Lateral patterning: The SiH substrate was covered with a mask, the aldehyde was added, and melted. For monolayer formation and cleaning see above.

Visualization of structures: The substrate with the monolayer was wetted under a microscope (Carl Zeiss, 20-fold enlargement) with a few drops of a mixture of 2-propanol and universal oil (Lubricant Consult GmbH). The pattern formed after a few seconds was photographed.

XPS measurements: XPS measurements were made with a Leybold MAX-200 spectrometer with $Mg_{K\alpha}$ radiation (300 W) under a pressure of 10^{-9} mbar. The superimposed signals were separated and assigned by calculations with the manufacturer's software (DS 100). The signals observed were O 1s (532.1 BeV), C 1s C–C (284.6 BeV), C 1s C–O (286.3 BeV), Si–O $2p_{3/2}$ (100.9 BeV), Si–O $2p_{1/2}$ (101.6 BeV).

Received: April 3, 1998
Revised version 12. June 1998 [Z11679]
German version: *Angew. Chem.* **1998**, *110*, 2651–2654

Keywords: monolayers · photochemistry · self-assembly · silicon

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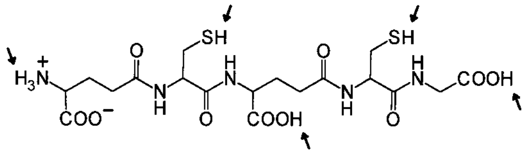
A Coordination-Chemical Basis for the Biological Function of the Phytochelatins[**]

Jens Johanning and Henry Strasdeit[*]

Dedicated to Professor Bernt Krebs
on the occasion of his 60th birthday

Organisms respond to heavy-metal stress, caused by toxic metals, with different detoxification strategies.[1] Thus plants synthesize short peptides of the composition $(\gamma\text{-Glu-Cys})_n$-Gly ($n = 2-11$), the phytochelatins ($PC_n$),[2] which coordinate to metal ions and thereby decrease the toxicity of these ions.[3] The best characterized phytochelatin complexes are those of cadmium.[4] However, the bioinorganic chemist also has only a rough picture even in this case because of the heterogenic character of the native complexes. Furthermore there is a lack of data on the thermodynamic stabilities, which are essential for a better understanding of the biological function.

We have therefore examined the system Cd^{II}/PC_2 by potentiometric titration[5] under exactly defined conditions (see Experimental Section) to gain information about the complexes occurring there. PC_2, the simplest phytochelatin, is a pentabasic acid (H_5L). Starting from the fully protonated form HPC_2^+ ($= H_6L^+$), the protonation constants $pK_1 = 2.39(5)$, $pK_2 = 3.18(3)$, $pK_3 = 4.01(7)$, $pK_4 = 8.75(9)$, $pK_5 = 9.03(3)$, and $pK_6 = 10.04(12)$ were obtained for the free ligand.[6] The complex species that were detected are listed in Table 1. The lgβ values given therein include the protonation equilibria of the ligands [Eq. (1)]. They are those quantities that have been directly determined in the refinements. In coordination-chemical considerations, however, lg$K$ values calculated from them are more useful. They allow equilibria such as that given in Equation (2) to be described quantitatively.



$PC_2 = H_5L$

$$2\,Cd^{2+} + 4\,H^+ + 3\,L^{5-} \rightleftarrows [Cd_2(H_2L)(HL)_2]^{7-} \quad (1)$$

$$2\,Cd^{2+} + H_2L^{3-} + 2\,HL^{4-} \rightleftarrows [Cd_2(H_2L)(HL)_2]^{7-} \quad (2)$$

The species models were developed and assessed on the basis of general criteria.[5] In addition some special guidelines were available:

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[**] This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We thank Prof. M. H. Zenk for donations of phytochelatin trifluoroacetate.

a) The pronounced tendency of Cd^{II} to bind thiolates under formation of an S_4 environment. A deficiency of thiolate results in multinuclear complexes such as $[Cd_2(SR)_6]^{2-}$ and $[Cd_4(SR)_{10}]^{2-}$, which contain doubly bridging ligands.[7]

b) Cd-EXAFS results that prove an S_4 coordination for isolated Cd^{II}/PC_n complexes as well as for Cd^{II} in plant cells exposed to cadmium.[4a, b] In some cases there are indications of multinuclear complexes.[4b] The EXAFS data also indicate an O_6 environment of cadmium in moderately acidic solutions.

c) Analyses with a cadmium-selective electrode carried out parallel to the potentiometric titrations. In this way the predictions that species models made about the pH-dependent concentration distribution of the free Cd^{II} could be experimentally checked.

