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Deuterium NMR Study of Amino Acid Coordination to Chromium(III)

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A series of bis(ethylenediamine)Cr(III)-amino acid complexes, synthesized with deuterium-labeled alanine, glycine, homoserine, leucine, methionine, phenylalanine, serine, and threonine, was characterized by ^2H NMR spectroscopy. The spectra show that these bidentate-coordinated amino acid complexes decompose via monodentate species. In addition, the diastereomeric isomers of alanine and leucine can be distinguished in the spectra. This was confirmed by the isolation of one of the L-leucine isomers. The bis(1,3-propanediamine)Cr(III) complexes of glycine and alanine were also synthesized, and the NMR spectra of these complexes and *α-cis*-[Cr(ethylenediaminediacetate)(glycinate)] show changes due to variation in the ligand complement. The crystal structure of *α-cis*-[Cr(ethylenediaminediacetate)(glycinate)]·$2H_2O$ ($CrO_6N_3C_8H_{14}\cdot 2H_2O$) was determined and supports the NMR observations. This complex crystallizes in the space group $P2_1/c$ of the monoclinic crystal system with a = 8.9231 (19) Å, b = 10.1889 (22) Å, c = 15.4180 (30) Å, β = 102.657 (17)°, and Z = 4. An improved method for the synthesis of bis(diamine)Cr(III)-amino acid complexes is also reported.

Introduction

Complexes of amino acids with transition metals are often cited as models for metal ion coordination in biological systems. In particular, bis(ethylenediamine)cobalt(III) complexes containing amino acids have been used to study the stereochemistry and reactions of amino acids bound to metal ions.[1,2]

The study of amino acid coordination to chromium(III) complements the cobalt(III) studies. Both metal ions are substitution-inert, but the stereochemistry and reactivity of the Cr(III) complexes are expected to be significantly different from those of Co(III).[3] The paramagnetism of Cr(III) limits NMR spectral investigation, and studies of Cr(III) complexes have been based primarily on visible and circular dichroism spectra.[4-7] Deuterium NMR spectroscopy provides an effective method for determining the structure of Cr(III) complexes in solution.[8,9] This technique has facilitated the characterization of the Cr(III)-amino acid complexes reported in this study.

A series of bis(ethylenediamine)chromium(III)-amino acid dithionate complexes was synthesized with deuterium-labeled (2-carbon) amino acids. The amino acids (alanine, glycine, homoserine, leucine, methionine, phenylalanine, serine, and threonine) coordinate to Cr(III) in a bidentate fashion through the primary amine and the carboxylate group. An objective of this work was to determine the solution structure of these complexes and to correlate the position of the deuteron resonance with the side chain of the amino acid. The bidentate amino acid complexes [Cr(1,3-pn)$_2$(gly)](S_2O_6), [Cr(1,3-pn)$_2$(ala)](S_2O_6), and *α-cis*-[Cr(edda)(gly)] (where 1,3-pn = 1,3-propanediamine, gly = glycinate, ala = alaninate, and edda = ethylenediaminediacetate), were also synthesized in order to determine the effect of the ligand complement on the ^2H NMR spectrum. The crystal structure of *α-cis*-[Cr(edda)(gly)]·$2H_2O$ was determined to confirm the bidentate coordination of glycine and the configuration of the edda ligand.

The use of deuterium NMR spectroscopy allows the mode of α-amino acid coordination to Cr(III) to be directly identified in solution. The bidentate-coordinated amino acid complexes were observed to decompose in aqueous solution via monodentate species. The identification of these species is based on the ^2H NMR spectra of [Cr(NH_3)$_5$(Hgly-*O*)](ClO_4)$_3$ and [Cr(NH_3)$_5$(Hala-*O*)](ClO_4)$_3$ (where Hgly-*O* and Hala-*O* are carboxylate-coordinated, deuterium-labeled glycine and alanine with protonated amine groups). In addition, it was found that ^2H NMR can be used to distinguish diastereomers.

Experimental Section

Preparation of [Cr(diamine)$_2$(H_2O)(OH)](S_2O_6) Complexes. The *cis*-[Cr(en)$_2$(H_2O)(OH)](S_2O_6) complex (where en = ethylenediamine) was prepared from *cis*-[Cr(en)$_2$$Cl_2$]Cl.[10] The method reported by Pedersen[11] was used to prepare *cis*-[Cr(en)$_2$$Cl_2$]Cl. The analogous preparation of *cis*-[Cr(1,3-pn)$_2$(H_2O)(OH)](S_2O_6) was found to be difficult since *cis*-[Cr(1,3-pn)$_2$$Cl_2$]Cl could not be readily isolated by the reported procedure. However, *trans*-[Cr(1,3-pn)$_2$$Cl_2$]Cl was converted to *cis*-[Cr(1,3-pn)$_2$(H_2O)$_2$](NO_3)$_3$ and then to *cis*-[Cr(1,3-pn)$_2$(H_2O)(OH)](NO_3)$_2$·0.5H_2O as reported.[12] A solution of sodium dithionate (0.45 g in 2 mL of H_2O) was added to a solution of *cis*-[Cr(1,3-pn)$_2$(H_2O)(OH)](NO_3)$_2$·0.5H_2O (0.70 g, 1.9 mmol) in 5 mL of H_2O at room temperature. The mixture was cooled in ice for 30 min. The dark pink precipitate was filtered, washed with ethanol, and air-dried. This yielded 0.55 g of *cis*-[Cr(1,3-pn)$_2$(H_2O)(OH)](S_2O_6)·0.5H_2O (72%).

Deuteriation of Ligands. The amino acids were deuterium-labeled on the 2-carbon by using [Co(en)$_2$(amino acid)]$^{2+}$ complexes in a manner similar to that previously reported.[13] The method reported by Casella et al.[2] for the preparation of [Co(en)$_2$(ser)]Cl_2 and [Co(en)$_2$(thr)]Cl_2 was used to prepare the Co(III)-amino acid complexes. The reactions were carried out at pH 7.2-7.4, and reaction times varied from 2 to 16 h. The temperature (initially 40 °C) was increased to 50-55 °C if an orange solution was not formed after 8 h. The resulting solutions were purified on a column of Sephadex G-10 and then rotary evaporated to dryness. The residues were dissolved in ^2H_2O, and the pH was adjusted to 9.5 by the addition of anhydrous K_2CO_3. The flasks were stoppered and placed in a 45 °C water bath for several weeks. Each deuterium-labeled amino acid was isolated by neutralizing the solution with glacial acetic acid and then passing the solution through a column of Dowex AG1-X8 (acetate form) to remove chloride. The resulting solution was saturated with H_2S, the flask stoppered, and the mixture allowed to stand for several hours. This process was repeated until the flask contained a colorless solution over the precipitated cobalt sulfide, which was then removed by filtration through Celite on Whatman No. 2 paper. The filtrate was rotary evaporated to a small volume, and ethanol was added to precipitate the deuterium-labeled DL-amino acid.

