

Electrochemistry of Nitrite Reductase Model Compounds. 3. Formation and Characterization of a Bis(hydroxylamine)(tetraphenylporphyrinato)iron(II) Complex

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Received July 1, 1986

The reaction of hydroxylamine with $\text{Fe}^{\text{III}}(\text{TPP})\text{Cl}$ and $\text{Fe}^{\text{II}}(\text{TPP})$ (TPP = tetraphenylporphyrinate(2-)) at room temperature and at lower temperatures ($< -20^\circ\text{C}$) was studied in this work. At room temperature, hydroxylamine reacted with both Fe^{III} - and $\text{Fe}^{\text{II}}(\text{TPP})$ to form $\text{Fe}(\text{TPP})(\text{NO})$. But, when the temperature was reduced to -40°C in methylene chloride, $\text{Fe}(\text{TPP})(\text{NH}_2\text{OH})_2$ was stable. This complex was characterized by visible and NMR spectroscopy and by electrochemistry. The visible and NMR spectra of $\text{Fe}(\text{TPP})(\text{NH}_2\text{OH})_2$ were similar to other low-spin six-coordinate ferrous TPP complexes. As was consistent with this low spin ($S = 0$) formulation, the chemical shifts of the porphyrin and hydroxylamine resonances were independent of temperature. In addition, the resonances due to the coordinated hydroxylamine were observed upfield from Me_4Si . The same $\text{Fe}(\text{TPP})(\text{NH}_2\text{OH})_2$ complex can be formed from $\text{Fe}(\text{TPP})\text{Cl}$ and hydroxylamine, where the excess hydroxylamine can reduce the iron. The $\text{Fe}(\text{TPP})$ -hydroxylamine complex was stable at low temperatures, but it decomposed at room temperature to $\text{Fe}(\text{TPP})(\text{NO})$. Conversely, when $\text{Fe}(\text{TPP})\text{NO}$ and hydroxylamine were mixed together at low temperatures, $\text{Fe}(\text{TPP})(\text{NH}_2\text{OH})_2$ was formed. Cyclic voltammetry of this complex was carried out at -35°C . The first reduction wave was observed with an $E_{1/2}$ value of -1.22 V vs. a Ag/AgNO_3 reference electrode, while the first oxidation wave occurred at an $E_{1/2}$ value of -0.18 V vs. Ag/AgNO_3 . Coulometric oxidation of the complex at -35°C at 0.1 V vs. Ag/AgNO_3 gave a coulometric n value of 3, and the final oxidation product was $\text{Fe}(\text{TPP})(\text{NO})$. The voltammetric data were consistent with an initial formation of a ferric complex, which underwent subsequent reaction to form the nitrosyl complex.

Introduction

The chemical transformations in the nitrogen cycle have been the subject of considerable interest in recent years.¹ There are several enzymes in this cycle that either utilize hydroxylamine or are thought to generate hydroxylamine as an intermediate. One such enzyme is the assimilatory nitrite reductase, which reduces nitrite to ammonia. This enzyme contains two metal groups: a heme (an iron isobacteriochlorin)^{2,3} and a 4Fe-4S cluster.⁴ The mechanism of this six-electron reduction process is still not understood; however, it is known that nitrite coordinates to the heme and is reduced to an iron nitrosyl⁵ and then to ammonia. There is considerable evidence that a coordinated hydroxylamine is an intermediate in this reaction.^{1,2} Another enzyme that utilizes hydroxylamine is hydroxylamine oxidoreductase,⁶ which catalyzes the oxidation of hydroxylamine to nitrite, a reaction that is nearly the reverse of the assimilatory nitrite reduction. This enzyme contains a yet uncharacterized heme P460 group that is a unique prosthetic group, different from other known hemes,^{7,8} along with seven or eight hemes *c* per heme P460.⁹