In the Cd^{II}/PC_2 system relatively stable complexes are only formed by ligands in which at least one of the sulfhydryl functions is deprotonated. Two categories can be distinguished (Table 1): complexes that contain the same number of ligands and Cd^{II} ions ($\lg K^{Cd} = 9.9-13.3$) and those having more ligands than metal ions ($\lg K^{Cd} = 16.3-19.3$). The consequences resulting from that under biological pH and concentration conditions can be inferred from the species distribution diagram shown in Figure 1. The cysteinyl:Cd^{II} ratio of 3:1 given occurs in native complexes of short-chain phytochelatins ($n = 2-4$);[4c] the total concentration of 50 μM cadmium is of the same order of magnitude as that found in highly burdened plant cells. At pH 7.3, a typical value in the cytoplasm of higher plants,[8] $[Cd_2(H_2L)(HL)_2]^{7-}$ ($L_3Cd_2H_4$) is the predominant complex. It should have mainly a sulfur coordination, but perhaps not exclusively, because in H_2L^{3-} one of the two SH groups is not yet deprotonated. Thus, on the one hand a $\{S_2Cd(\mu\text{-}S)_2CdS(O)_x\}$ structural fragment is conceivable that does not contradict the EXAFS results (see above), which are unable to exclude a small contribution of oxygen to the coordination. On the other hand, a $\{S_2Cd(\mu\text{-}S)_2CdS(SH)\}$ center with thiolate and thiol coordination may be present. The coordination of thiols to Cd^{II} is rarely observed; however the complex $[Cd_3(SR)_6(HSR)]$ ($R = 2,4,6\text{-}iPr_3C_6H_2$), for example, has been reported, which has a mixed thiol–thiolate ligand sphere.[9]

Table 1. Complex species in the system Cd^{II}/PC_2.[a]

Complex formula	Composition	$\lg\beta$	$\lg K^{Cd}$	$PC_2:Cd^{II}$ 1:1	1.5:1	2:1
$[Cd(H_6L)]^{3+}$	($LCdH_6$)	40.3	2.9	+	+	+
$[Cd(H_5L)]^{2+}$	($LCdH_5$)	37.4	2.4	+	+	+
$[Cd_3(H_2L)_3]^{3-}$	($L_3Cd_3H_6$)	87.0	9.9	+	+	+
$[Cd_3(H_2L)_2(HL)]^{4-}$	($L_3Cd_3H_5$)	82.4	11.4	+	+	
$[Cd_3(H_2L)(HL)_2]^{5-}$	($L_3Cd_3H_4$)	77.4	12.8	+	+	
$[Cd_2(HL)_2]^{4-}$	($L_2Cd_2H_2$)	45.6	12.8	+		
$[Cd_2(HL)L]^{5-}$	(L_2Cd_2H)	36.7	13.3	+		
$[CdL]^{3-}$	(LCd)	11.7	11.7	+		
$[Cd_2(H_2L)(HL)_2]^{7-}$	($L_3Cd_2H_4$)	71.8	16.3		+	
$[Cd_2(HL)_3]^{8-}$	($L_3Cd_2H_3$)	63.1	16.5		+	
$[Cd_2(HL)_2L]^{9-}$	($L_3Cd_2H_2$)	53.7	16.8		+	
$[Cd_2(HL)L_2]^{10-}$	(L_3Cd_2H)	44.1	17.0		+	
$[Cd_2L_3]^{11-}$	(L_3Cd_2)	33.0	16.5		+	
$[Cd_2(H_2L)_4]^{8-}$	($L_4Cd_2H_8$)	108.8	16.3			+
$[Cd_2(H_2L)_3(HL)]^{9-}$	($L_4Cd_2H_7$)	102.4	17.6			+
$[Cd_2(H_2L)_2(HL)_2]^{10-}$	($L_4Cd_2H_6$)	95.5	18.6			+
$[Cd_2(H_2L)(HL)_3]^{11-}$	($L_4Cd_2H_5$)	87.7	19.3			+
$[Cd(HL)_2]^{6-}$	(L_2CdH_2)	37.7	17.6			+
$[Cd(HL)L]^{7-}$	(L_2CdH)	28.6	18.5			+
$[CdL_2]^{8-}$	(L_2Cd)	18.0	18.0			+

