Note

Metal Specificities on Induction and Binding Affinities of Heavy Metal-binding Complexes in Water Hyacinth Root Tissues

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Various kinds of Cd-containing complexes a metallothionein-like Cd-binding protein have been found in higher plants.¹⁻⁵ Fujita,⁶ however, reported that a Cd-binding complex, which resembles fission yeast Cd-BP1⁷ consisting of cadystin peptides $[(\gamma\text{-Glu-Cys})_n\text{-Gly } n=2 \text{ and } 3]^8$ rather than mammalian Cd-thionein, was formed in the root tissues of water hyacinth (*Eichhornia crassipes* Solms) when the plant was cultivated in a Cd-containing medium. Fujita and Kawanishi⁹ also reported that fission yeast Cd-BP1-like Cd-binding complexes were formed in the root tissues of various kinds of vegetables. On the other hand, Grill *et al.*¹⁰ independently demonstrated that diverse plant cells absorbed various heavy metals and produced a series of phytochelatins, $(\gamma\text{-Glu-Cys})_n\text{-Gly } n=3\sim7$, which could form heavy metal-binding complexes. Recently, they¹¹ reported the details of their biosynthesis using plant cell suspension cultures.

In this paper, we report that the metal specificities on induction and binding affinities of heavy metal-binding complexes, constructed with cadystin peptides and/or phytochelatins, in the root tissues of water hyacinth are extremely specific to Cd, and also suggest that the complexes might not be important for the metabolism of Zn, differing from in case of mammalian metallothioneins.

Water hyacinths were cultivated in a greenhouse in Yoshimura's solution.¹² The plants were cultivated in the nutrient solution supplemented with 10 μM of a metal salt for 7 days, except that the Ag, Fe and Mn salts were supplemented at 20, 30 and 43 μM , respectively. After cultivation, the roots were washed with tap water and then with deionized water. The roots were homogenized with 1.5-fold tissue weight of 25 mM Tris-HCl buffer, pH 8.0, containing 0.25 M sucrose, 1% (w/v) ascorbate and 10% (w/v) Polyclar AT in a pestle and mortar. The homogenate was squeezed through two layers of nylon cloth and the filtrate was centrifuged at 22,000 $\times g$ for 1 hr. The supernatant was designated as the tissue extract.

A Sephadex G-50 column (4 $\times$ 59 cm) equilibrated with 10 mM Tris-HCl buffer, pH 8.0, containing 100 mM KCl

was used to fractionate the tissue extract. The tissue extract, 40 ml, was applied to the column and fractions of 6.3 ml each were collected. The extract was eluted with the buffer used to equilibrate the column and the void volume was determined using Blue Dextran.

Heavy metals were determined by atomic absorption analysis with a Hitachi 308 Two Wavelength Atomic Absorption Spectrophotometer.

In order to determine whether the induction of heavy metal-binding complexes in water hyacinth root tissues is specific to Cd or not, plants were grown in mediums containing various kinds of heavy metals and each tissue extract was applied to a Sephadex G-50 column (Table I). In the case of Cd, all Cd contained in the tissue extract was eluted at around fraction 65 as a heavy metal-binding complex (mol wt of about 4,000), as previously reported by Fujita and Kawanishi.¹³ In the cases of both Cu and Pb, though a small amount of each corresponding heavy metal-binding complex was detected at around fraction 65, most of the metal was eluted as the free ion (around fraction 93; bed volume fraction), a nonspecific protein-binding form (around fraction 18; void volume fraction) and an unknown form (around fraction 81), which was found only in the case of Cu (data not shown). In the cases of other heavy metals, all of the metal eluted was fractionated as the free ion and a nonspecific protein-binding form.