It has been the aim of our research to study the redox chemistry of iron porphyrin complexes with ligands that are part of the nitrogen cycle. The electrochemistry and spectroscopy of iron porphyrin complexes with nitrite¹⁰⁻¹² and nitric oxide¹³⁻¹⁵ have

been previously reported. In addition, iron porphyrin complexes with aliphatic amines have been characterized.^{16,17} Generally, the ferric state is not stable and is reduced to a low-spin bis-(amine)iron(II) by the excess ligand.^{16,17} The electrochemistry of these complexes, though, is dominated by changes in the iron oxidation state, with the identity of the ligand changing the relative stability of these oxidation states.^{18,19} The reduction of the ferric state by the excess amine is too slow to be observed on the electrochemical time scale. Unlike complexes of the above ligands, no complexes of hydroxylamine with metal porphyrins have been reported, in spite of the fact that hydroxylamine complexes have been observed for iron and other metals.²⁰ In this paper, we wish to report the characterization and electrochemistry of an iron porphyrin hydroxylamine complex.

Experimental Section

Equipment. Cyclic voltammetric data were obtained by using an ECO 553 potentiostat with an EG&G Princeton Applied Research (PARC) 175 waveform generator and a Hewlett-Packard 7045 X-Y recorder. The ECO 553 potentiostat was also used for coulometry along with a PARC 379 digital coulometer and a strip chart recorder. A three-electrode IBM cell was used, which consisted of a platinum working electrode, a platinum auxiliary electrode, and a $\text{Ag}/0.1\text{ M AgNO}_3$ in acetonitrile reference electrode (SRE). A coulometric cell with a mercury-pool (for reduction) or a carbon-cloth (for oxidation) working electrode was used for coulometry. Low temperatures were achieved with a water/alcohol/dry ice bath. The visible spectra were obtained on a Perkin-Elmer 320 UV-visible spectrophotometer with a Perkin-Elmer 3600 data station. A Dewar flask was constructed with a 1-cm visible cell for low-temperature visible spectroscopy. The NMR data were obtained on a 60-MHz (proton) JEOL FX60Q Fourier transform NMR spectrometer.

Chemicals. Tetraphenylporphyrin (H_2TPP) was purchased from Aldrich Chemical Co. $\text{Fe}(\text{TPP})\text{Cl}$,²¹ $\text{Fe}(\text{TPP})(\text{pip})_2$,²² $\text{Fe}(\text{TPP})(\text{NO})$,²³ $\text{Fe}(\text{TPP})(\text{NO})$,²⁴ and their deuteriated analogues²⁵⁻²⁷ were synthesized

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Table I. Chemical Shifts of $\text{Fe}^{\text{II}}(\text{TPP})(\text{NH}_2\text{OH})_2$ and Related Compounds (in ppm)

compd	solvent	spin state	pyrrole	phenyl			NH_2OH		ref
				ortho	meta	para	OH	NH_2	
$\text{Fe}(\text{TPP})(\text{NH}_2\text{OH})_2$	CDCl_3^a		8.71	8.11	7.74	7.74	-3.04	-2.50	tw ^b
$\text{Fe}(\text{TPP}-d_8)(\text{NH}_2\text{OH})_2$	CDCl_3^a			8.15	7.81	7.81	-3.00	-2.52	tw
$\text{Fe}(\text{TPP}-d_{20})(\text{NH}_2\text{OH})_2$	CDCl_3^a		8.71				-3.09	-2.61	tw
$\text{Fe}(\text{TPP})(\text{NOHpip})_2$	CDCl_3	0	8.9	7.8	7.5	6.8			17
$\text{Fe}(\text{TPP})(\text{CN})_2^{2-}$	$\text{Me}_2\text{SO}-d_6$	0	7.89	7.64	7.60	7.60			31
$\text{Fe}(\text{TPP})(\text{CNCOPh})_2$	CD_2Cl_2^c	0	8.79	8.03	7.74	7.74			32
$\text{Fe}(\text{TPP})$	C_6D_6	1	4.7	20.8	12.5	12.5			33
$\text{Fe}(\text{TPP})(2\text{-MeIm})$	toluene- d_8	2	52.5	9.5	8.8	8.7			34
$\text{Fe}(\text{TPP})$	$\text{THF}-d_8$	2	50.2						34

^aTemperature -30°C . ^btw = this work. ^cTemperature 0°C .