[a] $\lg K^{Cd}$ gives the stability per Cd^{II} ion of a complex of nuclearity m: $\lg K^{Cd} = 1/m \lg K$. The mean estimated standard deviation of the $\lg\beta$ and the $\lg K^{Cd}$ values is ±0.2 each. The last three columns state which complexes occur at the respective overall composition $PC_2:Cd^{II}$ of the system.

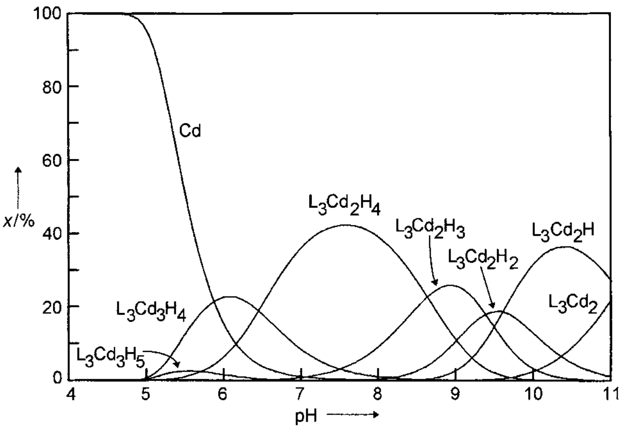

Figure 1. pH-Dependent distribution of the complex species for an overall composition of $PC_2:Cd^{II} = 1.5:1$ and a total concentration of cadmium of 50 μM (corresponding to 100%).

At pH 7.3 0.5% of the cadmium is still uncomplexed. Even though one can expect slightly higher stabilities for the other phytochelatins ($n \geq 3$),[10] this value nevertheless shows that the Cd^{II}/PC_n complexes are by no means extremely stable. Consequently, they are not the long-term storage form of cadmium in plant cells, but they are transported from the cytosol into the vacuole, where the Cd^{II} remains in a binding state that has not been finally clarified yet.[11]

Our stability data prove that Cd^{II}/PC_n complexes dissociate considerably to entirely in the acidic environment of the vacuole. In the system Cd^{II}/PC_2 (Figure 1), 62% of the cadmium is liberated at pH 5.4, a typical value in the vacuoles of higher plants.[8] At the same pH value, this amount rises to 100%, when the total concentration of cadmium is only 5 μM instead of 50 μM. This dissociation allows the plant cell to recover the phytochelatins and should therefore be of substantial biological importance.[12] The coordination-chemical basis for that lies in the absence of complexes with the potential carboxylate ligands H_3L^{2-} and H_4L^- as well as in the low stability of complexes with equimolar ligand and metal portions (see above). Thus, in the 1.5:1($PC_2:Cd^{II}$) system only the complex $[Cd_3(H_2L)(HL)_2]^{5-}$ ($L_3Cd_3H_4$) has a significant influence in the weakly acidic region. Its $\lg K^{Cd}$ value of 12.8, however, is far lower than that of $[Cd_2(H_2L)(HL)_2]^{7-}$ ($\lg K^{Cd} = 16.3$). A $Cd_3(\mu\text{-}S)_3$ six-membered ring, which also occurs in the β-"cluster" of the metallothioneins,[1] appears plausible as the central moiety of the trinuclear complexes $L_3Cd_3H_p$ ($p = 4-6$).

The results presented herein account for fundamental observations in connection with the biological function of the phytochelatins. Moreover, they can be useful for possible applications of these peptides, for example in heavy-metal biosensors.