Resolved, deuterium-labeled (2-carbon) alanine and leucine were synthesized by the method of Upson and Hruby.[14] The L-amino acids

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were made by the reaction of the deuterated *N*-acetyl-DL-amino acids with hog renal acylase.[15]

Deuterium labeling of edda was accomplished by dissolving *α-cis*-$[Co(edda)(en)](NO_3)$[16] (10 g, 28 mmol) in 80 mL of ^2H_2O on a steam bath. The pH was adjusted to 10 with K_2CO_3, and the solution was heated at 80 °C for 4–5 h. After the addition of 75 mL of water, KCN (11 g, 170 mmol) was added to the mixture. The solution was heated again on a steam bath (the color rapidly changed to a pale yellow), and the volume was reduced to about 60 mL. *In the hood*, 6 M HCl was added to obtain a pH of 4. The mixture was then placed in an ice bath for several hours. The precipitate that formed was filtered and washed with ethanol/H_2O (50/50) and ethanol. Concentration of the filtrate yielded additional crops of crystals. In all cases, the product was recrystallized from small volumes of hot water. The combined yield was 3.5 g (70%). Proton NMR of the recrystallized product showed >95% deuteriation of the methylene group on the acetate arms.

Preparation of $[Cr(en)_2(\text{amino acid})](S_2O_6)$ Complexes. The preparation of the chloride and iodide salts of several of these complexes from *cis*-$[Cr(en)_2Cl_2]Cl$ was reported by Kaizaki and Ito.[4] When one starts with a *cis*-aquahydroxybis(diamine)chromium(III) complex and uses dithionate as the counterion, these complexes can be synthesized and isolated in a more direct manner. Although many of the following preparations are similar, the variations are required to obtain reasonable yields of the pure products.

$[Cr(en)_2(gly)](S_2O_6)$ (gly = Glycinate). Glycine (0.10 g, 1.3 mmol) and *cis*-$[Cr(en)_2(H_2O)(OH)](S_2O_6)$ (0.40 g, 1.1 mmol) were mixed with 2 mL of H_2O. The suspension was heated, with stirring, on a steam bath to form a red-orange solution. Methanol (2 mL) was added, and the mixture was heated for 1 min on the steam bath. After the mixture stood for 1 h at room temperature, the orange precipitate that formed was collected on a filter, washed with methanol/H_2O (60/40) and with methanol, and then air-dried. The product was recrystallized from 7 mL of warm H_2O by the addition of 3 mL of methanol. After standing for 1 h, the product was collected as before; yield 0.094 g (21%).

$[Cr(en)_2(\text{DL-ala})](S_2O_6)$ (ala = Alaninate). Alanine (0.12 g, 1.3 mmol) and *cis*-$[Cr(en)_2(H_2O)(OH)](S_2O_6)$ (0.40 g, 1.1 mmol) were mixed with 3 mL of H_2O, and the mixture was heated on a steam bath to form a red solution. Methanol (2 mL) was added, and the mixture was heated for 3 min. After the mixture stood at room temperature for 1 h, the orange precipitate that formed was filtered, washed with 10 mL of methanol/H_2O (20/80), methanol/H_2O (60/40), and methanol, and then air-dried. The product was recrystallized from 10 mL of warm H_2O by the addition of 10 mL of methanol and recovered as before; yield 0.079 g (17%).

$[Cr(en)_2(\text{L-ser})](S_2O_6)$ (ser = Serinate). L-Serine (0.16 g, 1.5 mmol) and *cis*-$[Cr(en)_2(H_2O)(OH)](S_2O_6)$ (0.40 g, 1.1 mmol) were mixed with 2 mL of H_2O. The suspension was heated on a steam bath to form a red solution. Methanol (2 mL) was added, and the mixture was heated for 2 min. After the mixture stood at room temperature for 1 h, the orange precipitate was filtered, washed with methanol/H_2O (60/40) and methanol, and air-dried. The product was recrystallized from 15 mL of H_2O by the addition of 10 mL of ethanol. After standing 1–2 h, the product was collected as before; yield 0.072 g (15%).

$[Cr(en)_2(\text{DL-thr})](S_2O_6)\cdot 0.5H_2O$ (thr = Threoninate). Threonine (0.20 g, 1.7 mmol) and *cis*-$[Cr(en)_2(H_2O)(OH)](S_2O_6)$ (0.40 g, 1.1 mmol) were reacted in the same manner as described above for the synthesis of the serine-containing complex. The orange precipitate was filtered, washed with 5 mL of methanol/H_2O (60/40) and methanol, and then air-dried. The crude product was dissolved in 12 mL of H_2O, and 5 mL of methanol was added. The mixture was cooled in ice for several minutes, and the precipitated impurities were removed by filtration. Methanol (5 mL) was added to the filtrate, and after 1 h at ice temperature, the orange crystals were collected as before; yield 0.068 g (14%).

$[Cr(en)_2(\text{DL-hsr})](S_2O_6)\cdot H_2O$ (hsr = Homoserinate). Homoserine (0.20 g, 1.7 mmol) and *cis*-$[Cr(en)_2(H_2O)(OH)](S_2O_6)$ (0.40 g, 1.1 mmol) were mixed in 2 mL of H_2O. The solids were dissolved by heating the mixture on a steam bath. Methanol (2 mL) was added, and the mixture was heated for 2 min. After the mixture stood for about 1 h, the pinkish impurities were filtered from the orange solution. Methanol (2 mL) was added to the filtrate, and the mixture was warmed for 5 min. After the mixture stood at room temperature for 1 h, the orange precipitate was filtered, washed with methanol/H_2O (80/20) and methanol, and then air-dried. The product was recrystallized from 5 mL of H_2O by adding 8 mL of ethanol. After standing 1–2 h, the crystals were collected as before; yield 0.078 g (15%).