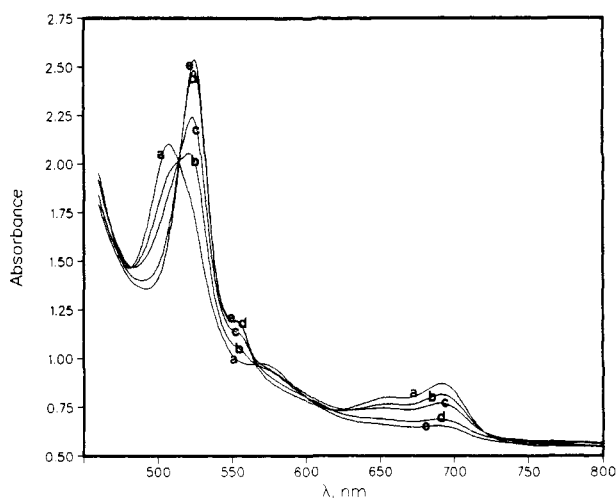


Figure 1. Visible spectral changes during the reaction between 0.08 mM $\text{Fe}(\text{TPP})\text{Cl}$ and 0.4 mM hydroxylamine in methylene chloride at -64°C after (a) 7, (b) 16, (c) 26, (d) 50, and (e) 101 min.

by literature procedures. $\text{Fe}^{\text{II}}(\text{TPP})$ was obtained by the reduction of $\text{Fe}(\text{TPP})\text{Cl}$ in methylene chloride with an aqueous sodium dithionite solution^{28,29} on a vacuum line. Complete reduction was verified spectroscopically, and the $\text{Fe}^{\text{II}}(\text{TPP})$ was used immediately after isolation. Hydroxylamine (or hydroxylamine- d_3) was obtained from the neutralization of hydroxylamine hydrochloride (or hydroxylamine- d_3 hydrochloride- d , 98% D, MSD Isotopes)³⁰ and was stored at dry ice temperatures. For hydroxylamine- d_3 , $n\text{-C}_4\text{H}_9\text{OD}$ (98% D, Aldrich Chemical Co.) was used instead of $n\text{-C}_4\text{H}_9\text{OH}$. All NMR measurements were obtained in CDCl_3 (99% D, Aldrich Chemical Co.). The solvents for spectroscopic and electrochemical measurements were obtained from Aldrich Chemical Co. at the highest purity available. Tetrabutylammonium perchlorate (TBAP) was obtained from G. F. Smith Chemical Co. All reactions were carried out under argon.

Results and Discussion

When hydroxylamine was added to $\text{Fe}(\text{TPP})\text{Cl}$, the visible spectrum that was obtained was identical with that of $\text{Fe}(\text{TPP})(\text{NO})$.²⁴ Cyclic voltammetry of the reaction product showed a one-electron reduction wave at -1.28 V vs. Ag/AgNO_3 , which was also the same as for $\text{Fe}(\text{TPP})(\text{NO})$.^{13,14} Quantitative analysis of the spectral changes indicated complete conversion of $\text{Fe}(\text{TPP})\text{Cl}$ to $\text{Fe}(\text{TPP})(\text{NO})$. The nitrosyl complex was also formed when $\text{Fe}(\text{TPP})(\text{NO}_3)$, $\text{Fe}(\text{TPP})$, and $\text{Fe}(\text{OEP})\text{Cl}$ were used. When hydroxylamine was added to $\text{Fe}(\text{TPP})(\text{pip})_2$ (pip = piperidine) or $\text{Fe}(\text{TPP})(\text{NO})$ in methylene chloride at room temperature, no spectral changes were observed.