Experimental Section

Measuring equipment: High-performance pH meter pMX 2000 (WTW, reading accuracy: 0.001 pH units) with temperature sensor, proportioning system ACCUSTEP (Tri-Continent Scientific), single-rod glass electrode 2GE1-DOKA (Jumo) with double-compartment system (salt bridge), cadmium-selective electrode Cd500 (WTW), titration cell with jacket for thermostatting. Chemicals: Analytically pure $HPC_2^+CF_3COO^-$ (C/H/F/N/S), $Cd(NO_3)_2 \times 4H_2O$ p. a., 0.1M aqueous solution of KOH (Aldrich, volumetric standard), 0.1M nitric acid (Aldrich, volumetric standard), KNO_3 (Fluka, MicroSelect, >99.5%). Measuring conditions: Aqueous solution, $T = 25.00 \pm 0.02\,^{\circ}C$, ionic strength $I = 1.00$M kept constant with KNO_3 as supporting electrolyte during the entire titration, humidified N_2 protective atmosphere. The pK_w value was determined to be 13.76. Calibration of pH meter and electrode system by strong acid/strong base titration.[13] Test system: Ni^{II}/glycine.[14] Titration example: $Cd(NO_3)_2$ (67.1 μmol) and $HPC_2^+CF_3COO^-$ (65.8 mg, 100.7 μmol) in supporting electrolyte solution (50.0 mL), adjusted to pH 2.5 with a known amount of nitric acid, titration with carbonate-free 0.1016M KOH solution (adjusted to $I = 1.00$M) up to pH 10.5. pK_a values: five titrations with a total of 336 experimental points ($\sigma_{fit} = 0.0039-0.0086$). Complex formation constants: seven titrations with different PC_2:Cd^{II} ratios and a total of 677 experimental points ($\sigma_{fit} = 0.0021-0.0086$). Program used: BEST.[5]

Received: April 7, 1998 [Z11694IE]
German version: *Angew. Chem.* **1998**, *110*, 2642–2644

Keywords: bioinorganic chemistry · cadmium · complex stabilities · phytochelatins · potentiometry

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[12] It has still to be studied in detail to which degree processes play a role that are coupled to the dissociation equilibrium, such as the complex formation of Cd^{II} with ligands of the vacuole.

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Colloid-Bound Catalysts for Ring-Opening Metathesis Polymerization: A Combination of Homogenous and Heterogeneous Properties[**]

Marcus Bartz, Jörg Küther, Ram Seshadri, and Wolfgang Tremel[*]

The advantages and disadvantages of molecular or homogenous versus surface-bound or heterogeneous catalysts are well known. The properties of the two kinds of catalyts have been combined by attaching catalytically active species to soluble polymers, which can be bound to highly branched macromolecules.[1] The use of transition metal ions as an intrinsic part (as "supramolecular glue") of highly branched systems also provides potential for the construction of hybrid catalytic systems.[2] Exploiting the fact that thiol-functionalized gold colloids behave like molecules (they are volatile, soluble, can be chromatographed) and possess a surface similar to the (111) surface of bulk gold, we have used a substituted, long-chain thiol to tether a Ru catalyst for ring-opening metathesis polymerization (ROMP) to gold colloids. The colloid-bound Ru species heterogeneously catalyzes the polymerization of norbornene in dichloromethane. At the same time, the catalyst system is readily soluble in and recoverable from acetone and therefore behaves like a molecular species. An interesting feature of the present catalytic system is the observation of much higher turnover frequencies (TOF) of the bound catalyst compared to the unbound molecular form.

Gold salts can be reduced in toluene in the presence of long-chain thiols [3] to give a material that almost perfectly bridges the world of molecular chemistry and the macroscopic world of extended solids and has opened up an exciting new area of nanomaterials.[4] The thiol-coated gold colloids (usually in the 2–10 nm size range) behave like molecules in that they can be dissolved, precipitated, redissolved, sublimed, crystallized, etc. This permits the use of characterization techniques such as solution NMR and time-of-flight MS that are not normally suitable for the study of solids. On the other hand, these colloids possess a distinct solid surface, not unlike the (111) surface of gold that has been the subject of many studies.[5,6]

We have been examining the binding of catalysts to gold (111) surfaces with ω-functionalized thiols with the intention of obtaining specific nanostructured materials, including functionalized atomic force microscopy (AFM) tips. One of the problems associated with multiple chemical modifications on a surface is the difficulty of characterizing the intermediates. This encouraged us to attempt the successive function-

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[**] We thank Professor Dr. Wolfgang Knoll for encouragement and for a generous loan of the SPS apparatus. The research group of Professor R. Zentel is thanked for the GPC measurements and Degussa, Hanau, for a gift of some chemicals. Dr. Christoph Janiak made some very helpful suggestions.