Cadmium-thiolate protein from the grass *Agrostis gigantea*

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A cadmium-binding protein rich in cysteine and acidic amino acid residues was isolated from roots of *Agrostis gigantea*. The molar ratio of cysteine to cadmium was 2.7:1. Electronic absorption and circular dichroism measurements were characteristic of cadmium-thiolate coordination. The cadmium-binding centre in the plant protein was strikingly similar to that of the well characterised vertebrate cadmium-thioneins.

Plant metallothionein *Cadmium-thiolate* *Circular dichroism*

1. INTRODUCTION

Metallothionein belongs to a group of ubiquitous metal-thiolate-rich polypeptides. The low M_r near 6000, the high cysteine content of up to 33% of the total amino acid residues, the lack of aromatic amino acids, and inducibility by Zn, Cd or Cu are the most striking common features (reviews [1–4]). The vertebrate Cd,Zn-thioneins are the most studied species. Homogeneous Cu-thioneins were isolated from yeast [5,6] and *Neurospora crassa* [7]. Only limited data are available on the occurrence of metallothioneins in plants [8–12].

Thus, it was of special interest to characterise the plant cadmium-binding proteins in more detail. Emphasis was placed on the question of whether cadmium was bound arbitrarily to the protein portion or specifically. The proof of a specific cadmium-thiolate chromophore similar to that of the vertebrate Cd-thionein would lend considerable support for the presence of Cd-thionein-like proteins in plants.

A cadmium-thiolate protein was isolated from *Agrostis gigantea* and chemically characterised. The presence of a possible Cd-thiolate chromophore was measured using electronic absorption and circular dichroism.

2. EXPERIMENTAL

Tillers of clone 4 of *Agrostis gigantea* Roth were grown hydroponically for 4 weeks [13]. The nutrient solution was then augmented with 3 μM $CdSO_4$ for 7 days. Roots were harvested [8] and homogenized in 20 mM Tris–HCl (pH 8.0), 5 mM ascorbic acid and 1 mM 2-mercaptoethanol. The homogenate was heated to 60°C for 3 min. After centrifugation the supernatant was chromatographed on QAE-Sephadex A-25. The Cd-binding protein in the conductivity range of 3.4–5 $dS \cdot m^{-1}$ was concentrated by ultrafiltration (Amicon UM 2 membrane), chromatographed on Sephadex G-75 in 1 M KCl, desalted on Biogel P-2 and lyophilized. Molecular weight was determined by chromatography in 5 mM Tris–HCl (pH 8.0), 1 M KCl on Sephadex G-50 fine. All operations were performed with N_2-purged buffers under N_2 at 4°C. Cadmium was quantitated on a Zeiss M4 QIII atomic spectrometer; copper and zinc were measured on a Perkin Elmer atomic absorption spectrometer (model 400 S) furnished with an HGA-76B unit. Circular dichroism was recorded on a Jasco 20A polarimeter. Amino acids were assayed

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after 6 M HCl hydrolysis; cysteine was converted into cysteic acid following performic acid oxidation [14]. The amino acids were analysed as their *o*-phthaldialdehyde derivatives by reverse phase HPLC [15].

3. RESULTS AND DISCUSSION

After the final purification step the Cd-binding protein remained homogeneous and migrated as one symmetrical band through Sephadex G-50 like a protein of M_r = 3700. Polyacrylamide gel electrophoresis was not definitive and in our experience prone to loss of cadmium and subsequent oligomerization of the thiolate-rich protein. The cadmium concentration was 110 $\mu g \cdot mg^{-1}$ which was close to 4 Cd per mol protein. The Cd:cysteine ratio was 1:2.7 not too far away from the established 1:3 ratio of the vertebrate Cd-thionein. Zinc and copper were below the detection limit. As in the case of yeast Cu-thionein the concentration of acidic amino acid residues was very high (table 1). Glutamate was highest with very little lysine and the cysteine content approach that of the vertebrate metallothionein. Only negligible amounts of aromatic amino acid residues were found which was supported by the low ultraviolet absorption at 280 nm (fig.1).

