Synthesis, characterization and catecholase-mimetic activity of mononuclear copper(II) aspirinate complexes

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Abstract

Four mononuclear copper(II) complexes have been prepared by allowing copper(II) aspirinate to react with benzimidazole, 2-methylbenzimidazole, metronidazole or 2-methyl-5-nitrobenzimidazole ligands. Elemental analyses, UV–Vis, IR, EPR and magnet moment data are consistent with mononuclear square planar complexes that contain two aspirinate ligands and two N-containing ligands to give a CuO_2N_2 chromophore. The benzimidazole, 2-methylbenzimidazole and metronidazole complexes catalyze the oxidation of catechol to *o*-quinone. This reaction was monitored spectrophotometrically by following the increase in intensity of the *o*-quinone band at 390 nm with time. Enzyme-mimetic activity of these copper complexes was determined and expressed as micromoles of substrate per mg catalyst per min. The activities were found to be 0.314 for the benzimidazole complex, 0.0998 for the 2-methylbenzimidazole complex, and 0.129 for the metronidazole complex. In the case of the 2-methyl-5-nitrobenzimidazole complex the initial formation of *o*-quinone is very rapid; however, after 2–3 min the catalyst is poisoned and the concentration of *o*-quinone drops slightly and remains constant.

Introduction

Salicylic acid and its derivatives including aspirin (acetylsalicyclic acid) have been used for many years as antiinflammatory, antipyretic and analgesic drugs. Copper(II) complexes of aspirin and other salicylic acid derivatives have been found to be more potent and desirable drugs than their constituent ligands [1, 2]. In addition, animal studies have shown that copper complexes exhibit a variety of pharmacological effects such as antiinflammatory, analgesic, antiulcer, antidiabetic, anticancer, anticonvulsant and radiation recovery activities [1–6]. X-ray analysis of copper(II) aspirinate has shown that it contains binuclear units with bridging carboxylate ligands [7], similar to other Cu(II) carboxylates including copper(II) acetate [8, 9]. Reaction of copper(II) aspirinate with pyridine produces the more soluble mononuclear adduct bis(aspirinato)bis-(pyridine)copper(II) [10] which exhibits enhanced antitumor activity [11] and has been shown by X-ray analysis to contain the CuN_2O_2 chromophore in a *trans* square-planar arrangement [10]. Pyridine adducts and other mononuclear adducts of copper(II) aspirinate that contain picoline or imidazole derivatives have recently been reported to have superoxide dismutase-mimetic activity [12].

As a continuation of our work on the interaction of copper(II) carboxylates with imidazoles [13, 14] as models for copper proteins that contain both the imidazole and carboxylate functionalities in the side chain [15], one of us recently reported the characterization of mononuclear tetrakis-adducts of copper(II) aspirinate with imidazole or *N*-methylimidazole and the bis-adduct with 1,2-dimethylimidazole [13]. Since benzimidazoles and nitroimidazoles are used as chemotherapeutic agents, particularly nitroimidazoles, in the treatment of bacterial infections and as radiosensitizer agents [16, 17], we have extended our studies to include interactions of these and other imidazole derivatives with copper(II) aspirinate and other copper(II) carboxylates.

Interest in developing small molecular weight copper(II)complexes as models for copper oxidase enzymes have led to the synthesis of many dinuclear [18–21]

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and mononuclear [22, 23] copper(II) complexes; the idea being that these complexes might mimic the behavior of various metalloproteins, such as the copper-containing protein tyrosinase. One reaction catalyzed by this metalloenzyme is the oxidation of catechols to their respective *o*-quinones [24]. It therefore is of interest to investigate the potential of metalloenzyme model complexes to catalyze the oxidation of catechols to quinones. This article reports the synthesis, spectroscopic characterization and catecholase-mimetic activity of four mononuclear copper(II) aspirinate benzimidazole or metronidazole bis-adducts.

Experimental

All chemicals were of high purity grade (Aldrich Chemical Co.) and used without further purification. All solvents were anhydrous. Tetrakis-μ-aspirinatodicopper(II) [$Cu_2(asp)_4$] was prepared according to a published procedure [1].