$[Cr(en)_2(\text{L-leu})](S_2O_6)\cdot 2H_2O$ (leu = Leucinate). L-Leucine (0.20 g, 1.5 mmol) and *cis*-$[Cr(en)_2(H_2O)(OH)](S_2O_6)$ (0.40 g, 1.1 mmol) were mixed with 3 mL of H_2O. The mixture was heated on a steam bath to form a red solution containing some undissolved leucine. Methanol (3 mL) was added, and the suspension was heated for about 4 min. More methanol (1 mL) was then added, and the mixture was cooled to room temperature. Heating the mixture for 3–5 min with stirring caused an orange precipitate to form. After the mixture stood at room temperature for 1 h, the product was collected on a filter, washed with 10 mL of methanol/H_2O (60/40) and methanol, and then air-dried. The product was purified by dissolving it in 15 mL of H_2O, adding 40 mL of ethanol, and letting the mixture stand for 2 h. The product was filtered, washed with methanol/H_2O (80/20) and methanol, and air-dried; yield 0.10 g (18%).

$[Cr(en)_2(\text{DL-met})](S_2O_6)\cdot H_2O$ (met = Methioninate). Methionine (0.35 g, 2.3 mmol) and *cis*-$[Cr(en)_2(H_2O)(OH)](S_2O_6)$ (0.60 g, 1.6 mmol) were mixed with 3 mL of H_2O. The mixture was heated on a steam bath to form a red-orange solution. Methanol (2 mL) was then added, and the mixture was heated for 2 min. After the mixture was cooled to room temperature, methanol (3 mL) was added and the mixture was again heated for 1 min. After the mixture stood for 1 h, the orange precipitate was filtered, washed with methanol/H_2O (60/40) and methanol, and air-dried. The product was recrystallized from 25 mL of H_2O by the addition of 10 mL of ethanol. After standing for 1–2 h, the product was collected as before; yield 0.23 g (29%).

$[Cr(en)_2(\text{DL-phe})](S_2O_6)\cdot 0.5H_2O$ (phe = Phenylalaninate). Phenylalanine (0.18 g, 1.1 mmol) and *cis*-$[Cr(en)_2(H_2O)(OH)](S_2O_6)$ (0.40 g, 1.1 mmol) were mixed with 2.5 mL of H_2O. The mixture was heated on a steam bath to form a red solution containing some undissolved phenylalanine. Methanol (3 mL) was added, and the mixture was heated for 3 min. After the mixture stood at room temperature for 1 h, the orange precipitate was filtered, washed with 10 mL of methanol/H_2O (40/60), methanol/H_2O (60/40), and methanol, and then air-dried. The product was recrystallized from 10 mL of H_2O by adding 5 mL of methanol. After standing for 1 h, the product was collected as before; yield 0.038 g (7%).

Preparation of $[Cr(1,3\text{-pn})_2(\text{amino acid})](S_2O_6)$ Complexes. $[Cr(1,3\text{-pn})_2(gly)](S_2O_6)\cdot H_2O$. Glycine (0.06 g, 1.1 mmol) and *cis*-$[Cr(1,3\text{-pn})_2(H_2O)(OH)](S_2O_6)\text{-}0.5H_2O$ (0.25 g, 0.62 mmol) were mixed with 0.75 mL of H_2O in a small beaker. The mixture was warmed with stirring on a steam bath to dissolve the solids. Methanol (1 mL) was added, and the solution was warmed for about 0.5 min. The mixture was allowed to cool, 1 mL of methanol was added, and the mixture was again heated for about 0.5 min. Ethanol (0.5–1 mL) was added, and the mixture was warmed for 3 min. After standing at room temperature for 30 min, the product was filtered, washed with ethanol/H_2O (50/50) and methanol, and air-dried; yield 0.10 g (36%).

$[Cr(1,3\text{-pn})_2(\text{DL-ala})](S_2O_6)\cdot 0.5H_2O$. Alanine (0.10 g, 1.1 mmol) and *cis*-$[Cr(1,3\text{-pn})_2(H_2O)(OH)](S_2O_6)\cdot 0.5H_2O$ (0.25 g, 0.62 mmol) were mixed with 0.75 mL of H_2O. The mixture was warmed with stirring on a steam bath to dissolve the solids. Methanol (2 mL) was added, and the mixture was warmed about 0.5 min with stirring. Methanol (1 mL) was then added, and the mixture was heated for 1 min. Ethanol (0.5–1 mL) was added to the warm solution to cause it to cloud slightly. The mixture was again heated on a steam bath for 1–2 min. The orange product was precipitated by scratching the inside of the beaker and allowing the mixture to cool slowly to room temperature. The precipitate was filtered, washed with ethanol/H_2O (50/50) and methanol, and air-dried; yield 0.050 g (18%).

Preparation of $[Cr(NH_3)_5(\text{Hamino acid-}O)](ClO_4)_3$ Complexes. Both $[Cr(NH_3)_5(\text{Hgly-}O)](ClO_4)_3$ and $[Cr(NH_3)_5(\text{Hala-}O)](ClO_4)_3$ were prepared by the method previously reported for the carboxylate-coordinated glycine complex.[17] Attempts to use the reported preparation of these complexes from $[Cr(NH_3)_5Cl]Cl_2$[18] were unsuccessful.

Preparation of *α-cis*-$[Cr(edda)(gly)]\cdot 2H_2O$. A solution of *α-cis*-$[Cr(edda)(H_2O)_2](NO_3)$[9] (0.43 g, 1.3 mmol) in 1.5 mL of H_2O was heated on a steam bath to about 80 °C. A solution of sodium glycinate (NaOH (0.053 g, 1.3 mmol), glycine (0.10 g, 1.3 mmol), and 1.5 mL of H_2O) was slowly added dropwise to the heated solution with stirring. The