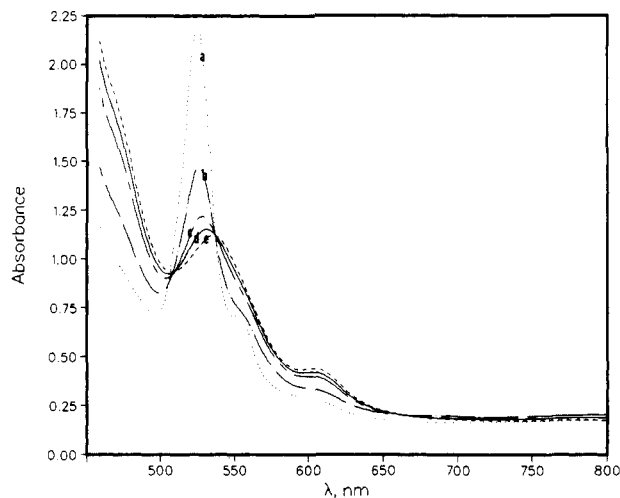


Figure 2. Visible spectral changes during the decomposition of 0.09 mM $\text{Fe}(\text{TPP})(\text{NH}_2\text{OH})_2$ in methylene chloride at 28°C : (a) initial spectrum; (b-e) spectra after 3, 13, 20, and 24 min, respectively. Initial concentration of hydroxylamine: 0.4 mM.

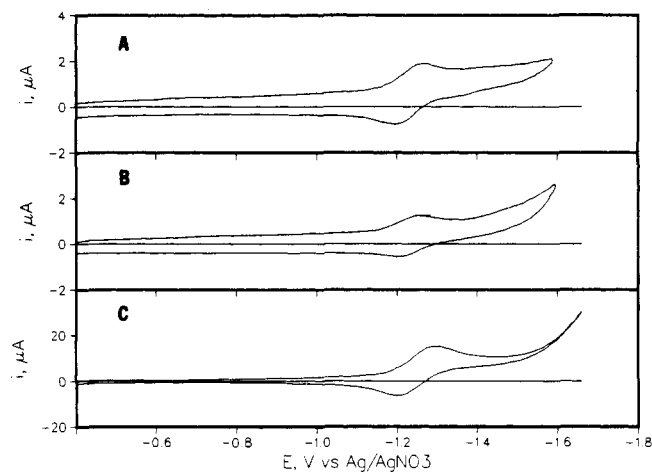


Figure 3. Cyclic voltammetry of (A) $\text{Fe}(\text{TPP})(\text{NH}_2\text{OH})_2$ at -35°C , (B) $\text{Fe}(\text{TPP})(\text{NO})$ at -35°C , and (C) $\text{Fe}(\text{TPP})(\text{NH}_2\text{OH})_2$, which was initially at -35°C and was then warmed to 25°C for 40 min. For all voltammograms: 0.3 mM iron porphyrin, 1.5 mM hydroxylamine, 0.1 M TBAP in methylene chloride; scan rate 0.10 V/s.

When the reaction was carried out at reduced temperatures with $\text{Fe}(\text{TPP})$, the results were substantially different. New bands at 526 and 556 nm were observed, while the 540-nm band for $\text{Fe}(\text{TPP})$ disappeared. The overall spectrum was very similar to the spectra of $\text{Fe}(\text{TPP})(\text{pip})_2$ (527 and 557 nm) or $\text{Fe}(\text{TPP})(\text{NOHpip})_2$ (528 and 556 nm).¹⁷ When $\text{Fe}(\text{TPP})\text{Cl}$ was used instead of $\text{Fe}(\text{TPP})$, the same product was obtained, but at a slower rate (see Figure 1). Once formed, this complex was stable at low temperatures. Only minimal spectral changes (<5%) were observed after 50 min at -30°C . When the temperature was raised, the hydroxylamine complex was converted to $\text{Fe}(\text{TPP})$ -

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Table II. $E_{1/2}$ Values for Ferrous Porphyrin Complexes^a

compd	$E_{1/2}$ ^b V		ref
	1st oxidn	1st redn	
Fe(TPP)	-0.11	-1.41	40
Fe(TPP)(NO)	+0.39	-1.28	13
Fe(TPP)(py) ₂	-0.31	-1.87	18
Fe(TPP)(NH ₂ OH) ₂	-0.18	-1.22	tw ^c

^aSolvent methylene chloride. ^bReference electrode Ag/AgNO₃. ^ctw = this work.

