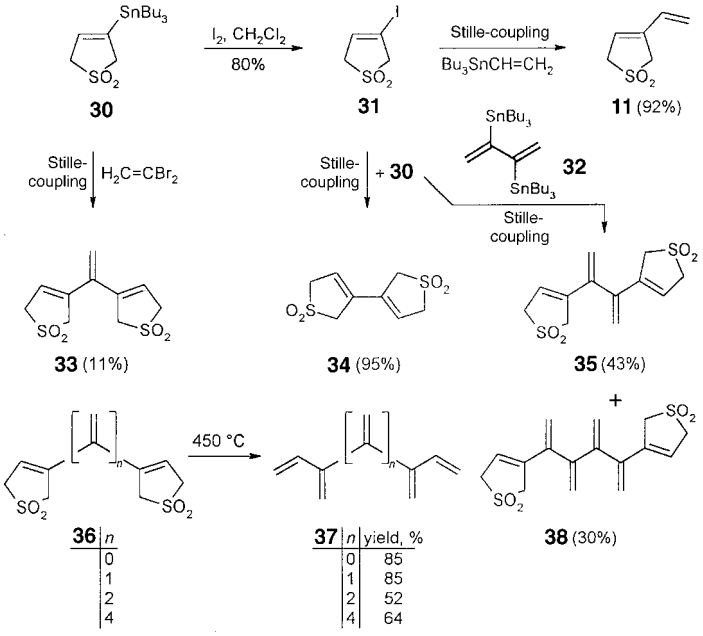

Scheme 4. General dendralene synthesis according to Sherburn et al.

hydrocarbons **37** are released on demand in good yields by high-temperature pyrolysis. As no solvent is required in these cheletropic reactions, the workup is clearly made easier. The dendralenes **37** obtained, up to [8]dendralene ($n = 6$), have been completely characterized by the usual spectroscopic and analytical methods and can, although they have a tendency to polymerize, be manipulated—the deciding prerequisite for the study of their reactivity.

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The First Cadmium-Specific Enzyme

Henry Strasdeit*

Is the Biochemistry of Cadmium More Similar to That of Zinc or Mercury?

From a biological point of view, group 12 of the periodic table is remarkable. Its lightest element, zinc, occurs as an essential constituent in numerous proteins. However, the situation is entirely different for the heaviest group homologue, mercury. This is considered one of the most toxic nonradioactive elements. Also in the case of cadmium, which is the middle element of the group, toxicity and carcinogenicity have been nearly exclusively in the foreground so far. Positive biological effects, for example, on the growth of a fungus species,[1] have been described only sporadically and remain biochemically unexplained. The recently published discovery of the first cadmium enzyme can therefore rank as a landmark in the biological chemistry of cadmium.[2]

Cadmium quite clearly differs chemically from mercury. This is true for many aspects of the toxicity as well. For example, the alkyl species MR_2 and RM^+ are only slowly degraded in the case of mercury and therefore show a characteristic symptomology of poisoning,[3] while in the case of cadmium the species are not of any special toxicological importance because of their fast hydrolysis to Cd^{2+}. On the other hand it has been known for a long time that, in its compounds, cadmium often resembles zinc. Against this background it should thus not be too surprising that cadmium can also have a defined function in organisms. However, nothing of that became visible in the biological career of this element during the first 140 years after its discovery (Table 1).

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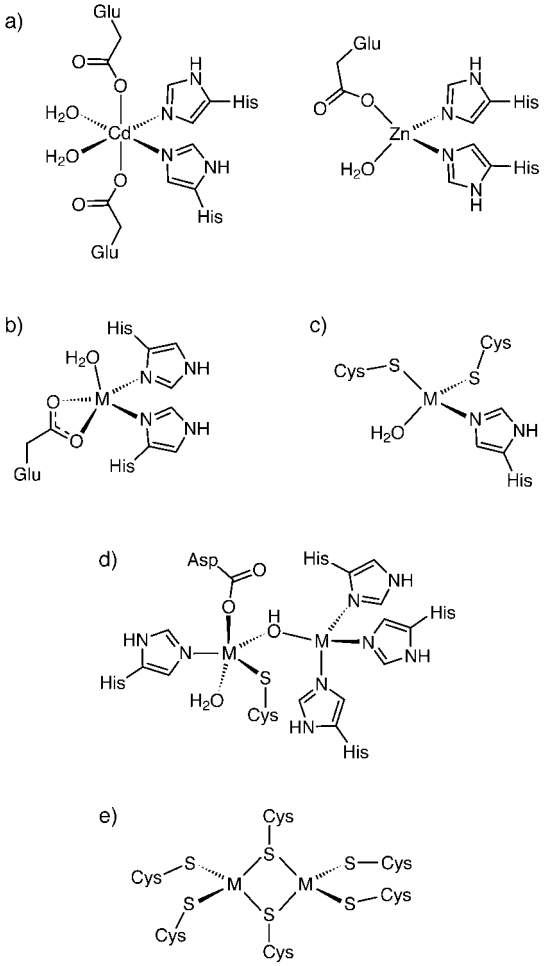
Table 1. A brief chronicle of the biological chemistry of cadmium.

