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EXAFS Studies of $[N(C_2H_5)_4]_2[M(SC_6H_5)_4]$ and $[N(C_2H_5)_4]_2[M_4(SC_6H_5)_{10}]$ (M = Zn, Cd): Model Compounds for Zn and Cd Metallothioneins

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Metallothioneins are metal-binding proteins found in a variety of mammals [1], invertebrates [2], birds, fish and microorganisms. These proteins are of low molecular weight (6500 daltons) and high cysteine content, containing 20 cysteines of a total of 61 amino acids [1]. One of the functions of this class of proteins seems to be heavy metal detoxification. They are commonly isolated with bound Zn, Cd or Cu [3–5]. Absorption spectroscopy [6–10], NMR [11–16] and X-ray photoelectron methods [17] have been used to examine the nature of the metal binding sites. For metals such as Zn, Cd, Hg and Co an MS_4 coordination sphere is indicated. Otvos *et al.* in a series of papers [12–16], have described ^{113}Cd NMR data that reveal the presence of metal–thiolate clusters in crab and rabbit Cd–metallothionein. The structures of these clusters were proposed to contain either three or four metal atoms bridged by thiolates. A recent presentation of preliminary crystallographic results [18] of rat liver (5 Cd, 2 Zn) metallothionein appears to confirm the presence of both three and four metal atom thiolate clusters. Distances of 3.6, 4.0, 4.3 and 5.0 Å were reported [18] for the Cd–Cd separations in the four metal atom, tetrahedral-like cluster.

In previous EXAFS studies of sheep [19], rabbit [20] and rat [21] metallothionein, Garner *et al.* have characterized the metal atom binding site as having an MS_4 coordination sphere. No evidence for metal atom clustering was prepared. Clearly, distinguishing between a single metal-thiolate center and a metal–thiolate cluster by EXAFS requires the observation of either multiple M–S bond distances (arising from the difference in the bridging and terminal M–S bond lengths) or the observation of metal....metal back scattering. The Zn and Cd complexes of the form $M(SC_6H_5)_4^{2-}$ and $M_4(SC_6H_5)_{10}^{2-}$ which have been structurally characterized [22–25], are suitable models to investigate the utility of EXAFS for distinguishing monometallic *versus* metal cluster environments in metallothioneins. We have studied the EXAFS of these compounds at both 300 and 77 K. The results of this work are described and the implications for EXAFS studies of metal clusters in metallothionein are discussed.

Experimental

The compounds $Zn(SC_6H_5)_4^{2-}$ (**1**), $Zn_4(SC_6H_5)_{10}^{2-}$ (**2**), $Cd(SC_6H_5)_4^{2-}$ (**3**) and $Cd_4(SC_6H_5)_{10}^{2-}$ (**4**) were prepared by known methods [22–25]. The Cd and Zn K-shell X-ray absorption spectra of **1**–**4** were recorded with the C_1 and C_2 stations at the Cornell High Energy Synchrotron Source (CHESS). Standard absorption detection techniques were employed on powdered samples consisting of enough material to provide one absorption length after being diluted with Li_2CO_3 and packed to a uniform density in a 5 X 25 mm slot cut in 0.5 mm plastic. The near-edge regions of the absorption spectra of the four compounds are shown in Fig. 1. The EXAFS signal was isolated from the full range of the recorded spectra (1000 eV from the edge) by subtraction of a four section cubic spline fitted to the data. The origin of the wavenumber (k) scale was selected as the mid-point of the edge jump. The k^3-weighted EXAFS of **1** and **2** recorded at 77 and 300 K as well as the magnitudes of the Fourier transforms of these spectra are shown in Fig. 2. The corresponding 77 and 300 K EXAFS and transforms of the Cd compounds **3** and **4** are shown in Fig. 3.

Structural information was extracted from the EXAFS spectra by the Fourier filtering method. The signal from each shell was isolated by a Hanning filter, reverse transformed and separated into phase and amplitude components. The mean radial distance was obtained from the slope of the derived phase

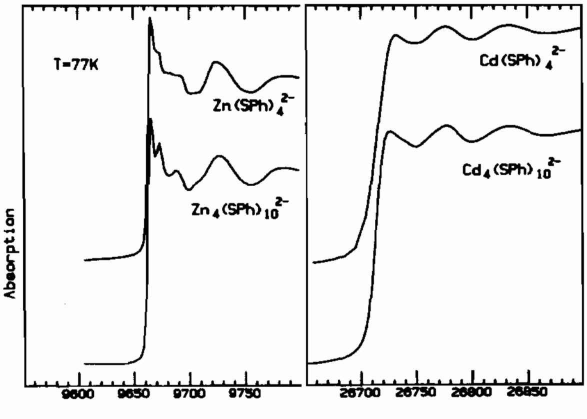

Fig. 1. The near edge regions of the X-ray absorption spectra of **1** and **2** (Zn K); **3** and **4** (Cd K).