The electronic absorption below 300 nm closely resembles that observed for the vertebrate Cd,Zn-thionein [17]. A shoulder at 257 nm is indicative of cadmium-thiolate bonding. Upon titration with HCl to pH 0.4 this absorption leveled off and no significant absorption at 260 nm attributable to cystine remained [18]. More specific evidence for Cd-thiolate bonding is demonstrable using circular dichroism measurements (fig.2). An intriguing similarity between the Cotton extrema of the plant protein and those of the vertebrate Cd-thionein is seen.

Although the Cotton bands of the plant protein are somewhat dislocated, the characteristic form attributable to a cadmium-thiolate chromophore is evident [17]. This dislocation was almost expected and is largely explicable through the substantial differences in polypeptide sequence between the two protein types. The magnitudes of the Cotton bands are, like in the case of yeast copper-thionein, approximately twice as high when compared to the vertebrate Cd-thionein. Stepwise titration to pH 2

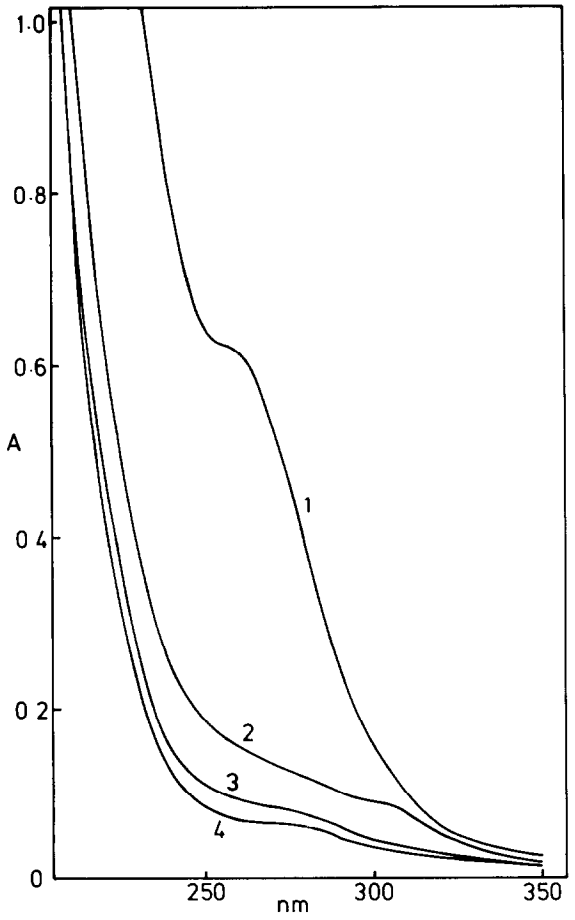

Fig.1. Electronic absorption of *A. gigantea* Cd-thiolate protein. Native protein at pH 7.1 (1) was titrated to pH 3.0 (2); pH 2.0 (3) and pH 0.4 (4) with microlitre amounts of 3 N HCl; the light path was 10 mm.

resulted in the complete disappearance of all Cotton bands. On adjustment to pH 7.0 the original Cotton extrema reappeared to 60% of the initial value suggesting complete cadmium dislocation from and rebinding to the cysteine sulphur.

For the first time conclusive evidence is presented that cadmium is bound to thiolate sulphurs in a protein of plant origin. Although the amino acid composition is much more related to the yeast type Cu-thionein, the Cd-thiolate binding centre appears to be identical to that of the tetrahedrally arranged mononuclear $Cd(SR)_4$-units of vertebrate metallothionein [17,19,20]. As in the case of the iron-sulphur proteins the metal-thiolate binding centres of Zn,Cd- and Cu-thioneins appear to enjoy the same ubiquitous distribution.