Physical measurements

Magnetic moments were determined at 298 K by the Evans method [25] with an NMC/NMR-1280 (200 MHz) spectrometer (Bruker). Methanol was used as the solvent and benzene as the reference. The effective magnetic moment is related to the reference shift, $\Delta\nu$ (Hz), at any temperature by the expression: $\mu_{eff}=0.0618(\Delta\nu T/\nu M)^{1/2}$, where ν is the NMR frequency in MHz and M is the molarity of the paramagnetic substance.

Electronic spectra of methanol solutions were obtained with a Hewlett Packard 8452A diode array spectrophotometer. Nujol mulls sealed between polyethylene sheets were used to obtain IR spectra in the 4000–450 cm^{-1} region with a FTS-7 Bio-Rad SPC 3200 Fourier transform IR spectrometer. X-band EPR spectra of polycrystalline material and of methanol/toluene solutions were obtained at room temperature and 77 K with and ESPIT-330 VO1.501 spectrometer. Diphenylpicrylhydrazide (DPPH, $g=2.0036$) was used as the calibrating field marker.

The catecholase-mimetic activities of these complexes in air were followed spectrophotometrically by monitoring the increase in the *o*-quinone absorbance at 390 nm as a function of time. Methanol solutions of the copper(II) complex (0.3 ml of a 3×10^{-3} M) and 2.0 ml of a methanol solution (1.5×10^{-1} M) of catechol were combined in a 1 cm quartz cell at 298 K and the absorbance changes at 390 nm were recorded.

*Preparation of bis(aspirinato)bis(benzimidazole)$_2$-copper(II) (**1**), $Cu(asp)_2(bnz)_2$*

A solution of 0.287 g (2.43 mmol) of benzimidazole in 100 ml of methanol was added to 0.5 g (0.593 mmol) of $Cu_2(asp)_4$. The mixture was stirred at about 50–60 °C for 1 h. The green solution was filtered and left in the hood to evaporate. The green precipitate was collected, washed with anhydrous ether, and dried in a desiccator over anhydrous calcium chloride. *Anal.* Calc. for $C_{32}H_{26}N_4O_8Cu$: C, 58.40; H, 3.95; N, 8.52. Found: C, 58.22; H, 3.75; N, 8.39%.

*Preparation of bis(aspirinato)bis(2-methylbenzimidazole)-copper(II) (**2**), $Cu(asp)_2(2mbnz)_2$*

This bluish green complex was prepared by the method described for **1** except that 2-methylbenzimidazole 0.321 g (2.43 mmol) was used in place of benzimidazole. *Anal.* Calc. for $C_{34}H_{30}N_4O_8Cu$: C, 59.51; H, 4.37; N, 8.17. Found C, 59.39; H, 4.52; N, 8.44%.

*Preparation of bis(aspirinato)bis(metronidazole)-copper(II) (**3**), $Cu(asp)_2(mtnd)_2$*

A solution of 0.416 g (2.43 mmol) metronidazole in 100 ml of ethanol and 25 ml of methanol was added to 0.5 g (0.593 mmol) of $Cu_2(asp)_4$. The mixture was stirred and refluxed for about 4 h. The olive green solution was filtered while hot and left in the hood to evaporate. The green precipitate that formed was washed with anhydrous ether and dried in a desiccator over calcium chloride. *Anal.* Calc. for $C_{30}H_{32}N_6O_{14}Cu$; C, 47.15; H, 4.19; N, 11.00. Found: C, 46.13; H, 4.22; N, 11.28%.

*Preparation of Bis(aspirinato)bis(2-methyl-5-nitrobenzimidazole)copper(II) (**4**), $Cu(asp)_2(2m5nbnz)_2$*

This green complex was prepared by the method described for **3** except that 2-methyl-5-nitrobenzimidazole 0.430 g (2.43 mmol) was used in place of metronidazole. *Anal.* Calc. for $C_{34}H_{28}N_6O_{12}Cu$: C, 52.61; H, 3.61; N, 10.83. Found: C, 52.51; H, 3.43; N, 10.55%.

Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, USA and Desert Analytic, Tucson, AZ, USA.

Results and discussion

Magnetic and spectroscopic results

The effective magnetic moments and electronic and IR spectral data are summarized in Table 1. The room-temperature magnetic moments for these four complexes are in the range 1.84–1.90 BM. These values are consistent with the presence of one unpaired electron in a mononuclear copper(II) complex.