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Table I. Elemental Analysis

complex	% C		% H		% N	
	calcd	found	calcd	found	calcd	found
$[Cr(en)_2(gly)](S_2O_6)$	17.7	17.8	4.96	4.89	17.2	17.1
$[Cr(en)_2(DL\text{-}ala)](S_2O_6)$	20.0	19.9	5.27	5.32	16.7	16.4
$[Cr(en)_2(L\text{-}leu)](S_2O_6)\cdot 2H_2O$	24.0	23.6	6.46	6.38	14.0	14.1
$[Cr(en)_2(L\text{-}ser)](S_2O_6)$	19.3	19.4	5.08	5.11	16.0	15.9
$[Cr(en)_2(DL\text{-}thr)](S_2O_6)\cdot 0.5H_2O$	20.9	20.9	5.48	5.47	15.2	15.2
$[Cr(en)_2(DL\text{-}hsr)](S_2O_6)\cdot H_2O$	20.5	20.5	5.59	5.49	15.0	15.0
$[Cr(en)_2(DL\text{-}met)](S_2O_6)\cdot H_2O$	21.7	21.7	5.66	5.61	14.0	14.0
$[Cr(en)_2(DL\text{-}phe)](S_2O_6)\cdot 0.5H_2O$	30.9	31.0	5.38	5.55	13.8	13.6
$[Cr(1,3\text{-}pn)_2(gly)](S_2O_6)\cdot H_2O$	21.2	21.4	5.79	5.80	15.5	15.1
$[Cr(1,3\text{-}pn)_2(DL\text{-}ala)](S_2O_6)\cdot 0.5H_2O$	23.6	24.0	5.95	6.06	15.3	15.0
α-cis-$[Cr(edda)(gly)]\cdot 2H_2O$	28.6	28.6	5.40	5.00	12.5	12.4
cis-$[Cr(1,3\text{-}pn)_2(H_2O)(OH)](S_2O_6)\cdot 0.5H_2O$	17.8	17.9	5.98	6.17	13.9	14.0

Table II. Visible Spectra in Aqueous Solution[a]

complex	λ_{max}, nm (ϵ, L mol^{-1} cm^{-1})	
$[Cr(en)_2(gly)](S_2O_6)$	359 (71.6)	478 (84.9)
$[Cr(en)_2(DL\text{-}ala)](S_2O_6)$	360 (72.4)	478 (87.5)
$[Cr(en)_2(L\text{-}leu)](S_2O_6)\cdot 2H_2O$	361 (76.8)	480 (92.6)
$[Cr(en)_2(L\text{-}ser)](S_2O_6)$	360 (70.2)	478 (84.2)
$[Cr(en)_2(DL\text{-}thr)](S_2O_6)\cdot 0.5H_2O$	360 (71.2)	478 (86.0)
$[Cr(en)_2(DL\text{-}hsr)](S_2O_6)\cdot H_2O$	360 (77.3)	478 (90.7)
$[Cr(en)_2(DL\text{-}met)](S_2O_6)\cdot H_2O$	360 (73.2)	478 (87.6)
$[Cr(en)_2(DL\text{-}phe)](S_2O_6)\cdot 0.5H_2O$	361 (70.8)	479 (85.0)
$[Cr(1,3\text{-}pn)_2(gly)](S_2O_6)\cdot H_2O$	361 (82.8)	481 (71.4)
$[Cr(1,3\text{-}pn)_2(DL\text{-}ala)](S_2O_6)\cdot 0.5H_2O$	361 (81.2)	482 (75.8)
α-cis-$[Cr(edda)(gly)]\cdot 2H_2O$	387 (60.9)	518 (79.8)

[a] 0.001 M HCl.

Table III. Data Collection and Structure Determination

compd name: *α-cis*-$[Cr(edda)(gly)]\cdot 2H_2O$
empirical formula: $CrO_6N_3C_8H_{14}\cdot 2H_2O$
mol wt: 336.24
diffractometer syst: Nicolet R3m/E
radiation: Mo Kα (0.710 69 Å), graphite monochromator
cryst class: monoclinic
space group: $P2_1/c$
systematic absences: $h0l$, l odd; $0k0$, k odd
lattice constants (based on 25 reflecns in the range $28° < 2\theta < 36°$): a = 8.8231 (19) Å; b = 10.1889 (22) Å; c = 15.4180 (30) Å; β = 102.657 (17)°
vol: 1352.36 Å^3
calcd density, ρ: 1.65 g/cm^3 (Z = 4)
cryst size: approx 0.26 × 0.10 × 0.09 mm, irregular shape
abs coeff: 8.45 cm^{-1}
$F(000)$ = 619.87
type of abs cor: empirical ψ scan
max transmission: 0.716
min transmission: 0.608
data collection technique: ω scan[a]
scan range: 1.0°
scan speed: 5°/min (min), 30°/min (max)
temp: 22 °C
check reflecns (monitored every 100 scans): 100; 114; −1,1,2
total reflecns: 2578 (2θ(max) = 45°)
data collected: for $h > 0$, $k > 0$, all l
unique reflecns: 1633 (1092 with $I > 3\sigma$)
R for equiv reflecns: 0.0201
structure soln package: Nicolet SHELXTL[b]
scattering factors: [c]
structure soln technique: direct methods
refinement: least-squares full-matrix minimizing $\sum w(|F_o| - |F_c|)^2$
$R = \sum||F_o| - |F_c||/|F_o| = 0.0555$
$R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2} = 0.0530$ with $w = 1/[\sigma^2(F) + g(F)^2]$ and $g = 0.001\,57$
total parameters refined: 211
thermal parameters: anisotropic on all non-H atoms
H atoms: constrained to C–H and N–H = 0.96 Å, thermal parameters fixed at 1.2 times isotropic heavy-atom parameters
largest peak on final difference map: 0.678 e/Å^3 near O(1)
extinction cor: none

[a] Reference 19. [b] Reference 20. [c] Reference 21.

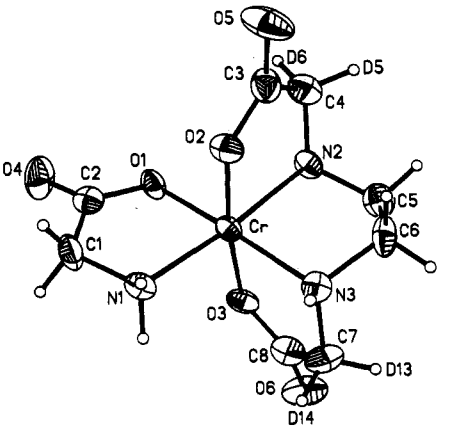

Figure 1. *α-cis*-$[Cr(edda)(gly)]\cdot 2H_2O$. The labels D5, D6, D13, and D14 are used in the text to describe the deuterium-labeled complex.