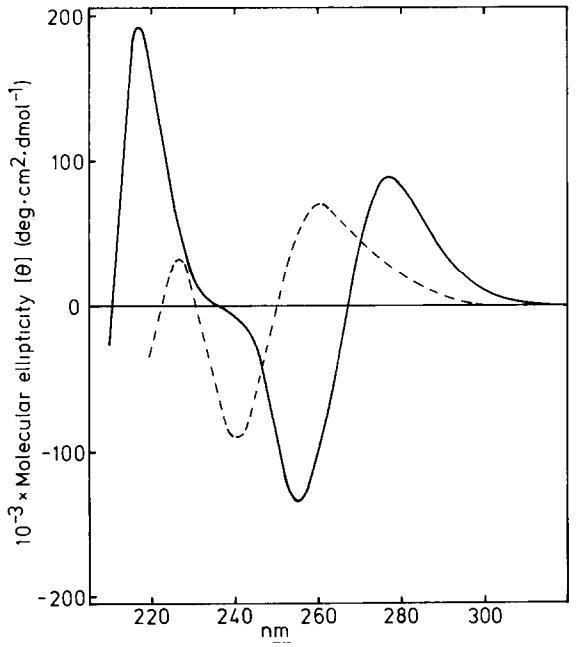

Fig.2. Circular dichroism of *A. gigantea* Cd-thiolate protein (——) and vertebrate 6 Cd-thionein taken from [17] (– – –). Molecular ellipticities were calculated using M_r = 3700 and 6600, respectively.

Table 1

Amino acid composition of different metal-thiolate proteins in % of total residues

Amino acid residue	Equine liver metallo-thionein [12]	Yeast Cu-thionein [6,16]	*A. gigantea* Cd-thiolate-protein
Cysteine*	33.2	20.0	29.0
Aspartate	5.0	13.8	2.5
Glutamate	4.5	17.6	41.8
Glycine	10.1	8.0	5.5
Serine	11.6	11.6	9.3
Threonine	3.9	4.1	0.8
Proline	5.1	5.3	–
Alanine	9.4	1.2	1.5
Valine	2.6	0.5	0.7
Methionine	1.5	–	–
Isoleucine	0.6	0.5	0.4
Leucine	0.6	0.7	0.6
Tyrosine	–	–	1.3
Phenylalanine	–	–	0.3
Lysine	10.4	11.1	1.7
Histidine	–	1.7	4.1
Arginine	2.2	–	0.4

*Determined as cysteic acid

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REFERENCES

[1] Kägi, J.H.R., Kojima, Y., Berger, C., Kissling, M.M., Lerch, K. and Vasak, M. (1979) in: Metalloproteins (Weser, U. ed) pp.194–206, Georg Thieme Verlag, Stuttgart.
[2] Kägi, J.H.R. and Nordberg, M. (1979) Metallothionein, Experientia Supplement 34, Birkhäuser Verlag, Basel.
[3] Webb, M. (1979) Top. Environ. Health 2, 195–266.
[4] Weser, U. and Rupp, H. (1979) Top. Environ. Health 2, 267–288.
[5] Prinz, R. and Weser, U. (1975) Z. Physiol. Chem. 356, 767–776.
[6] Weser, U., Hartmann, H.-J., Fretzdorff, A. and Strobel, G.J. (1977) Biochim. Biophys. Acta 493, 465–477.
[7] Lerch, K. (1980) Nature 284, 368–370.
[8] Rauser, W.E. and Curvetto, N.R. (1980) Nature 287, 563–564.
[9] Bartolf, M., Brennan, E. and Price, C.A. (1980) Plant Physiol. 66, 438–441.
[10] Wagner, G.J. and Trotter, M.M. (1982) Plant Physiol. 69, 804–809.
[11] Casterline, J.L. jr and Barnett, N.M. (1982) Plant Physiol. 69, 1004–1007.
[12] Kaneta, M., Hikichi, H., Endo, S. and Sugiyama, N. (1983) Agric. Biol. Chem. 47, 417–418.
[13] Hogan, G.D. and Rauser, W.E. (1981) J. Exp. Bot. 32, 27–36.
[14] Hirs, C.H.W. (1967) Methods Enzymol. 11, 59–62.
[15] Winspear, M.J. and Oaks, A. (1983) J. Chromatogr., in press.
[16] Kimura, M., Otaki, N., Hartmann, H.-J. and Weser, U. (1981) Regard Biochim. 3, 101.
[17] Rupp, H. and Weser, U. (1978) Biochim. Biophys. Acta 523, 209–226.
[18] Weser, U., Rupp, H., Donay, F., Linnemann, F., Voelter, W., Voetsch, W. and Jung, G. (1973) Eur. J. Biochem. 39, 127–140.
[19] Bordas, J., Koch, M.H.J., Hartmann, H.-J. and Weser, U. (1983) Inorg. Chim. Acta 78, 113–120.
[20] Garner, C.D., Hasnain, S.S., Bremner, I. and Bordas, J. (1982) J. Inorg. Biochem. 16, 253–256.