1858	40 years after the discovery of the element, the first cases of cadmium poisoning in humans are reported
1946	Beginning of the systematic study of the itai-itai desease in Japan; about 20 years later cadmium poisoning is suspected for the first time as being a coinducing factor
	In the zinc enzyme carboxypeptidase A, Zn^{2+} can be substituted by Cd^{2+}; the Cd-substituted enzyme continues to show esterase activity, but the peptidase activity is largely lost
1961	Results of animal experiments give the first indications of the carcinogenic potential of cadmium; in 1993 the International Agency for Research on Cancer classifies cadmium as a human carcinogen
	In the zinc enzyme carboxypeptidase A, Zn^{2+} can be substituted by Cd^{2+}; the Cd-substituted enzyme continues to show esterase activity, but the peptidase activity is largely lost
1981	It is reported that cadmium has a growth-promoting effect on a mushroom species, but it cannot compensate for zinc deficiency
1985	Crystallographic studies show that the structure of the active site of an alcohol dehydrogenase changes only slightly when the native Zn^{2+} is substituted by Cd^{2+}; nevertheless the enzymatic activity is greatly reduced
1989	The first cadmium-activated enzyme, phytochelatin synthase, is described
1990	A possible relation between the carcinogenicity of cadmium and the experimentally established substitution of Zn^{2+} by Cd^{2+} in gene-regulating "zinc-finger" proteins is discussed
	Under conditions where zinc is scarce, the marine diatom *Thalassiosira weissflogii* utilizes cadmium as an alternative bioelement
1994	It is assumed that *T. weissflogii* substitutes deficient zinc by cadmium in the zinc enzyme carbonic anhydrase
2000	The substitution hypothesis turns out to be incorrect; *T. weissflogii* synthesizes a novel cadmium-specific carbonic anhydrase—the first cadmium enzyme is discovered



Scheme 1. Schematic representation of the metal coordination in cadmium-substituted zinc proteins and in the corresponding native forms (M = Cd, Zn). a) The catalytic center of thermolysin; in the Zn form the carboxylate group can also be η^2-coordinating—this is probably dependent on the pH value. b) Carboxypeptidase A. c) The catalytic center of alcohol dehydrogenase. d) Metallo-β-lactamase. e) GAL4.

Cadmium-Substituted Zinc Proteins

The way for cadmium as a "bioelement" was essentially paved by investigations on zinc proteins. In 1961 Coleman and Vallee showed that the native metal ion can be removed from the zinc enzyme carboxypeptidase A by use of chelating agents and that Cd^{2+} ions can then be introduced into the apoenzyme.[4] Many other cadmium-substituted zinc proteins followed. In contrast to the situation with the spectroscopically "dead" zinc, it was now possible to utilize the potential of ^{113}Cd NMR and ^{111}Cd PAC spectroscopy (PAC = perturbed angular correlation of γ-rays) in order to study the structures of the metal centers.

The enzymatic activity of the cadmium forms, however, is mostly lower than that of the zinc enzymes and sometimes no longer present at all. This is caused by the different ionic radii of Cd^{2+} and Zn^{2+}. The markedly larger Cd^{2+} ion prefers higher coordination numbers and is a weaker Lewis acid. In the extreme case of thermolysin, the metal is six-coordinate in the cadmium form [**Cd**$(N \cdot His)_2(O_2C \cdot Glu)_2(OH_2)_2$] and four- or five-coordinate in the zinc form [**Zn**$(N \cdot His)_2(O_2C \cdot Glu)(OH_2)$] (Scheme 1a).[5] This difference obviously accounts for the fact that cadmium-substituted thermolysin is totally enzymatically inactive. With other zinc enzymes, such as carboxypeptidase A,[6] horse liver alcohol dehydrogenase,[7] and metallo-β-lactamase,[8] the metal environments remain intact in the cadmium forms (Scheme 1b–d). Nevertheless, the enzyme activities here are significantly reduced as well, probably because the less Lewis acidic Cd^{2+} ion has a weaker polarizing effect on its environment. This is the reason why, among other things, coordinated substrates are less activated and the pK_a values of coordinated water molecules are not as strongly decreased. Also, it is noteworthy that in gene-regulating proteins cadmium ions can substitute for the structure-stabilizing zinc ions without inducing drastic changes. For example, in the transcription factor GAL4 the dinuclear metal center continues to exist (Scheme 1e).[9] As gene-regulating proteins interact directly with DNA, a causal relation to the carcinogenicity of cadmium is conceivable.[10]