The electronic spectra for these complexes obtained in methanol solution exhibit one broad absorption band near 700–730 nm and a weak shoulder in the 400–420 nm region (Table 1). The broad band is assigned to the copper(II) d–d transition and the shoulder to ligand-

TABLE 1. Magnetic moments and spectral data for Cu(II) complexes

Compound	μ_{eff} (BM) (298 K)	λ_{max}(nm) (ϵ)[a]	ν_{as}(COO) (cm^{-1})	ν_{s}(COO) (cm^{-1})	$\nu_{acetoxy}$(C=O) (cm^{-1})
1	1.88	695 (86)	1598	1392	1745
2	1.90	706 (82)	1595	1403	1743
3	1.84	730 (72)	1602	1390	1745
4	1.85	714 (85)	1600	1390	1742

[a] ϵ in units of $dm^3\ mol^{-1}\ cm^{-1}$.

to-metal charge transfer. A similar shoulder also attributed to charge transfer was previously observed for mononuclear copper(II) salicylate complexes [26]. The position of the band of the copper(II) d–d transition and the magnitude of its molar absorptivity are in the range expected for mononuclear copper(II) carboxylate adducts [8, 12–14]. These values are indeed comparable to those found for mononuclear copper(II) aspirinate adducts with imidazoles or pyridines, and with those of other tetragonally distorted copper(II) complexes that contain a CuN_2O_2– – –O_2 chromophore [12–14, 26].

The IR frequencies for the carboxyl and acetoxy carbonyl vibrations are given in Table 1. X-ray analysis of $Cu_2(asp)_4$ revealed two types of acetoxy carbonyl groups [7]. One type is weakly bonded to a copper(II) atom of a neighboring copper(II) aspirinate molecule, while the other is not. As a consequence, the IR spectrum of $Cu_2(asp)_4$ shows two different acetoxy carbonyl stretching frequencies at 1758 and 1725 cm^{-1}. The IR spectra for complexes **1–4** show one strong acetoxy carbonyl band near 1745 cm^{-1} (Table 1). The asymmetric ν_{as}(COO) and the symmetric ν_{s}(COO) frequencies for $Cu_2(asp)_4$ occur at 1620 and 1415 cm^{-1}, respectively, and are similar to those reported for other dinuclear copper(II) carboxylates [8, 9, 27], in which the carboxylate acts as a 'bridging' bidentate ligand. The IR spectra for the four mononuclear complexes show strong ν_{as}(COO) at about 1600 cm^{-1} with a shoulder at about 1625 cm^{-1}. The ν_{s}(COO) in these complexes occurs between 1390 and 1403 cm^{-1} (Table 1). The positions of these carboxylate stretching vibrations and the separation between them are in the range expected for carboxylate groups that act essentially as a monodentate or asymmetric bidentate ligands [10, 13, 14, 27]. These parameters are comparable to those reported for mononuclear copper(II) aspirinate complexes that contain pyridines [10, 12] or imidazoles [12, 13]. The stretching vibrations of the NO_2 group in **3** and **4** occur at 1535 and 1521 cm^{-1}, respectively.