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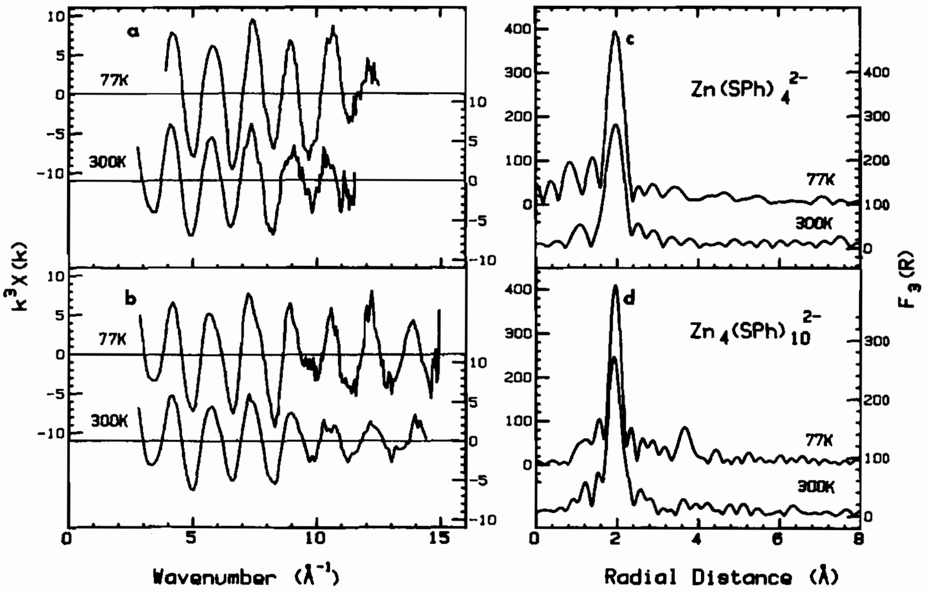

Fig. 2. The k^3-weighted Zn K-shell EXAFS of 1 (a), 2 (b) recorded at both 77 and 300 K. The magnitudes of the Fourier transforms of these spectra are shown in (c) and (d).

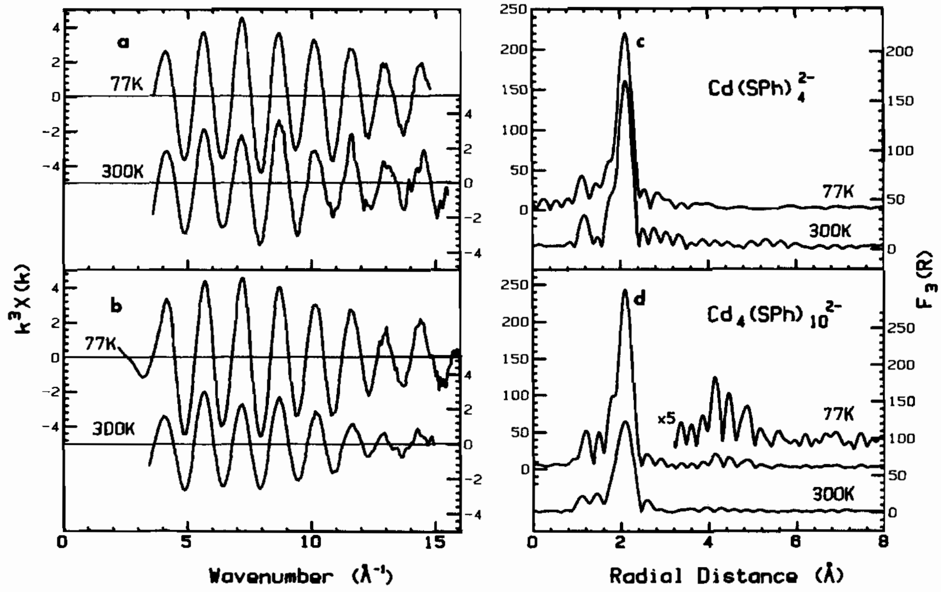

Fig. 3. The k^3-weighted Cd K-shell EXAFS of 3 (a) and 4 (b) recorded at both 77 and 300 K. The magnitudes of the Fourier transforms of these spectra are shown in (c) and (d).