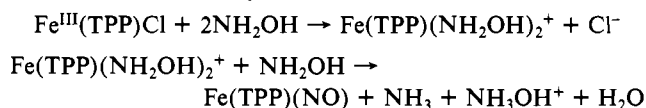
(NO) (see Figure 2). Conversely, if a solution of Fe(TPP)(NO), which was unreactive with hydroxylamine at room temperature, was cooled in the presence of hydroxylamine, a spectrum identical with curve e in Figure 1 was observed.

The NMR spectra obtained from the reaction of hydroxylamine with Fe(TPP) and its deuteriated analogues are summarized in Table I along with some related compounds. The observed chemical shifts were independent of temperature in the temperature range studied (-25 to -47 °C). The hydroxylamine assignments were based on the NMR integrations (integrated areas: pyrrole, 8 H; ortho, 8 H; meta and para, 12 H; OH, 2 H; NH₂, 4 H). Figure 3 shows the cyclic voltammetry of the Fe(TPP)-hydroxylamine and Fe(TPP)(NO) complexes at -35 °C as well as the voltammetry obtained when the Fe(TPP)-hydroxylamine solution was warmed to room temperature. At low temperatures, a single reversible reduction wave with an $E_{1/2}$ of -1.22 V vs. Ag/AgNO₃ was observed (Figure 3B). The half-wave potential of this wave was quite close to that of Fe(TPP)(NO) (Figure 3A), which has an $E_{1/2}$ value of -1.25 V vs. Ag/AgNO₃ at the same temperature. Furthermore, it should be noted that the $E_{1/2}$ for the hydroxylamine complex is positive of that observed for the Fe(II)/Fe(I) wave when no ligand is present ($E_{1/2}$ = -1.41 V). The voltammetric data are summarized in Table II. Unlike the case for the reduction, several waves were observed for the oxidation. We are not in a position to assign the redox processes for all these waves, so we have focused on the first oxidation wave. Coulometric oxidation of the hydroxylamine-Fe(TPP) complex gave an *n* value of 3. From the visible spectra, obtained during the electrolysis, the product was found to be Fe(TPP)(NO). Coulometric reduction also led to Fe(TPP)(NO) as the ultimate product. Cyclic voltammetry of hydroxylamine alone revealed no waves in the region between the first oxidation and reduction waves of the Fe(TPP)-hydroxylamine complex.

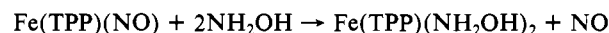
The visible spectrum of the Fe(TPP)-hydroxylamine complex is quite similar to those of other low-spin 6-coordinate amine complexes such as piperidine or *N*-hydroxypiperidine.¹⁷ In addition, the porphyrin chemical shifts and the independence of the chemical shifts as a function of temperature confirm the low-spin (*S* = 0) formulation of the complex. All the data are then consistent with a bis(hydroxylamine)iron(II) complex, Fe(TPP)(NH₂OH)₂. Finally, the hydroxylamine proton resonances, which are shifted upfield due to the porphyrin ring currents,^{35,36} are decreased significantly when deuteriated hydroxylamine is used. Complexes with *S* = 1 or 2 give rise to significantly different proton NMR spectra for the porphyrin, as can be seen in Table I. The interconversion of the hydroxylamine species, which is formed at low temperatures, into Fe(TPP)(NO) might lead one to speculate that the species formed at low temperature was a Fe(TPP)(NO)(NH₂OH) complex that loses coordination of hydroxylamine at room temperature. Arguing against this formulation is the fact that such a species would be paramagnetic (*S* = 1/2) (as is Fe(TPP)(NO) itself). As a result, the chemical shifts

should vary with temperature. In addition, pyridines and alkylamines form very weak complexes with Fe(TPP)(NO)³⁷ and have little effect on the visible spectrum of Fe(TPP)(NO) below 0.5 M pyridine.¹³