Grill *et al.*¹¹ reported that various heavy metals, such as Cu, Zn and Ni, induced the synthesis of phytochelatins (including cadystin peptides), components of plant heavy metal-binding complexes, in cell suspension culture of a high plant, *Rauvolfia serpentina*, but the concentrations of the heavy metals used in their experiment were much higher

TABLE I. FORMATION OF METAL-BINDING COMPLEXES IN WATER HYACINTH ROOT TISSUES TREATED WITH VARIOUS HEAVY METALS

Treatment		In Fraction 65 eluted from a Sephadex G-50 column	
Heavy metal	Concentration (μM)	A_{265}^a	Heavy metal
Cd	10	++	++
Cu	10	+	+
Zn	10	—	—
Ni	10	—	—
Ag	20	—	—
Hg	10	—	—
Pb	10	—	+
Fe	30	—	—
Mn	43.2	—	—
Bi	10	—	—

^a A_{265} is due to the heavy metal-mercaptide chromophore.

than those in ours. Their heavy metal concentrations caused some serious morphological damage to the water hyacinth roots and leaves, when no heavy metal-binding complexes occurred during the treatments. In order to determine whether or not other heavy metals than Cd have the ability to act as a trigger for the synthesis of phytochelatins in water hyacinth root tissues or binding affinity to them, though they can induce phytochelatins like Cd, an equimolar amount of Cd was added to the tissue extract of water hyacinth root treated with each heavy metal, and the mixture was applied to the Sephadex G-50 column. No new Cd-binding complex was detected in place of the free Cd ion on the chromatogram. These results suggest that the synthesis and formation of heavy metal-binding complexes is physiologically very specific to Cd in water hyacinth root tissues. Maita *et al.*¹⁴⁾ recently reported that a complex was specifically induced by Cd in a unicellular phytoplankton, *Phaeodactylum tricornutum*. Therefore, the induction of heavy metal-binding complexes might be specific to Cd not only in multicellular systems, such as tissues and/or organs, but also in single cell systems, though Grill *et al.*^{11,15)} reported that various heavy metals induced some phytochelatins in fission yeast and suspension cultures of *R. serpentina*. As described above, the Sephadex G-50 column chromatogram of water hyacinth root tissue treated with Cu showed a peak of an unknown Cu form between those of the Cu-binding complex and the free Cu ion. When this peak fraction was rechromatographed in the same way, all the Cu was eluted as the free ion. When an appropriate amount of Cu was added to the extract of the water hyacinth root tissues not treated with a heavy metal, Cu was eluted as the free ion on Sephadex G-50 column chromatography. As the amount of Cu used was gradually decreased, the free Cu ion first disappeared and then the unknown Cu peak disappeared, though the Cu-binding complex remained. It remains to be determined whether the unknown form of Cu is due to weak interaction with the Cu-binding complex itself or an unknown low molecular weight substance induced by Cu.

The transport of Cd, Cu and Zn from the roots to leaves of water hyacinth was examined for 6 days. Though the rate of absorption of Cu from the cultivation medium into the plant was apparently much greater than those of Cd and Zn, the metal content ratios, root to leaf, for Cd, Cu and Zn were 6.8, 5.3 and 3.7, respectively. This result led to the conclusion that the formation of a heavy metal-binding complex might play a role in the suppression of the diffusion of the heavy metal among tissues and/or organs.

In order to examine the substitution ability of heavy metals, as to the water hyacinth Cd-binding complex, an extract of root tissues of the plant treated with Cd was incubated with another heavy metal at 4°C for 1 hr and then applied to the Sephadex G-50 column as described above (Table II). Copper could substitute for all the Cd in the Cd-binding complex, in an equimolar ratio. Hg and Ag could also be substituted but the others including Zn and

TABLE II. SUBSTITUTION ABILITY OF HEAVY METALS AS TO THE WATER HYACINTH Cd-BINDING COMPLEX

Additive	In Fraction 65 eluted from a Sephadex G-50 column	
	A_{265}^a	Substitution of metals
Cu	+	+
Zn	+	—
Ni	+	—
Ag	+	+
Hg	—	+
Pb	+	—
Fe	+	—
Mn	+	—
Bi	+	—

^a A_{265} is due to the heavy metal-mercaptide chromophore.

Ni could not at all. These characteristics seem to resemble to those of mammalian metallothioneins, except that the binding affinity of Zn was too weak.

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