The EPR parameters, g and A, for the frozen solution and the polycrystalline forms of the complexes studied are given in Table 2. A representative frozen solution EPR spectrum is that of **2** shown in Fig. 1(A). Frozen solution EPR spectra of complexes **1–4** exhibit resolved structure with $g_{\parallel} > g_{\perp}$ and are consistent with a tetragonally elongated structure [28]. The $g_{\perp}$ regions of these spectra exhibit ^{14}N super-hyperfine structure consisting of five lines. This structure is attributed to the presence of two nitrogen atoms in a plane of the copper(II) atom. Spectral parameters for complexes **1–4** are comparable to those of previously reported complexes that essentially contain the CuN_2O_2 chromophore in a *trans* or *cis* square-planar arrangement [10, 12–14, 29], including those reported for mononuclear copper(II) aspirinate complexes of pyridines or imidazoles [10, 12, 13]. These complexes contain a copper(II) atom in a *trans* or *cis* arrangement of two ligand nitrogen atoms and one oxygen atom from each of the two carboxylate ligands. The second oxygen atom of each carboxylate ligand is weakly bonded in an axial arrangement. Solid-state EPR spectra of **2, 3** and **4** are anisotropic and contain $g_{\parallel}$ and $g_{\perp}$ components with the $g_{\parallel}$ component exhibiting Cu hyperfine coupling. The solid-state EPR spectrum of **3** is shown in Fig. 1(B) as being representative of these spectra. The solid-state EPR spectrum of **1** contains an asymmetric signal centered near 3150 G. Spectra similar to this are exhibited by bis-adducts of copper(II) acetate and copper(II) ferrocenecarboxylate with 1,2-dimethylimidazole. We have determined by X-ray analysis that these adducts contain the CuN_2O_2 chromophore in a *cis* square-planar arrangement [14, 30]. The lack of copper(II) hyperfine coupling in these complexes is likely due to dipole–dipole interactions between copper atoms of neighboring molecules. The spectral data strongly suggest the presence of the CuN_2O_2 (or $CuN_2O_2\cdots O_2$) chromophore in a *trans* or *cis* square-planar arrangement for the bis-adducts **1–4**.

Catecholase-mimetic activity

Since *o*-quinone shows a characteristic absorption band at 390 nm, the catalytic oxidation of catechol to *o*-quinone by copper(II) complexes can be easily followed spectrophotometrically. The change in absorbance at 390 nm versus time for the first 30 min of the reaction with complexes **1, 2** and **3** is depicted in Fig. 2 while the behavior of **4** is demonstrated in the insert in Fig. 2. Although *o*-quinone is produced in the

TABLE 2. EPR and kinetic data for the oxidation of catechol by Cu(II) complexes

Compound	State	$g_\perp$	$g_\parallel$	$A_\parallel$, Cu ($\times 10^4$ cm^{-1})	$A_\perp$, N ($\times 10^4$ cm^{-1})	Activity[a]
1	solid[b]					0.314
	[c]	2.07	2.29	171	14	
2	solid	2.09	2.29	167		0.0998
	[c]	2.07	2.30	166	15	
3	solid	2.07	2.30	168		0.129
	[c]	2.06	2.31	166	13	
4	solid	2.07	2.29	168		
	[c]	2.06	2.30	167	14	

[a]The activity is reported as moles of substrate per mg catalyst per min. [b]Only a broad peak is observed with $g = 2.13$. [c]Frozen solution.

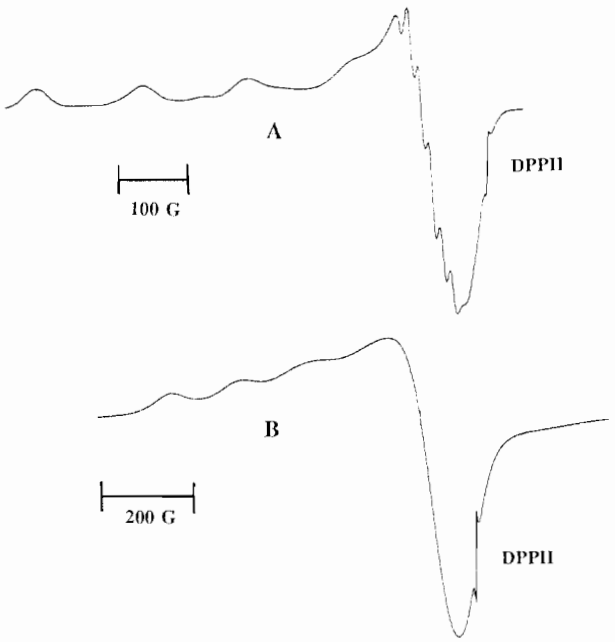

Fig. 1. (A) Frozen-solution EPR spectrum of **2**, (B) solid-state EPR spectrum of **3**.

