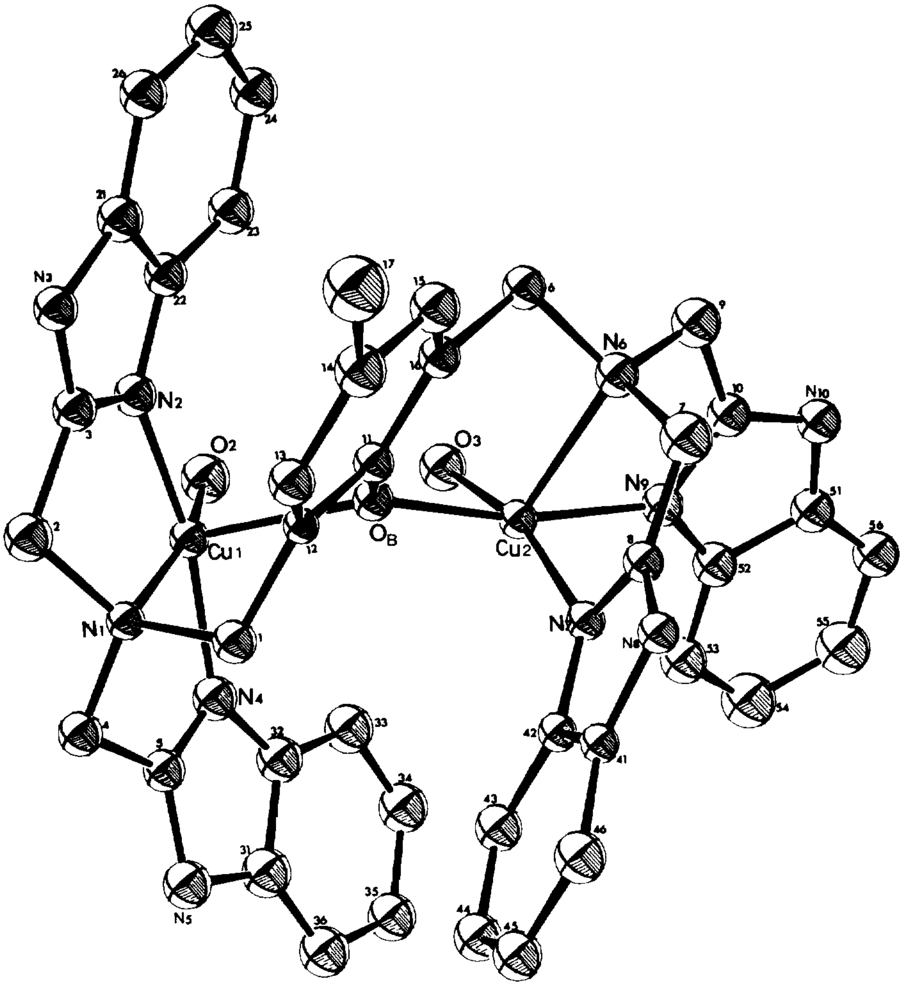

Fig. 3. An ORTEP drawing of the trication of (4), 50% thermal ellipsoids are shown and the hydrogen atoms have been omitted for clarity. Unlabeled numbered atoms are carbons.

**Anal.* calcd for $C_{41}H_{47}N_{10}O_{18}Cu_2Cl_3$, C: 40.99%, H: 3.94%, N: 11.66%; found C: 40.48%, H: 3.95%, N: 11.34%.

than the other Cu–N bonds reflecting the apical position of N1. The dissymmetry of the bridging phenolate moiety is clear from the Cu1–O1 and Cu2–O1 bond distances, 2.28(1) *vs.* 1.92(1) Å. The Cu1–O1–Cu2 angle is 128.4(6)° and Cu1–Cu2 distance is 3.875 Å. Bond distances and angles in the remainder of the cation are typical. Selected bond distances and angles of the trication are given in Table I. Positional parameters of the atoms are listed in Table II.

TABLE I. Selected Bond Distances and Angles.

Distances (Å)			
Cu1–N1	2.06(2)	Cu2–N6	2.26(2)
Cu1–N2	1.97(1)	Cu2–N7	1.96(2)
Cu1–N4	2.02(2)	Cu2–N9	1.98(2)
Cu1–O1	2.28(1)	Cu2–O1	1.92(1)
Cu1–O2	2.03(2)	Cu2–O3	1.99(2)
	Cu1..Cu2	3.875	
Angles (deg)			
N1–Cu1–n2	83.9(7)	N6–Cu2–N7	81.1(7)
N1–Cu1–N4	82.0(7)	N6–Cu2–N9	79.1(6)
N1–Cu1–O1	94.1(5)	N6–Cu2–O1	93.6(5)
N1–Cu1–O2	165.4(6)	N6–Cu2–O3	115.3(7)
N2–Cu1–N4	165.3(8)	N7–Cu2–N9	96.4(7)
N2–Cu1–O1	86.9(5)	N7–Cu2–O1	90.6(6)
N2–Cu1–O2	97.9(7)	N7–Cu2–O3	163.5(6)
N4–Cu1–O1	98.1(5)	N9–Cu2–O1	169.0(7)
N4–Cu1–O2	94.9(7)	N9–Cu2–O3	86.0(7)
O1–Cu1–O2	100.5(5)	O1–Cu2–O3	89.8(6)
	Cu1–O1–Cu2	128.4(6)	

TABLE II. Positional Parameters[a].

