Evidence for Discrete $Cd(SCys)_4$ Units in Cadmium Phytochelatin Complexes from EXAFS Spectroscopy

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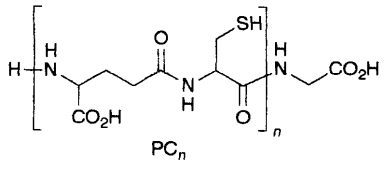
The principal structural features of multinuclear complexes of cadmium(II) with the plant-peptide phytochelatin have been deduced from Cd-EXAFS (EXAFS = extended X-ray absorption fine structure) data: (*i*) $Cd(SCys)_4$ coordination, (*ii*) in contrast to Cd-metallothioneins, no formation of cadmium 'clusters' and (*iii*) non-coordinating carboxylate groups.

Organisms synthesize special molecules to chelate heavy-metal ions like cadmium(II). The most widespread of these chelators are the 'metallothioneins' of animals[1] and the 'phytochelatins' of plants.[2] The metallothioneins contain cysteinyl–sulphur coordinated metal ions arranged in multinuclear centres ('clusters'). They are relatively well characterized. In contrast, there is a lack of structural information on phytochelatin complexes.

Phytochelatins are γ-glutamyl-cysteinyl peptides of the general formula PC_n(n = 2–11). They form cadmium(II) complexes whose molecular masses have been determined as 2500 and 3600.[2a,3] These compounds can not yet be crystallized, and their NMR spectra are extremely complex. Therefore, we carried out Cd-EXAFS measurements (EXAFS = extended X-ray absorption fine structure) to establish their principal structure.†

Our results show that the immediate environment of the metal in the native Cd–PC_n complexes (samples **1** and **2**) consists of four sulphur atoms. From the analytical data it is clear that sulphide is of no significance as a ligand. Thus, $Cd(SCys)_4$ centres must be present. Indeed, the observed Cd–S bond length of 2.52 ± 0.02 Å is typical of $[Cd(SR)_4]^{2-}$ thiolato complexes (see Fig. 1). The EXAFS data exclude the participation of lighter atoms, especially of carboxylate groups, in the coordination of cadmium (see Fig. 2). Clusters containing bridging cysteinyl sulphur atoms can also be excluded. For this type of cluster, Cd · · · Cd distances in the range 3.4–4.5 Å are expected. They are observed in the Fourier transformed spectra of model complexes and in simulations, but not for **1** and **2**. Furthermore, in clusters



PC_n

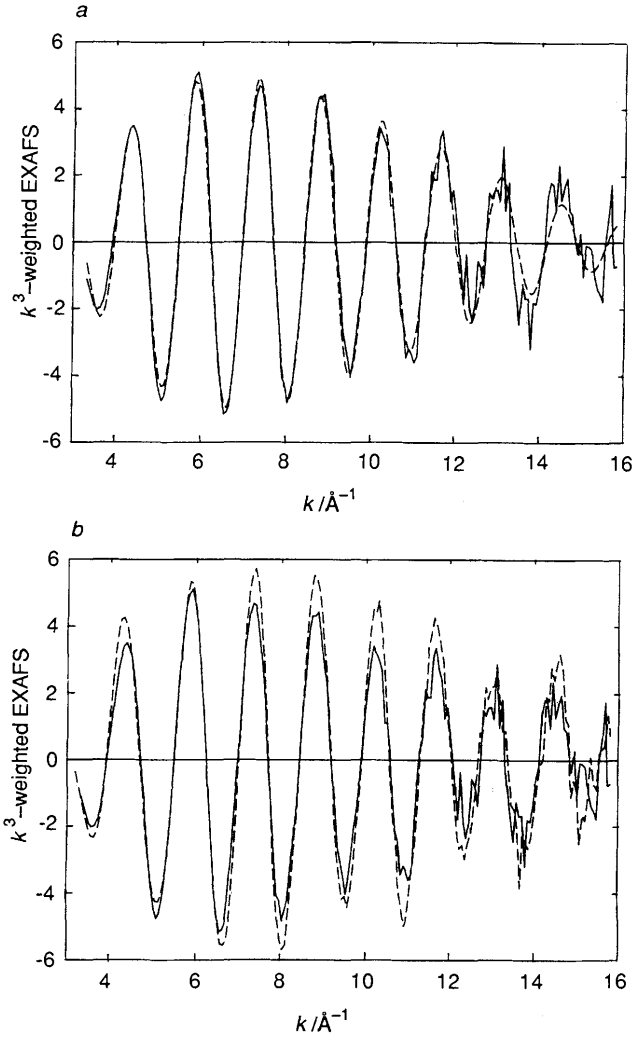


Fig. 1 (*a*) Experimental k^3-weighted EXAFS for **2** (solid line) compared with calculated data for the same sample and (*b*) with experimental data for $(Et_4N)_2[Cd(SCH_2CH_2S)_2]$. k is the wave vector of the photoelectron.

† X-Ray absorption data (transmission, 20 K) were recorded on the EXAFS spectrometer of the European Molecular Biology Laboratory at HASYLAB (DESY, Hamburg). Typically 5–10 spectra of each sample were averaged after individual energy calibration.[4] A four-segmented cubic spline routine was used for background subtraction. No Fourier filtering was applied to the data. Structural parameters were derived from a curved-wave single-scattering analysis based on theoretical amplitude and phase functions.[5]

For **1**: native Cd–PC_n complexes isolated from cadmium-treated cell suspension cultures of *Rauvolfia serpentina*;[6] composition (μmol): Cd 89.4, PC_2 1.7, PC_3 71.0, PC_4 22.9, PC_5 3.9, PC_6 1.1, glutathione 3.9, S^{2-} 1.0; molar ratio Cd:SCys = 1:3.8; lyophilized. **2**: native Cd–PC_n complexes as above; aqueous solution (pH 7.6). **3**: solid prepared from **2** by addition of $CdSO_4$; composition (μmol): Cd 91.7, PC_2 0.6, PC_3 18.7, PC_4 6.8, PC_5 1.0, PC_6 0.4, glutathione 0.7; Cd:SCys = 1:1.0. **4**: aqueous solution (*ca.* pH 4) prepared from $CdSO_4$ and PC_3; Cd:SCys = 1:2.5.