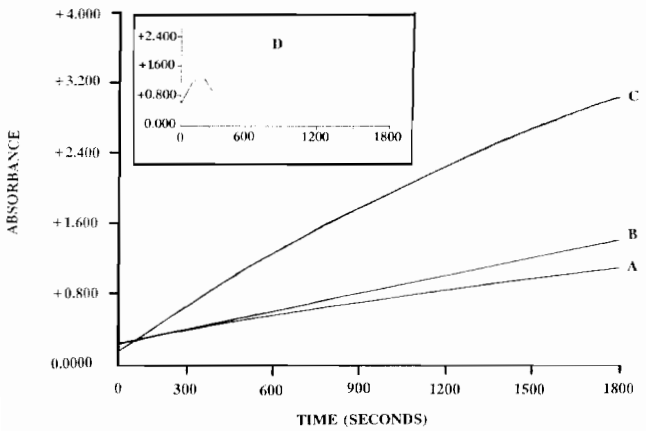

Fig. 2. Plot of absorbance vs. time for the oxidation of catechol catalyzed by **2** (A), **3** (B), **1** (C) and **4** (D, insert).

presence of **1**, **2** and **3**, the rate at which *o*-quinone is produced is clearly dependent on the type of imidazole ligand present in the complex. Calculated activities for these three complexes are given in Table 2. It was not possible to compare the activity for **4** since the *o*-quinone produced during the first 2–3 min of the catalyzed reaction appears to poison the catalyst (see insert in Fig. 2).

A number of dinuclear and mononuclear copper(II) complexes have been studied with regard to their catalytic hydroxylation of monophenols and oxidation of catechols to *o*-quinones [18–23, 31]. In tyrosinase and in synthetic copper(II) dinuclear models, it is believed that two proximate metal atoms are needed to bond to the two hydroxyl oxygen atoms of catechols in the oxidation to *o*-quinones [31]. For non-planar mononuclear copper(II) complexes, it has been proposed that the two copper(II) atoms must be located at a distance of less than 5 Å for bonding to the catechol hydroxyl groups and the subsequent two-electron transfer to dioxygen [31a]. In addition, Thompson and Calabrese [32] isolated and characterized a series of mononuclear Cu(II) di-tert-butyl-*o*-semiquinone complexes from reactions of the corresponding catechol or benzoquinone with dinuclear and mononuclear Cu(II) or Cu(I) complexes, respectively. It was concluded that the single-step two-electron oxidation of catechol by Cu(II) complexes is not observed and *o*-benzoquinone was obtained only after exposure of the Cu(II)–*o*-semiquinone to dioxygen or by the addition of small molecules such as pyridine. This study indicates that the formation of mononuclear Cu(II)–*o*-semiquinone complexes as an intermediate should be considered in the catecholase-mimetic activity of Cu(II) complexes.

In a recent study of the catecholase-mimetic activity of mononuclear five-coordinate copper(II) complexes Malachowski *et al.* [23] showed that the rate of oxidation of catechol to *o*-quinone is dependent on the ease of loss of the fifth ligand. This permits the formation of a Cu(II) catecholate complex. For complexes reported in this paper the geometry is purported to be a severely

distorted octahedron that contains the $CuN_2O_2\cdots O_2$ chromophore in which the fifth and the sixth donor atoms are the weakly coordinated second carboxylate oxygen atoms. The weakly interacting oxygen atoms are likely to dissociate to provide sites on Cu(II) for catechol bonding. Dissociation of the weakly bonding donors would also facilitate any necessary ligand rearrangement induced by catechol bonding. The catecholase-mimetic activity of the benzimidazole adduct **1** is higher than that of the 2-methylbenzimidazole adduct **2** and metronidazole adduct **3** (Table 2). This may be due to steric hindrance caused by the proximity of the methyl group to the nitrogen donor atom in **2** and **3**. The presence of methyl groups could render the approach of catechol to Cu(II) sites more difficult in these two adducts when compared to the benzimidazole adduct.

Conclusions

Four new copper(II) monomers have been prepared and characterized. Spectral data suggest that these complexes contain the CuO_2N_2 chromophore. Oxidation of catechol to *o*-quinone by these four complexes has been studied. These copper complexes catalyze the oxidation of catechol, however, the rate of reaction is dependent on the nature of the N-containing ligand. Furthermore, the 2-methyl-5-nitrobenzimidazole complex which initially is the most reactive complex quickly poisons the catalyst and the oxidation ceases. The exact manner in which the catalyst is poisoned is not known at this time but is under investigation.

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