function using both calculated [26] and model compound phase shifts (1 for 2, 3 for 4, and $CdCl_2 \cdot 2.5H_2O$ for the Cd–S distances). Coordination numbers (N) and relative Debye-Waller factors ($\Delta\sigma^2$) were derived from the isolated amplitude functions using experimental models (77 K data for 300 K, monomer for tetramer) according to standard procedures [27]. $\Delta\sigma^2$ is a measure of the difference in mean square deviations in radial distances of the model and unknown. These variations arise because of both thermal motion and disorder. For the tetrameric species 2 and 4, where the first shell of EXAFS signal contains contributions from both terminal and bridging metal–sulfur distances, nonlinear least-squares curve fitting was used to fit the Fourier filtered first shell signal to the sum of two components. Further details of the data acquisition and analysis procedures have been presented elsewhere [28]. The R, N and $\Delta\sigma^2$ parameters derived from our analysis are summarized in Table I.

TABLE I. Radial Distances, Debye–Waller Factors and Coordination Numbers Derived from Zn and Cd K-shell EXAFS of 1–4

Compound	EXAFS						X-ray diffraction				
	T	R (Å)	Phase model[a]	$\Delta\sigma^2$ (Å^2)	N	Amplitude model[b]	R_t[c]	N_t	R_b	N_b	Reference
(a) Zn–S Distances											
1	77, 300[d]	2.34(1)	CuS–calc[e]	0.0055(4)	5.0(6)	1 (77 K)	2.353(3)	4			20
2	77, 300	2.33(1)[f]	CuS–calc[e]	0.0026(2)	3.9(2)	2 (77 K)	2.29(1)	1	2.37(1)	3	21
	77	2.35(1)	1 (77 K)	0.0025(4)	3.4(2)	1 (77 K)					
	300	2.34(1)	1 (300 K)	0.0022(4)	3.0(6)	1 (300 K)					
(b) Cd–S distances											
3	77, 300[d]	2.53(2)	$CdCl_2 \cdot 2.5H_2O$[g]	0.0030(2)	3.6(3)	3 (77 K)	2.535(3)	4			20
4	77, 300	2.54(2)[f]	$CdCl_2 \cdot 2.5H_2O$[g]	0.0023(1)	3.0(3)	4 (77 K)	2.467(9)	1	2.56(2)	3	19
	77	2.53(1)	3 (77 K)	0.0023(4)	3.8(3)	3 (77 K)					
	300	2.53(1)	3 (300 K)	0.0031(3)	4.7(4)	3 (300 K)					

Compound	Terminal			Bridging			
	R_t (Å)	N_t	$\Delta\sigma^2$ (Å^2)	R_b (Å)	N_b	$\Delta\sigma^2$ (Å^2)	Model
(c) Fits to two M–S distances in 2 and 4							
2	2.32	0.9	0.0006	2.37	2.6	0.0005	1 (77 K)
4	2.49	0.5	−0.0008	2.54	2.5	0.0011	3 (77 K)

Compound	EXAFS					X-ray diffraction		
	R (Å)	Phase model[a]	$\Delta\sigma^2$ (Å^2)	N	Amplitude model[b]	R	N	Reference
(d) Metal–metal distances (77 K data)								
2	4.00(3)	Cu–calc[e]				4.0	3	21
	4.03(2)	Zn (77 K)[g]	0.0033(7)	3.6(3)	Zn metal			
4	4.32(1)	Cd–calc[e]						
	4.34(2)	Cd (77 K)[g]	0.0062(25)	3(2)	Cd metal	4.3	3	19
	4.34(2)	CdO (77 K)[g]	0.0050(19)	1(1)	CdO			

[a] Source of phase function used to derive distance. The unit charge of Z between Cu and Zn or Cl and S is expected to have little effect on the phase function. When the monomers 1 and 3 phase was used to model the tetramers 2 and 4, the distances from X-ray diffraction were used. [b] Source of model amplitude function used to derive N and $\Delta\sigma^2$. [c] t = terminal, b = bridging. [d] The values derived from data at both temperatures were the same within the quoted errors. [e] From *ab initio* calculations [26]. [f] This should be compared to the coordination number-weighted average of the R_t and R_b values which are 2.35 Å for 2 and 2.54 Å for 4. [g] Structure of model compounds is known from X-ray diffraction [30].

Results and Discussion

The X-ray absorption features at the Cd K-edge are considerably broader than those at the Zn K-edge because of both increased instrumental broadening and the shorter lifetime of the Cd K-hole [29] (Fig. 1). Although essentially the same sets of features are observed for the monomeric and tetrameric species (**1** and **2**; **3** and **4**), the spectra of the tetramers **2** and **4** show somewhat improved definition.