EXAFS results (N: number of backscatterers, $2\sigma^2$: Debye–Waller-type factor): **1**: N(S) = 4.2, Cd–S 2.52 Å, $2\sigma^2$ = 0.009 Å^2. **2**: N(S) = 4.0, Cd–S 2.52 Å, $2\sigma^2$ = 0.007 Å^2. **3**: N(S) = 1.0 (fixed), Cd–S 2.54 Å, $2\sigma^2$ = 0.001 Å^2; N(O) = 3.7, Cd–O 2.29 Å, $2\sigma^2$ = 0.013 Å^2. **4**: N(O) = 6.8, Cd–O 2.27 Å, $2\sigma^2$ = 0.012 Å^2. The estimated accuracy is ±10% for coordination numbers and ±0.02 Å for bond distances. A residual index was defined as $\Sigma[k^3\chi(k)_{exp} - k^3\chi(k)_{fit}]^2/\Sigma[k^3\chi(k)_{exp}]^2$, and the following values were obtained: **1**: 0.048, **2**: 0.047, **3**: 0.044 and **4**: 0.077.

In addition to the results presented in this communication, we have measured EXAFS spectra of two cadmium glutathione samples and of model complexes of known molecular structures. The tripeptide glutathione is the parent compound of the phytochelatins (n = 1).

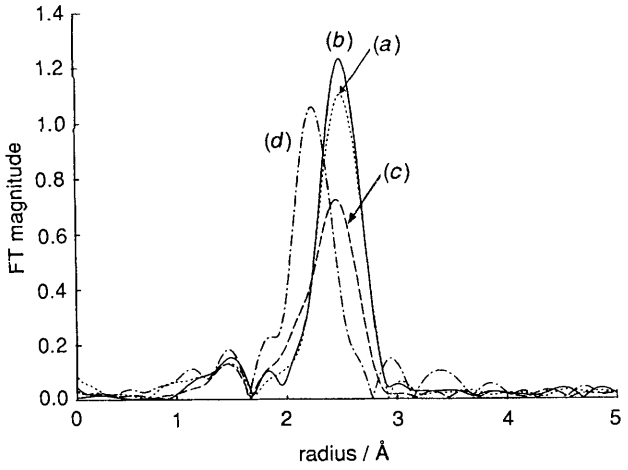


Fig. 2 Fourier-transforms of the k^3-weighted EXAFS for (*a*) **1**, (*b*) **2**, (*c*) **3** and (*d*) **4**. The data are phase corrected for sulphur **1–3** and oxygen **4**, respectively.

terminal and bridging Cd–S bonds differ by *ca.* 0.10 Å. The observed Debye–Waller factor is much too small for a shell of backscatterers split to such an extent.

Increasing the Cd^{2+} concentration in a solution of native Cd–PC_n complexes resulted in the precipitate **3**. The average cadmium environment in **3** is a mixed one and consists of one S atom and three to four O,N atoms. Compared with that, solution **4** obtained from Cd^{2+} and PC_3 at a low pH value contains all the cadmium ions in a 'light-atom' environment. The EXAFS parameters of **4** are those expected for *e.g.* CdO_6 coordination units. Obviously, at *ca.* pH 4 the phytochelatin molecules are no longer capable of coordinating through their sulphur atoms. From Fig. 2 one can clearly recognize the close similarity between **1** and **2** as well as the different cadmium coordinations in **3** and **4**.

Based on the EXAFS results, and consistent with the molecular sizes (see above), the analytical data and the extremely hydrophilic character of the Cd–PC_n complexes,[2a] we propose structural models as the one shown for $[Cd_3(PC_3)_4]$ in Fig. 3.‡ The Cd–PC_n molecules contain

‡ The maximum possible charge of this complex is −22 resulting from 16 $-CO_2^-$, 12 $CysS^-$ and 3 Cd^{2+}. Its molecular mass is 3400.

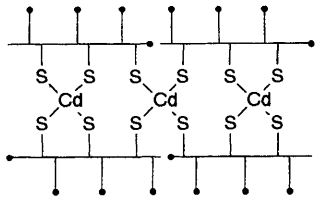


Fig. 3 Structural model of $[Cd_3(PC_3)_4]$. Carboxylate groups are shown as filled circles. Analogous structures are possible for other Cd–PC_n complexes, *e.g.* $[Cd_2(PC_2)_4]$ and $[Cd_3(PC_4)_3]$.

discrete $Cd(SCys)_4$ units. The carboxylate groups are non-coordinating; their location at the surface leads to a high negative surface charge. Within the framework of this model, several isomers and higher oligomers (M_r observed up to 10000[2a,3]) are possible. Cd-EXAFS measurements on plant cells should show whether this concept is also applicable to phytochelatin complexes *in vivo*.

This study was supported by the Deutsche Forschungsgemeinschaft and the Körber-Stiftung, Hamburg. H. S. thanks the Stiftung Stipendien-Fonds des Verbandes der Chemischen Industrie for the award of a Liebig fellowship. Helpful discussions with Professor S. Pohl are gratefully acknowledged.

Received, 21st March 1991; Com. 1/01369F

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