Note

Isolation of a Cadmium-binding Protein from Cadmium-treated Rice Plants (Oryza sativa L.)

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Recent concern over cadmium pollution of rice paddy soil has stimulated our research on the response of rice plants to cadmium. Several recent studies have reported the isolation and characterization of metal-binding proteins from plants.1-4 The present study reports the isolation of a cadmium-binding protein from cadmium-treated rice plants. Rice ‘nihonbare’ was seeded on 13 April, 1980. Approximately 4 weeks-old rice plants were grown in culture solution in a greenhouse. After 2 weeks, they received a daily application of CdCl₂ at 0.3 μg Cd²⁺/ml for one month. The plants were harvested, rinsed with deionized water, cut into small pieces, and homogenized in 0.05M-sodium phosphate buffer, pH 7.8, in a chilled electric blender. The homogenate was extracted with the same buffer for 1 hr with continuous stirring and stored at 4°C for 2 weeks. The homogenate was squeezed through cloth. The filtrate was lyophilized and centrifuged at 5000 rpm for 30 min. The supernatant fluid was applied to a Sephadex G-75 column (5.4 x 120 cm). After elution with 0.05 M-sodium phosphate buffer, pH 7.8, at a flow rate of 30 ml/h, cadmium-rich fractions were combined and concentrated by ultrafiltration on a UP-20 (TOYO), while the heavier and lighter fractions containing a small amount of cadmium were discarded. Cadmium was determined by an atomic absorption method. The cadmium-rich fraction was rechromatographed on a Sephadex G-50 column as shown in Fig. 1. The cadmium-rich fractions after concentration and lyophilization were purified further by passage through a CM-cellulose column as shown in Fig. 2. Further purification was performed by column

Fig. 1. Rechromatography of a Cadmium-binding Protein on a Sephadex G-50 Column.

The cadmium-rich fraction from the Sephadex G-50 column was rechromatographed on a Sephadex G-50 column (5.4 x 118 cm) equilibrated with 0.05 M-sodium phosphate buffer, pH 7.8. Fraction (10 ml) were collected at a flow rate of 30 ml/hr.

Fig. 2. Chromatography of a Cadmium-binding Protein on a CM-cellulose Column.

The cadmium-rich fraction from the Sephadex G-50 column was applied to a CM C-50 cellulose column (2.9 x 92 cm) equilibrated with 0.01 M-sodium phosphate buffer, pH 7.0. Elution was carried out with a linear gradient of 0.01 to 0.3 M-sodium phosphate buffer, pH 7.0. Fractions (10 ml) were collected at a flow rate of 20 ml/hr.

Fig. 3. UV-Absorption Spectrum of a Cadmium-binding Protein.

Absorption spectrum at pH 7.0 (——) and pH 2.0 (-----) adjusted with HCl.
Fig. 4. Determination of Molecular Weight of a Cadmium-binding Protein.

The molecular weight of a cadmium-binding protein was determined by gel-permeation chromatography on a TSK-G 3000 SW column (0.75 x 60 cm). The column was equilibrated with 0.2 M-sodium chloride, 1/30 M-sodium phosphate buffer, pH 7.0. The flow rate was maintained at 1 ml/min. The effluent fractions were monitored at 280 nm to determine the elution volume of proteins. The proteins used as standards were: A, thyroglobulin (mol. wt. 669,000); B, ferritin (440,000); C, catalase (210,000); D, albumin (67,000); E, ovalbumin (43,000); F, chymotrypsinogen A (25,000); G, ribonuclease A (13,700); H, bacitracin (1,400); Δ, cadmium-binding protein.

electrophoresis. The cadmium-containing material from the CM-cellulose column was mixed with 3% saccharose and separated on a 6 x 10 cm polyacrylamide gel column, equilibrated with 0.02 M-sodium phosphate buffer, pH 7.8, at 10°C and 35 mA. After 40 hr of electrophoresis, 5 ml fractions were collected at a flow rate of 10 ml/hr. The cadmium-containing material moving toward the anode was recovered in a single peak. Homogeneity of the purified cadmium-containing material was evident on analytical polyacrylamide-disc-gel electrophoresis. This material contains 12 mg Cd g⁻¹ protein. It reacts positively to the biuret and ninhydrin tests. The UV-absorption spectrum of this material showed absorptions at 280 and 250 nm (Fig. 3). Absorption at 250 nm was significantly reduced upon acidification to pH 2. This observation suggests that this material is a cadmium-binding protein like metallothionein. By means of gel-permeation chromatography, the molecular weight of the material was estimated to be 33100 (Fig. 4), which differs from that of metallothionein in animals. This material was not eluted even at a very high ionic strength from the DEAE-cellulose column. But it was eluted at a very low ionic strength from a CM-cellulose column, indicating a highly anionic molecule. On the basis of these results, this cadmium-containing material isolated from cadmium-treated rice plants can be classified as a type of metallothionein but differs from that in animals. Characterization of this material in detail is in progress.

REFERENCES

3) C. Nr. We Rauser, Plant Physiol., 63, S-59 (1979).