Phytochelatin Synthase, a Cadmium-Activated Enzyme

Phytochelatins are short peptides of the composition (γ-Glu-Cys)$_n$-Gly ($n = 2-11$), which in plants serve, inter alia, for cadmium detoxification. They are biosynthesized from the tripeptide glutathione (γ-Glu-Cys-Gly) by the enzyme γ-glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase, PCS) [Eq. (1)].

$$(\gamma\text{-Glu-Cys})_n\text{-Gly} + \gamma\text{-Glu-Cys-Gly} \xrightarrow{\text{PCS}} (\gamma\text{-Glu-Cys})_{n+1}\text{-Gly} + \text{Gly}\ (n \geq 1) \quad (1)$$

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Interestingly, the PCS molecules are initially inactive and are activated only by cadmium ions that have entered the plant cell.[11] The subsequently synthesized phytochelatins form stable Cd^{2+} complexes,[12] so that after some time the enzyme reaction breaks down for lack of cadmium. Thus PCS is self-regulating. The molecular mechanism of the activation is still unclear. It seems uncertain whether the cadmium is located in the catalytic center of the enzyme because, although Cd^{2+} is the most effective activator, various other metal ions can, in some cases considerably, activate PCS too.[11, 13]

Cadmium Carbonic Anhydrase, the First Cadmium Enzyme

The starting point for the discovery of the cadmium carbonic anhydrase was the observation that the concentration of cadmium in sea water follows that of nutrients such as phosphate. That is, dissolved cadmium in water layers near the surface is often strongly depleted, obviously because cadmium is taken up by the phytoplankton. Zinc behaves completely analogously, and thus its availability is a growth-limiting factor for many marine microorganisms. The assumption seemed obvious that these organisms possibly utilize cadmium as a substitute for zinc. Price and Morel could indeed demonstrate on artificially zinc-limited cell cultures of the marine diatom *Thalassiosira weissflogii* that the drastic growth limitations which are caused by zinc deficiency can be largely compensated by addition of Cd^{2+}.[14] However, when zinc is sufficiently available, cadmium does not act in a growth-promoting manner.

Soon a connection between these observations and the carbonic anhydrase activity was discovered.[15] The zinc-containing carbonic anhydrases catalyze the hydration of CO_2 highly efficiently [Eq. (2)]. They play a key role, for example, in the photosynthetic CO_2 fixation in many photoautotrophic plants.[16] Cadmium-substituted zinc carbonic anhydrases can exhibit significant enzymatic activity, although only in the alkaline pH region. It therefore seemed plausible to attribute the positive effect of cadmium in *T. weissflogii*, at least partly, to metal substitution in the zinc carbonic anhydrase (TWCA1). On the basis of more recent findings this hypothesis was, however, dismissed. Instead, Lane and Morel arrived at the surprising result that under conditions where zinc is scarce *T. weissflogii* can synthesize a special cadmium carbonic anhydrase (Cd-CA).[2] This marks the discovery of the first defined biological function of cadmium. As zinc limitation generally occurs in the natural habitat of *T. weissflogii* (see above), cadmium must be regarded as an essential element for this organism.

$$CO_2 + H_2O \rightleftarrows H^+ + HCO_3^- \qquad (2)$$

Cd-CA is definitely not a cadmium-substituted TWCA1. The authors were able to give several proofs for this including, among other things, the different molar masses (Cd-CA: 43 kDa, TWCA1: 27 kDa). The function of Cd^{2+} as part of the catalytic center of the Cd-CA can hardly be doubted because of the analogy with the zinc carbonic anhydrases. As soon as sufficient protein material is available, the full characterization will commence. One may be especially curious about the three-dimensional structure and particularly about the coordination of the cadmium. Recently, even the zinc carbonic anhydrases have offered surprises with respect to metal coordination: instead of the "classic" $\{\mathbf{Zn}(N \cdot His)_3(OH_2)\}$ center seen in the α- and γ-carbonic anhydrases, $\{\mathbf{Zn}(N \cdot His)(S \cdot Cys)_2L\}$ centers ($L = OH_2$ or $O_2C \cdot Asp$) were discovered in β-carbonic anhydrases of plants.[17]

In contrast to cadmium carbonic anhydrase, into whom investigation has only recently begun, the characterization of phytochelatin synthase has already proceeded a step further. At virtually the same time, three research groups reported on the first identification of PCS genes and the amino acid sequences belonging to them.[18] Thus the goal of elucidating the mechanism of the activation by Cd^{2+} ions has moved a good deal closer. Not least by the new role of cadmium as a bioelement are the coordination chemists challenged to contribute to the understanding of the catalytic and structure-directing properties of Cd^{2+} ions in enzymes.

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