The metal–sulfur distances derived from the first shell EXAFS of the monometallic species **1** and **3**, are all in good agreement with the values measured by single crystal X-ray diffraction methods [21, 22]. The EXAFS results show no significant difference in the M–S distances of the corresponding monomeric and tetrameric complexes. The average M–S distances derived from **2** and **4** are in agreement with the coordination number weighted average of the bridging and terminal M–S distances reported by single crystal diffraction [23, 25]. However, the Debye–Waller factors for signals from the tetrameric species treated in this fashion are significantly larger than those for the corresponding monomeric compounds (at the same temperature). The $\Delta\sigma^2$ values indicate that the single shell analysis treats the signals arising from the M–S bonds in the tetrameric species as ones containing 0.05 Å additional disorder relative to the signal observed for the monomeric species. These

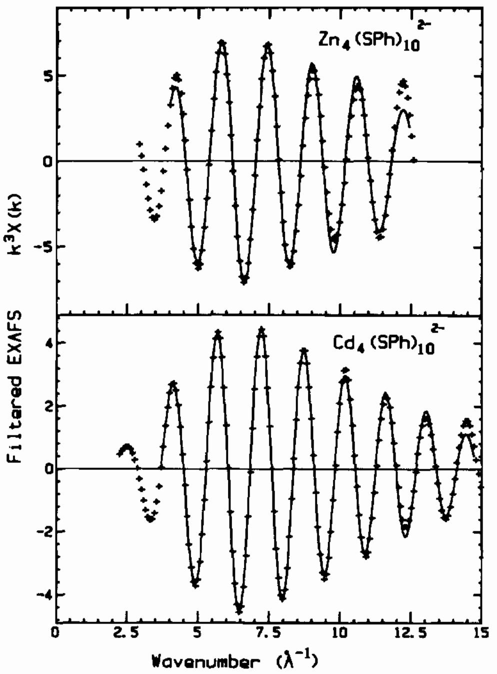

Fig. 4. The Fourier-filtered metal–sulfur signal from the 77 K EXAFS of the tetrameric species 2 and 4 (crosses) compared to a least-squares of the EXAFS from two metal distances (solid line) using the EXAFS data of the monomeric species 1 and 3 as models.

results are expected as crystallographic data show that the average bridging and terminal M–S distances differ by 0.09 Å. When the first shell signals from 2 and 4 are fitted to two M–S distances the derived distances and coordination numbers are in good agreement with the crystallographic results. In addition, the Debye–Waller factors are reduced from the single shell analysis, as expected. The calculated fitted functions are compared to the 77 K Fourier filtered M–S EXAFS for 2 and 4 in Fig. 4.

Beyond the primary coordination shell, the results show that a metal–metal distance can be observed in the 77 K EXAFS of the tetrameric species 2 and 4 (Figs. 2, 3). For the Zn compound 2, the Zn backscattering signal is clearly observed at 4.0 Å. This agrees well with the Zn–Zn distance observed in the crystallographic study [24]. In the Cd K-EXAFS of 4, the Cd backscattering signal is very weak, but the derived distance, the amplitude shape and the absence of signal in the same location in the spectrum of 3 are positive evidence that the signal observed in the transform spectrum of 4 does correspond to the Cd–Cd distance.

The results presented here demonstrate that although monometallic and thiolate cluster compounds yield similar first shell M–S EXAFS spectra, these species can be distinguished on the basis of metal–metal backscattering. Detection of a metal–metal distance in the EXAFS of a metallothionein would provide direct evidence in support of the M_4-cluster-type binding site proposed on the basis of ^{113}Cd NMR data [12, 15]. The results presented here indicate that if the thiolate clusters are good models for the metal binding site in metallothionein then a low temperature EXAFS study of fully-loaded Zn–thionein would offer the best chance for observing a metal–metal EXAFS signal. This is especially true in light of the large range of Cd–Cd distances reported by Stout *et al.* [18]. It is interesting to note that a signal was observed at 3.6 Å in the EXAFS studies by Garner *et al.* [20] of rabbit liver metallothionein. However, no comment regarding this signal was made. Clearly, this signal suggests the possibility of Zn....Zn backscattering. Our own efforts to observe and verify the presence of Zn backscattering in the EXAFS of Zn–thionein and thus clarify the nature of the Zn–thionein binding site are in progress and will be reported in due course.

Acknowledgements

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