Table IV. Atomic Coordinates[a] for *α-cis*-$[Cr(edda)(gly)]\cdot 2H_2O$

	x	y	z
Cr	−174 (1)	2581 (1)	5812 (1)
O(1)	1054 (6)	3447 (5)	5060 (3)
C(1)	2622 (9)	1522 (8)	5402 (6)
C(2)	2271 (9)	2849 (7)	4952 (5)
O(4)	3168 (7)	3297 (6)	4505 (4)
N(1)	1596 (7)	1231 (6)	6007 (4)
O(2)	−1374 (6)	1533 (5)	4845 (3)
C(3)	−2583 (9)	2072 (8)	4336 (6)
O(5)	−3384 (7)	1524 (6)	3688 (4)
N(2)	−2055 (7)	3831 (6)	5472 (4)
C(5)	−2996 (10)	3692 (8)	6158 (6)
C(6)	−2997 (8)	2279 (8)	6436 (6)
N(3)	−1375 (7)	1790 (6)	6700 (4)
C(7)	−573 (10)	2206 (8)	7615 (5)
C(8)	435 (9)	3410 (7)	7595 (5)
O(3)	798 (5)	3665 (5)	6847 (3)
O(6)	824 (7)	4060 (5)	8277 (3)
C(4)	−2923 (10)	3457 (8)	4571 (5)
O(7)	5853 (11)	−418 (9)	7639 (7)
O(8)	−6361 (7)	1537 (6)	7727 (5)

[a] Multiplied by 10^4.

resulting mixture was heated for approximately 40 min and removed from the steam bath, and H_2O (2 mL) was added. After the mixture was stirred briefly, ethanol (3 mL) was added. Scratching the inside of the beaker yielded a crude red precipitate, which was collected on a filter. The product was dissolved in a small volume of H_2O, and impurities were filtered from the solution. Ethanol was added to the filtrate to precipitate the product; yield 0.15 g (34%).

Physical Measurements. Elemental analyses (by Galbraith Laboratories, Inc., Knoxville, TN) are shown in Table I.

The visible spectra (recorded on a Varian/Cary 219 spectrophotometer) are summarized in Table II. The sample concentrations were in the range of 10^{-2}–10^{-3} M.

The crystal structure determination was done with use of a crystal of *α-cis*-[Cr(gly)(edda)] grown in aqueous solution by slowly cooling a saturated solution. The parameters for the data collection and the structure determination are given in Table III. A view of *α-cis*-[Cr-

Table V. Bond Lengths (Å) and Bond Angles (deg) for α-*cis*-[Cr(edda)(gly)]·2H_2O

Cr–O(1)	1.961 (6)	Cr–N(1)	2.053 (6)
Cr–O(2)	1.947 (5)	Cr–N(2)	2.066 (6)
Cr–N(3)	2.070 (6)	Cr–O(3)	1.975 (5)
O(1)–C(2)	1.278 (10)	C(1)–C(2)	1.520 (11)
C(1)–N(1)	1.465 (12)	C(2)–O(4)	1.243 (11)
O(2)–C(3)	1.299 (9)	C(3)–O(5)	1.225 (10)
C(3)–C(4)	1.504 (12)	N(2)–C(5)	1.488 (12)
N(2)–C(4)	1.482 (9)	C(5)–C(6)	1.502 (11)
C(6)–N(3)	1.486 (9)	N(3)–C(7)	1.494 (9)
C(7)–C(8)	1.519 (12)	C(8)–O(3)	1.290 (10)
C(8)–O(6)	1.226 (9)		
O(1)–Cr–N(1)	83.5 (2)	O(1)–Cr–O(2)	94.1 (2)
N(1)–Cr–O(2)	90.8 (2)	O(1)–Cr–N(2)	95.1 (2)
N(1)–Cr–N(2)	173.2 (2)	O(2)–Cr–N(2)	82.7 (2)
O(1)–Cr–N(3)	174.5 (2)	N(1)–Cr–N(3)	97.2 (3)
O(2)–Cr–N(3)	91.3 (2)	N(2)–Cr–N(3)	84.8 (2)
O(1)–Cr–O(3)	91.6 (2)	N(1)–Cr–O(3)	94.3 (2)
O(2)–Cr–O(3)	172.8 (2)	N(2)–Cr–O(3)	92.4 (2)
N(3)–Cr–O(3)	83.0 (2)	Cr–O(1)–C(2)	116.9 (5)
C(2)–C(1)–N(1)	112.2 (7)	O(1)–C(2)–C(1)	117.2 (7)
O(1)–C(2)–O(4)	123.5 (7)	C(1)–C(2)–O(4)	119.3 (7)
Cr–N(1)–C(1)	109.1 (5)	Cr–O(2)–C(3)	117.9 (5)
O(2)–C(3)–O(5)	123.4 (7)	O(2)–C(3)–C(4)	115.6 (7)
O(5)–C(3)–C(4)	120.9 (7)	Cr–N(2)–C(5)	107.7 (4)
Cr–N(2)–C(4)	107.5 (5)	C(5)–N(2)–C(4)	112.8 (6)
N(2)–C(5)–C(6)	109.2 (7)	C(5)–C(6)–N(3)	109.8 (6)
Cr–N(3)–C(6)	106.8 (5)	Cr–N(3)–C(7)	108.0 (5)
C(6)–N(3)–C(7)	113.1 (6)	N(3)–C(7)–C(8)	111.6 (6)
C(7)–C(8)–O(3)	116.2 (6)	C(7)–C(8)–O(6)	118.3 (8)
O(3)–C(8)–O(6)	125.4 (7)	Cr–O(3)–C(8)	117.4 (4)
C(3)–C(4)–N(2)	112.2 (6)		

Table VI. ^2H NMR Data for Bidentate Amino Acid Complexes[a]

complex[b]	–R group	δ[c]
$[Cr(en)_2(gly)]^{2+}$	–H (D)	–61
$[Cr(en)_2(DL\text{-ala})]^{2+}$ [d]	$-CH_3$	–28, –31
$[Cr(en)_2(DL\text{-ser})]^{2+}$	$-CH_2OH$	–44
$[Cr(en)_2(DL\text{-thr})]^{2+}$	$-CH(CH_3)OH$	–41
$[Cr(en)_2(DL\text{-hsr})]^{2+}$	$-CH_2CH_2OH$	–28
$[Cr(en)_2(DL\text{-met})]^{2+}$	$-CH_2CH_2SCH_3$	–28
$[Cr(en)_2(DL\text{-leu})]^{2+}$ [d]	$-CH_2CH(CH_3)_2$	–26, –37
Λ-$[Cr(en)_2(L\text{-leu})]^{2+}$	$-CH_2CH(CH_3)_2$	–26
$[Cr(en)_2(DL\text{-phe})]^{2+}$	$-CH_2C_6H_5$	–34
$[Cr(1,3\text{-pn})_2(gly)]^{2+}$	–H (D)	–59
$[Cr(1,3\text{-pn})_2(DL\text{-ala})]^{2+}$	$-CH_3$	–28
α-*cis*-[Cr(edda)(gly)]	–H (D)	–41, –44

[a] Deuterium label on the 2-carbon of the amino acid. [b] Complexes are mixtures of Δ and Λ isomers unless otherwise indicated. [c] Shift relative to $CDCl_3$ assigned as +7.26 ppm. [d] Spectrum identical when L-amino acid used in synthesis.