The room-temperature formation of Fe(TPP)(NO) from Fe(TPP)Cl or Fe(TPP) no doubt occurs by the metal-catalyzed disproportionation of hydroxylamine. The mechanism of this reaction was not studied in this work, but similar reductive nitrosylation reactions have been reported previously.²⁰ Nitrosyls with a number of transition metals can be generated by their reaction with hydroxylamine,³⁸ often with the concurrent reduction of the metal. This reaction is quite general and can be used to synthesize a wide variety of metal nitrosyls, though its occurrence in metal porphyrin chemistry has not yet been reported. In these reactions, the hydroxylamine disproportionates to nitric oxide and ammonia, and in some cases, the metal may also be reduced. On the basis of this precedent, the following reaction can be written for the reduction nitrosylation:



The formation of the bis(hydroxylamine) complex from Fe(TPP)(NO) at low temperatures probably occurs by way of ligand substitution:



The voltammetric oxidation of this complex is not straightforward due to the presence of excess hydroxylamine. By use of the known relationship between $E_{1/2}$ values of the ferric/ferrous wave and the $\text{p}K_a$ of this ligand ($\text{p}K_a = 5.8^{39}$), an $E_{1/2}$ of -0.30 V vs. Ag/AgNO₃ was calculated for this wave,¹⁸ which is reasonably close to the experimental value (-0.18 V vs. Ag/AgNO₃). The final oxidation product was not the ferric complex, but Fe(TPP)(NO). Therefore, while a ferric complex may be initially formed, the ligand is probably oxidized rapidly. The net reaction can be written as



The acid that is generated can react readily with the excess hydroxylamine. If sufficient time is allowed with an excess hydroxylamine, the bis(hydroxylamine) complex could be regenerated by the mechanism discussed earlier. This reaction, though, is relatively slow.

The reduction wave, though, is quite unusual. On the basis of the $\text{p}K_a$ of hydroxylamine, the $E_{1/2}$ for the reduction of the Fe(TPP)(NH₂OH)₂ complex can be estimated to be about -1.90 V vs. Ag/AgNO₃, compared to the observed value of -1.22 V. In any case, if the wave is due to the reduction of Fe(II) to Fe(I), the $E_{1/2}$ must occur at a potential more negative than the Fe^{II}(TPP)/Fe^I(TPP) wave (-1.41 V), where the ferrous complex has no axial ligands. It appears that both the reduction and oxidation processes catalyze the disproportionation of hydroxylamine. In both cases, Fe(TPP)(NO) is formed. At this time it is not known if the disproportionation of hydroxylamine occurs via a homogeneous redox reaction between free hydroxylamine and a transient, reduced iron species or by means of an internal redox transfer.

Conclusions

Stable complexes of hydroxylamine with iron porphyrins can be generated at reduced temperatures. At room temperature,

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reductive nitrosylation will occur, leading to the nitrosyl complex. Surprisingly, hydroxylamine can displace NO at low temperatures, leading to $\text{Fe(TPP)(NH}_2\text{OH)}_2$. With excess hydroxylamine, this reaction is reversible, and the iron porphyrin can be cycled to and from the nitrosyl or hydroxylamine complex by changing the temperature. The electrochemistry of this complex is dominated by the redox behavior of the axial ligand, which is quite different from the electrochemistry of other iron porphyrin complexes. Apparently, the nitrosyl complex is a well into which both nitrite¹⁰ and hydroxylamine easily fall. This can readily be seen in the enzyme itself, where the dominant species during turnover is the nitrosyl complex.⁴¹ In addition, the ease of oxidation of hydroxylamine to NO is quite advantageous for the hydroxylamine

oxidoreductases. By contrast, though, the reduction of hydroxylamine for ammonia will require the cleavage of the N-O bond, a two-electron process, which does not occur in these studies. But, it should be noted that the active site of nitrite reductases has two redox centers, in addition to a relatively easily oxidized isobacteriochlorin. This active-site structure could facilitate the two-electron process and generate the desired product, ammonia.