atom	*x*	*y*	*z*
Cu1	256.7(2)	609.8(1)	321.5(1)
Cu2	8.4(2)	516.4(1)	209.7(1)
N1	349(1)	613.1(7)	284.0(7)
N2	353(1)	547.0(7)	382.1(7)
N3	511(2)	493.0(8)	406.8(8)
N4	198(2)	679.1(8)	255.4(8)
N5	246(2)	741(1)	184(1)
N6	21(1)	410.8(7)	212.1(9)
N7	45(1)	503.3(6)	127.1(7)
N8	136(1)	440.3(8)	72.4(8)
N9	−157(2)	493.0(8)	175.0(9)
N10	−301(2)	424(1)	152.6(9)
O1	170(1)	527.7(5)	256.6(6)
O2	141(1)	626.0(6)	371.3(7)
O3	−33(1)	555.0(8)	284.5(8)
C1	369(2)	569.0(9)	229(1)
C2	498(2)	592(1)	338(1)
C3	460(2)	543(1)	378(1)
C4	400(2)	678(1)	263(1)
C5	280(2)	697(1)	237(1)
C6	125(2)	398(1)	269(1)
C7	34(2)	392(1)	147(1)
C8	76(2)	444.3(9)	117.8(9)
C9	−89(2)	387(1)	217(1)
C10	−182(2)	437(1)	181(1)
C51	−353(2)	479(1)	127(1)
C53	−280(2)	583(2)	121(1)
C55	−481(3)	551(3)	75(2)
Cl1	392.4(9)	766.8(3)	427.1(4)
Op1	330(2)	786(1)	469(1)
Op2	349(2)	795.8(8)	364.7(7)
Op3	511(1)	785(1)	455(1)
Op4	387(2)	701.0(5)	418.4(9)
Cl2	201.7(9)	955.0(5)	140.0(5)
Op5	248(2)	956(1)	87(1)
Op6	249(2)	1004(1)	185(1)
Ow1	92(3)	744(2)	385(2)
Ow3	249(3)	339(2)	40(2)
C11	249(2)	485.2(9)	251.8(9)
C12	349(2)	502.3(7)	239.6(8)
C13	432(2)	459(1)	236(1)
C14	420(2)	396(1)	246(1)
C15	313(2)	379(1)	256(1)
C16	229(2)	421.8(9)	260.6(9)
C17	502(2)	346(1)	239(2)
C21	437(2)	460(1)	433(1)
C22	336(2)	496(1)	416.6(9)
C23	241(3)	475(1)	435(1)
C24	245(3)	421(2)	467(2)
C25	342(4)	487(2)	477(1)
C26	445(2)	403(1)	462(1)
C31	130(4)	744(1)	171(1)
C32	97(3)	707.8(9)	219(1)
C33	−13(2)	699(1)	222(1)
C34	−102(3)	737(1)	170(2)
C35	−72(4)	773(1)	128(2)
C36	45(4)	777(2)	128(2)
C41	152(2)	499(1)	53(1)
C42	92(2)	540(1)	87(1)
C43	93(2)	604(1)	80(1)
C44	148(2)	629(1)	39(1)
C45	205(2)	588(2)	5(1)
C46	204(2)	521(2)	10(1)
C52	−262(3)	520(1)	142(1)
C54	−405(3)	600(2)	84(2)
C56	−467(3)	494(2)	93(2)
Op7	230(3)	897(1)	174(2)
Op8	80(1)	961(2)	116(1)
Cl3	224(2)	200.6(7)	152(2)
Op9	294(5)	190(3)	218(2)
Op10	296(7)	213(2)	123(4)
Op11	150(3)	252(1)	150(2)
Op12	157(4)	147(1)	128(3)
Ow2	334(3)	784(1)	87(1)

[a] Multiplied by 10^3, all Op atoms are oxygen atoms of perchlorate anions, all Ow atoms are oxygen atoms of ocluded water molecules.

The complex nature of the EPR spectrum of **(4)** is reminiscent of the spectrum of the EPR detectible Met hemocyanin [10]. There too the absorption in the g = 2 region is broad and the forbidden transition in the g = 4 area is observed. Although these gross features are similar, computer simulations of the dimer protein spectrum are consistent with a Cu–Cu distance of about 6 Å [10] while in the present complex the Cu–Cu distance is only 3.875 Å. The closer proximity of the two metals in **(4)** accounts for the stronger intensity of the half field transition. This metal–metal interaction is presumably mediated through the bridging oxygen atom. In the oxygen bound protein where the interaction is antiferromagnetic, theoretical calculations have shown that such coupling will only result in one atom bridged systems when the square planes of the two copper coordination spheres are coplanar [10]. The unusual form of metal–metal interaction observed here arises because the square planes of the two copper coordination spheres are far from coplanarity. The dihedral angle between the Cu1, N1, N2, N4, O2 plane and the Cu2, N7, N9, O1, O3 plane is 64.7°.

The ligand system described herein provides a set of donors which are biologically relevant to the synthesis of hemocyanin model systems. It yields a synthetic route to a novel dicopper complex in which an unusual magnetic metal–metal interaction is observed. A question that now arises is whether the present system is capable of undergoing a reorientation upon interaction with a bridging substrate to yield a diamagnetic copper dimer, thus modelling the active site of oxy-hemocyanin. This aspect is under current investigation.

Acknowledgement

The authors thank the NSERC of Canada for the financial support of this research.

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