(edda)(gly)] is shown in Figure 1. The atom positions are given in Table IV, and the bond lengths and bond angles are shown in Table V. The bond lengths and angles associated with the glycinate ligand are similar to those found in $[Cr(gly)_2OH]_2$[22] and *fac*-$[Cr(gly)_3]\cdot H_2O$.[23] The torsion angle of the glycinate (N(1), C(1), C(2), O(1)) is –8.5°.

The ^2H NMR spectra (Tables VI and VII) were obtained with a Nicolet NT-200WB spectrometer operating with a field of 4.7 T. Data were collected with a 12 000-Hz sweep width (8K block size) by use of a quadrature detection system. The spectrometer was operated with an acquisition time of 0.338 s, a pulse rate of 2.283 s^{-1}, and a flip angle of 30°. The spectra are reported relative to $CDCl_3$ at +7.26 ppm (by sample replacement). The samples (0.05–0.1 M) were usually prepared by exchanging at ice temperature (with QAE Sephadex, Cl^- form) the dithionate ions for chloride ions. Data were collected for 30 min to 1 h in order to obtain reasonable signal to noise ratios.

Results and Discussion

The Cr(III) complexes containing bidentate-coordinated glycine are distinct since glycine does not have a side chain. Due to the lack of symmetry in these complexes, two resonances might be expected from the glycinate deuterons. The ^2H NMR spectra of these complexes are shown in Figure 2. Only a single resonance, which is shifted to about –60 ppm, is observed in the spectra of the bis(diamine) complexes. However, the spectrum of the edda complex does show resonances at –41 and –44 ppm. The shift from about –60 ppm to about –40 ppm is attributed to the change from CrN_5O^{2+} to CrN_3O_3.

The two resonances observed for the edda complex (containing gly-d_2) were originally accepted as consistent with the β-cis-facial configuration reported for this complex.[7] However, the ^2H NMR spectrum of [Cr(edda-d_4)(gly)] shows peaks at –23, –32, and –72 ppm with integration ratios of 1:1:2, respectively. This spectrum is quite similar to the spectra of α-*cis*-$[Cr(edda\text{-}d_4)(H_2O)_2]^+$ and α-*cis*-$[Cr(edda\text{-}d_4)(mal)]^-$ and does not support a β-cis complex of edda.[9] The determination of the structure by single-crystal x-ray crystallography confirmed the α-cis configuration as well as the bidentate coordination of the glycine. On the basis of the assignment of the deuteron resonances for α-*cis*-[Cr(edda-d_4)(mal)]$^-$[9] the –72 ppm resonance is due to the acetate deuterons, D5 and D13, which point toward the ethylenediamine backbone

Table VII. ^2H NMR Data for Monodentate Amino Acid Complexes[a]

complex	δ[b]
cis-$[Cr(en)_2(Hgly\text{-}O)(OH)]^{2+}$	+37
cis-$[Cr(en)_2(Hala\text{-}O)(OH)]^{2+}$	+32
cis-$[Cr(en)_2(Hser\text{-}O)(OH)]^{2+}$	+30
cis-$[Cr(en)_2(Hthr\text{-}O)(OH)]^{2+}$	+27
cis-$[Cr(en)_2(Hhsr\text{-}O)(OH)]^{2+}$	+29
cis-$[Cr(en)_2(Hmet\text{-}O)(OH)]^{2+}$	+28
cis-$[Cr(en)_2(Hleu\text{-}O)(OH)]^{2+}$	+19
cis-$[Cr(en)_2(Hphe\text{-}O)(OH)]^{2+}$	+20
cis-$[Cr(1,3\text{-pn})_2(Hgly\text{-}O)(OH)]^{2+}$	+31
cis-$[Cr(1,3\text{-pn})_2(Hala\text{-}O)(OH)]^{2+}$	+25
α-*cis*-[Cr(edda)(Hgly-*O*)(OH)]	+32
$[Cr(NH_3)_5(Hgly\text{-}O)]^{3+}$	+36
$[Cr(NH_3)_5(Hala\text{-}O)]^{3+}$	+31

[a] Deuterium label on the 2-carbon of the amino acid. [b] Shift relative to $CDCl_3$ assigned as +7.26 ppm.

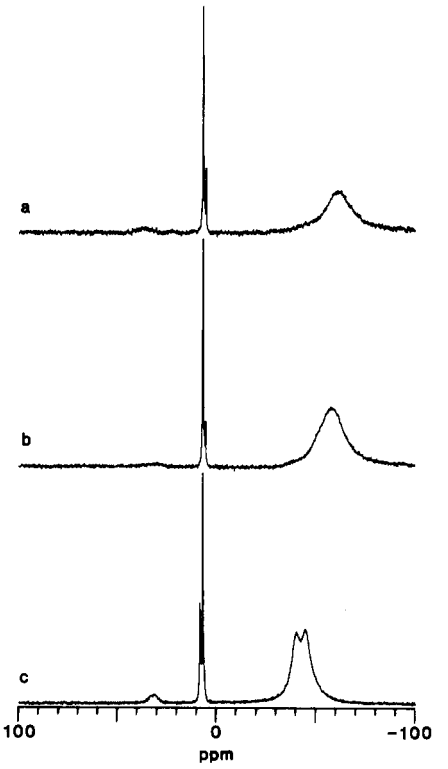

Figure 2. ^2H NMR spectra (obtained in H_2O at pH 6–7): (a) $[Cr(en)_2(gly\text{-}d_2)]^{2+}$; (b) $[Cr(1,3\text{-pn})_2(gly\text{-}d_2)]^{2+}$; (c) α-*cis*-[Cr(edda)(gly-d_2)].

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(Figure 1). The acetate deuterons D6 and D14 which point toward the glycinate ligand, are associated with the −23 and −32 ppm resonances. It is not yet possible to assign these resonances to specific deuterons.