Acknowledgment. We wish to acknowledge the National Science Foundation for the purchase of electrochemical equipment (Grant No. PRM-8106653) and spectroscopic equipment (Grant No. PRM-8201885). We also wish to acknowledge the Computer Service Division of Marquette University for computer time and Committee on Research of Marquette University and Arthur J. Schmitt Foundation for partial support of this research. We also thank Daniel T. Haworth for helpful discussions and help in the use of low-temperature NMR.

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Crystal and Molecular Structure of $\Delta\Delta\Delta(+)$ -[*N,N'*-1,2-Ethanedylbis[*N*-(carboxymethyl)glycyl-L-methionine] ethyl esterato]copper(II) Sesquihydrate. Asymmetric Induction in Synthesis and Amide Carbonyl Coordination to Copper

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Received August 4, 1986

The ligand *N,N'*-1,2-ethanedylbis[*N*-(carboxymethyl)glycyl-L-methionine] ethyl ester (H_2L) has been synthesized and its binding to Cu(II) in aqueous solution studied by a variety of methods including circular dichroism. These indicate that asymmetric induction at Cu(II) occurs. Crystals of the blue complex $\text{C}_{24}\text{H}_{40}\text{O}_{10}\text{N}_4\text{S}_2\text{Cu}\cdot\frac{3}{2}\text{H}_2\text{O}$ were studied by X-ray diffraction: orthorhombic, $a = 8.962$ (2) Å, $b = 11.595$ (2) Å, $c = 31.491$ (2) Å, $Z = 4$, space group $P2_12_12_1$, $R = 0.038$. The single $\Delta(+)$ enantiomer in the crystal contains tetragonally distorted N_2O_4 Cu(II) ions with trans N,O equatorial coordination from ethylenediamine nitrogens and $-\text{CH}_2\text{COO}^-$ oxygens. The ethylenediamine ring adopts a skew-boat conformation. The axial ligands are amide carbonyl oxygens. The average axial Cu-O distance (2.39 Å) is 0.4 Å longer than the average equatorial Cu-ligand bond length. The methionine sulfurs are not coordinated.

Introduction

The role of methionine in controlling the binding and activation of metal ions by proteins is not well understood.

In cytochrome *c*, octahedral Fe(II) or Fe(III) is tightly bound to methionine sulfur as an axial ligand.² In contrast, methionine is only a distant ligand to tetrahedral Cu(II) in "blue" (type 1) copper proteins such as plastocyanin³ and azurin.⁴ The Cu(II)-S(Met) bond in plastocyanin is long (2.9 Å) when determined by X-ray crystallography and undetectable by extended X-ray absorption fine-structure analysis.⁵

Copper(II) appears to have little affinity for the sulfur atom of methionine itself or its simple derivatives. For example, no such binding was detected for Cu(L-Met)_2 or Cu(DL-Met)_2 in the solid state^{6,7} or in solution.⁸

In previous studies of *N,N'*-1,2-ethanedylbis[*N*-(carboxymethyl)glycyl-L-cysteine] ethyl ester complexes of Cd(II), we found^{9a} that the EDTA remnant imparted a high thermodynamic and kinetic stability (NMR time scale). Reactions between Cu(II) and cysteinyl sulfur of this ligand also occur but are complicated by redox processes.^{9b} It seemed likely that reactions of Cu(II) with the methionine analogue might involve interactions of Cu(II) with pendent methionines. We report here the synthesis of the

new ligand *N,N'*-1,2-ethanedylbis[*N*-(carboxymethyl)glycyl-L-methionine] ethyl ester (**1**) and studies on its interaction with Cu(II) in aqueous solution and the solid state.

Experimental Section

EDTA bisanhydride was synthesized¹⁰ in 85% yield (10.9 g) by heating (65 °C, 24 h) EDTA (14.63 g) in pyridine (21 mL, dried with anhydrous Na_2SO_4) containing acetic anhydride (21.4 g). The solid product (mp 195-198 °C) was filtered off, washed with anhydrous ether, and dried (50 °C). The ligand **1** was synthesized by heating EDTA bisanhydride (666 mg, 2.7 mmol) in dry bis(2-methoxyethyl) ether (300 mL) with L-methionine ethyl ester hydrochloride (Sigma, 939 mg, 4.4

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