The ^2H NMR results for the bidentate-coordinated amino acid complexes are shown in Table VI. The presence of a side chain on the amino acid is expected to restrict the deuteron on the 2-carbon to an axial position on the ring formed by the amino acid and the Cr(III) atom. For the bis(diamine) complexes, this steric restriction may be partially responsible for the separation between the resonances observed for the glycine-containing complexes, at about −60 ppm, and the range of resonances, from −26 to −44 ppm, observed for the complexes containing amino acids that have side chains. The NMR data for these complexes were generally obtained from mixtures of diastereomers, ΔΛ-$[Cr(diamine)_2$(DL-amino acid)$]^{2+}$. These isomers can be divided into two diastereomeric sets, and since there are two chemically distinct deuterons, two resonances might be expected from a mixture of isomers.

Although it is difficult to establish correlations on the basis of the data available, the complexes can be grouped into several categories. Both serine and threonine have a hydroxy group attached to the 3-carbon. The bis(en) complexes of these amino acids gave only a single resonance at −41 and at −44 ppm, respectively (Table VI).

The complexes containing bidentate-coordinated methionine and homoserine both gave ^2H NMR spectra having a single resonance at −28 ppm. Both amino acids have a linear side chain with a functional group attached to the 4-carbon.

The bidentate-coordinated phenylalanine complex, with a single resonance at −34 ppm, is not readily grouped with any of the other complexes. Current investigations with β-phenylserine may provide some insight into the effect of the phenyl group relative to the shift observed.

The bidentate complexes of alanine and leucine might be grouped together since they have aliphatic side chains. Both the bis(en) and bis(1,3-pn) complexes of alanine show ^2H NMR shifts of about −28 ppm. However, the spectra of the bis(en) complexes of alanine and leucine each show two resonances (Figure 3). As expected, the analogous complexes of L-alanine and L-leucine gave the same spectra. Thus, for these complexes, a distinct resonance is observed for each diastereomer. The larger separation between the resonances observed for the leucine isomers may be a result of the increased bulk of the side chain.

The assignment of these resonances to diastereomers was confirmed by resolving the L-amino acid complexes. The isomers were separated on a column of SP-Sephadex (with use of potassium antimonyl tartrate) as reported by Kaizaki and Ito.[4] Concentration of the eluant by lyophilization was successful only for the first band obtained from the resolution of the L-leucinate complex. The ^2H NMR spectrum of this isomer gave a single resonance at −26 ppm, which corresponds to one of the two resonances observed for the unresolved isomers (Table VI). This isomer also gave a positive CD rotation at 470 nm and, on the basis of the work by Kaizaki and Ito,[4] is assigned as Λ-$[Cr(en)_2$(L-leu)$]^{2+}$. Since the bidentate-coordinated species decompose via monodentate, carboxylate-coordinated species (vide infra), the accessibility of the amine group of the amino acid to the solvent may determine the rate of decomposition. When space-filling models of the isomers are compared, it is the Λ (L) isomer that has the more protected amine group.

The ^2H NMR spectrum of each bidentate amino acid complex showed an additional resonance (initially of low intensity) located between +19 and +37 ppm. The spectra of $[Cr(NH_3)_5$(Hgly-*O*)$]^{3+}$ and $[Cr(NH_3)_5$(Hala-*O*)$]^{3+}$, where the amino acid is coordinated through the carboxylate group, showed resonances at +36 and +31 ppm, respectively. These resonances are quite close to the additional resonances observed in the $[Cr(en)_2(gly)]^{2+}$ spectrum at +37 ppm and in the $[Cr(en)_2(ala)]^{2+}$ spectrum at +32 ppm. Therefore, the resonances located between +19 and +37 ppm are assigned to monodentate, carboxylate-bound amino acid complexes (Table VII).

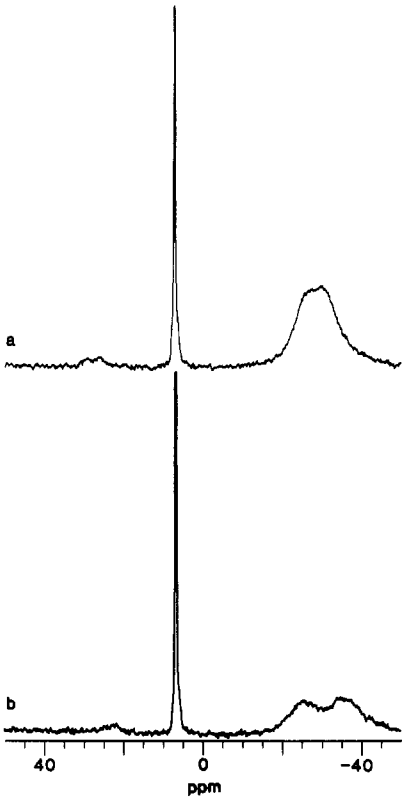

Figure 3. ^2H NMR spectra: (a) $[Cr(en)_2$(L-ala-*d*)$]^{2+}$ in 1 mM $HClO_4$ (pH changed during data collection from 3 to 6); (b) $[Cr(en)_2$(leu-*d*)$]^{2+}$ in 0.05 M phosphate buffer (pH 2.1).

The study of the aquation of $[Cr(ox)_2(gly)]^{2-}$ in acidic solution, reported by Kallen and Hamm, supports stepwise decomposition via a monodentate, carboxylate-bound amino acid complex.[5] Their study benefited from a rather long-lived intermediate that could be isolated. The bidentate amino acid complexes described in this paper decompose via carboxylate-bound amino acid complexes, which have not been isolated. The aquation of the $[Cr(diamine)_2(ala)]^{2+}$ complexes was followed by ^2H NMR spectroscopy. The $[Cr(en)_2(ala)]^{2+}$ complex was studied in acidic solution since this complex is not very stable at room temperature at pH 7. After 4 h at room temperature (in buffered solutions at pH 2.1 or 4.8), approximately 80% of the bidentate alanine complex remained, approximately 15% of the alanine was present as the monodentate, carboxylate-bound alanine complex, and about 5% of the alanine was free in solution (based on the free alanine resonance at 5–6 ppm). In contrast, the decomposition of $[Cr(1,3\text{-pn})_2(ala)]^{2+}$ could be followed in H_2O at pH 6–7. After 18 h at room temperature, approximately 70% of the bidentate alanine complex was still present, and almost all of the remaining alanine was free in solution. In a solution buffered at pH 2.1 (after 18 h), approximately 80% of the bidentate alanine complex was present, approximately 10% of the alanine was present as the monodentate species, and 10% was present as free alanine.

Summary

The range of isotropic shifts observed for the 2-carbon deuterium labeled amino acid complexes of Cr(III) shows that ^2H NMR is sensitive to small changes in environment. The decomposition of the bidentate amino acid complexes via monocoordinate species can be observed directly in the ^2H NMR spectra. The instability of these intermediates restricts the methods by which they can be characterized. The instability of many of the Cr(III)–amino acid complexes, as evidenced by the NMR studies, probably accounts for the difficulties in isolation and characterization that have been encountered in previous studies.

The isotropic shifts resulting from bidentate coordination of the amino acid are dependent on the steric effect of the side chain, which induces varying amounts of pucker into the ring formed by the amino acid and the Cr(III) center. The shifts are also

influenced by the number of type of substituent atoms in the side chain. However, these isotropic shifts, which result primarily from Fermi hyperfine contact interactions,[24] are not readily interpreted in terms of currently developed theory. Further work may determine the relative magnitudes of the steric and electronic contributions to the isotropic shifts.

Acknowledgment. We thank Don Appel for his assistance in using the Nicolet NT-200. We are also indebted to Dr. N. Koine (Ehime University) and to Dr. S. Kaizaki (Nara Women's University) for their assistance and encouragement during this project. The Nicolet 200-MHz spectrometer was acquired with the aid of the Boeing Co. The Nicolet X-ray diffractometer system was acquired with funds provided by the NSF (Grant CHE-8408407) and by the Boeing Co. Partial support of this study by the NIH (Grant GM 23081) and the USDA (Grant 82-CRCR-1-1005) is gratefully acknowledged.

Supplementary Material Available: A listing of anisotropic thermal parameters and a stereoview and packing diagram for α-*cis*-[Cr(edda)(gly)]·$2H_2O$ (2 pages); a listing of observed and calculated structure factors for the same compound (7 pages). Ordering information is given on any current masthead page.

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Structural Study of the Cu Sites in Metallothionein from *Neurospora crassa*

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The structure of the Cu centers in the low-molecular-weight metallothionein (MT) from *Neurospora crassa* has been investigated by X-ray absorption edge and EXAFS spectroscopy. Analysis of the EXAFS data by curve-fitting procedures yields results most consistent with an average Cu environment of 3–4 S atoms at 2.20 Å, and 1–2 Cu atoms at 2.71 Å. Comparison of the Cu–MT X-ray absorption edge with that of a structurally characterized Cu_4S_6 inorganic cluster reveals distinct similarities, suggesting significant congruence between the protein and the compact cluster structure found in the model. When they are taken together, the edge and the EXAFS data of Cu–MT are most consistent with three- or four-coordinate geometry around Cu and do not support the previously proposed two-coordinate linear-chain structure.

Introduction

Metallothioneins (MT) are low-molecular-weight, cysteine-rich proteins, binding high amounts of metal ions such as Zn, Cd, and/or Cu.[1] The mammalian proteins are characterized by a single polypeptide chain of 61 amino acids, with a total of 20 cysteine residues. On the basis of recent chemical, spectroscopic, and structural studies, mammalian MT's are thought to bind IIB (12)[23] metal ions in the form of two clusters with each ion tetrahedrally coordinated to four cysteinyl residues.[2-5] In contrast to the MT's of higher eucaryotic organisms that bind different metal ions, fungal MT's contain exclusively Cu.[6,7] *Neurospora* Cu–MT consists of only 25 amino acids and binds 6 Cu ions to 7 cysteinyl residues.[8] A recent spectroscopic study has indicated that the metal ions are coordinated in the form of a single Cu(I)–thiolate cluster.[9] To obtain further insight into the structure of this unique Cu(I) complex, an X-ray absorption study was undertaken.

In this paper, the edge, near-edge, and extended X-ray absorption fine structure (EXAFS) spectra of *Neurospora* Cu MT are presented and compared to those of a Cu_4S_6 cluster complex. The data are indicative of a compact and rigid cluster structure for *Neurospora* Cu MT.

Experimental Section

A solution of *Neurospora crassa* metallothionein (Cu–MT) was prepared in an inert-atmosphere glovebox by dissolving ~2 mg of a lyophilized powder of the protein[8] in 0.5 mL of degassed 60 mM phosphate buffer (pH 7.2). The resulting solution was ~10 mM in Cu. A 250-mL sample of the solution was loaded into an anaerobic, Mylar-lined cell and then frozen in dry ice.

Cu K-edge X-ray absorption spectra were collected at the Stanford Synchrotron Radiation Laboratory under dedicated ring conditions (3.5-GeV electron energy, 30–60-mA current) with use of a Si(220) two-crystal monochromator. Data were measured on the frozen solution, kept at −45 °C by a thermoelectric cooling module. EPR spectra taken before and after X-ray irradiation to monitor any sample degradation did not reveal any significant differences, each giving a Cu(II) signal consistent with ~0.7% Cu(II) impurity in the Cu MT solution.

The Cu K-edge absorption was monitored as the Kα excitation fluorescence signal detected by six NaI(Tl) scintillation detectors. Sixteen data scans, collected from 300 eV below to 600 eV above the absorption edge, were averaged to give the reported spectra. The spectra were energy-calibrated relative to the first inflection point (8980.3 eV) of a Cu-foil spectrum collected simultaneously.[10a] Established methods of data averaging, background removal, Fourier transformation, and curve fitting were used, which have been described previously in detail.[10] The edge spectrum presented has been normalized by fitting the smooth postedge region (9050–9650 eV) with a linear function and scaling the data to give a value of 1.0 for this smooth background absorption extrapolated to 9000 eV.

Results and Discussion

EXAFS Results. The EXAFS data are shown in Figure 1 and the Fourier transform of these data taken over the k range of 3.5–12.5 $Å^{-1}$ in Figure 2. The Fourier transform is characterized by a major peak at 1.75 Å and a peak on the high-R side of this main peak at 2.28 Å that is smaller in amplitude. Two peaks at higher R (3–4 Å) may also be above the noise level of the data. The peak positions in the Fourier transforms are shifted to lower R by an average of 0.35–0.4 Å from the true interatomic distance as a result of the pairwise atom phase shift. In order to obtain more accurate distances and coordination numbers, the EXAFS data were fit with use of S and Cu parameters obtained from

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