CONF

CONF-8508108--2

Los Alamos National Laboratory is operated by the University of California for the United States Department of Energy under contract W-7405-ENG-36

LA-UR--85-2944

DE85 017546

TITLE Characterization of Metal Binding Peptides
From Cadmium Resistant Plant Cells

AUTHOR(S) N. J. Robinson, K. Barton, C. M. Naranjo, L. O. Sillerud
J. Trewhella, K. Watt, P. J. Jackson

SUBMITTED TO 2nd International Meeting on Metallothionein
Zurich, Switzerland, August 20-23, 1985

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

By acceptance of this article, the publisher recognizes that the U.S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution or to allow others to do so for U.S. Government purposes.

The Los Alamos National Laboratory requests that the publisher identify this article as work performed under the auspices of the U.S. Department of Energy.

Los Alamos Los Alamos National Laboratory
Los Alamos, New Mexico 87545

MASTER

gsw

CHARACTERIZATION OF METAL BINDING PEPTIDES
FROM CADMIUM RESISTANT PLANT CELLS

N.J. Robinson¹, K. Barton², C.M. Naranjo¹, L.O. Sillerud¹,
J. Trehwella¹, K. Watt³, and P.J. Jackson¹

¹Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM
87545, USA, ²Agracetus Corp., 8520 University Green, Middleton, WI
53562, USA, ³Cetus Corp., 1400 Fifty-third Street, Emeryville, CA
94608, USA

Abstract: The majority of the cellular cadmium (<80%) in cadmium resistant *Datura innoxia* cells is bound to a small, metal induced peptide which is not metallothionein. This peptide consists of glutamate, cysteine and glycine in a ratio between 2:2:1 and 3:3:1 and has an apparent molecular weight of 776, under denaturing conditions. It is heat stable and complexes with cadmium to produce multimeric forms which are separable by gel filtration. Chemical analyses suggest that some amino acids are not joined by classical peptide linkages. This indicates that the synthesis of the peptide may not be directed by mRNA and that induction of its synthesis may not involve increased transcription from a putative gene corresponding directly to this peptide. A smaller proportion (>15%) of the cellular cadmium is bound to a larger compound which is also heat stable and binds copper more readily than cadmium *in vivo*. This larger compound has an amino acid composition similar, in some respects, to metallothioneins.

Introduction

Metallothioneins have been considered to be ubiquitous (1) but it is not known whether this class of proteins occurs in higher plants. Apparently inducible copper and cadmium complexes occur in several plant species (2-12). Amino acid compositions of partially purified complexes from angiosperms show some similarities to metallothioneins with high cysteine and low aromatic amino acid contents (5,7,12,15,16). However, the degree of homology between such phytochelatins and metallothioneins remains to be established.

Two low molecular weight, cysteine rich, cadmium induced metal complexes are involved in the mechanism of tolerance in cadmium resistant *Datura innoxia* cells grown in cell suspension culture (10,11). These two compounds are further characterized.

Results and Discussion

D. innoxia cells were selected for resistance to 12.5, 25, 50, 100, 160, 200, 250, and 300 μ M cadmium chloride. Variant cell lines retain their ability to grow in cadmium after growth in its absence for more than seven hundred generations. Resistance to increasing concentrations of cadmium correlates with both the rate of de novo synthesis and the maximum accumulation of two cadmium complexes. One of these (Fig. 1, peak 2) also binds copper in vivo, and addition of copper to cells growing in the presence of cadmium causes the displacement of cadmium from this complex. Addition of a normally non-toxic concentration of copper to cells growing in a sublethal concentration of cadmium leads to cell death. This suggests that production of this compound is a potentiator of, rather than a responder to, cadmium resistance. However, this cadmium/copper complex only binds a small proportion (>15%) of the cellular cadmium. The majority of the cadmium (<80%) is bound to a smaller compound which forms at least three multimeric aggregates in the presence of cadmium (Fig. 1, peaks 3, 4, 5). The apparent molecular weight of all three of these complexes is 776 under denaturing

Fig. 1. Gel filtration of an extract from cadmium resistant D. innoxia cells grown in cadmium and exposed to ¹⁰⁹Cd. Cells were extracted as described by Jackson et al. (10) and separated on a 150 x 5 cm column of Sephadex G-50. ¹⁰⁹Cd (—), absorbance at 280 nm (---).

conditions on a Sephadex G-50 column in the presence of 6 M guanidine hydrochloride following calibration with cytochrome c, α insulin, β insulin and cyanogen bromide fragments I, II, and III of cytochrome c. Fractions containing cadmium complex from peaks 3, 4, and 5 (Fig. 1) have been further purified by covalent chromatography on thiopropyl Sepharose 6B following the procedures of Ryden and Deutsch (17) and Riordan and Richards (18). The

Table I. Amino acid compositions of two cadmium complexes from cadmium resistant *D. innoxia*. Cadmium peptide corresponds to peaks 3, 4, and 5, and copper/cadmium complex corresponds to peak 2, Fig. 1. Pooled fractions were desalted and concentrated by ultrafiltration then further purified by covalent chromatography on thiopropyl Sepharose 6B.

Amino acid	Proportion of total residues (%)	
	Cadmium peptide	Copper/cadmium complex
Cys	39.5	21.6
Asx	0.0	6.1
Glx	41.9	19.6
Gly	18.5	10.7
Ser	0.0	4.1
Thr	0.0	4.5
Pro	0.0	2.3
Ala	0.0	4.9
Val	0.0	4.7
Met	0.0	0.8
Ile	0.0	3.1
Leu	0.0	5.0
Tyr	0.0	2.1
Phe	0.0	2.2
Lys	0.0	4.7
His	0.0	1.4
Arg	0.0	2.2

resulting isolate contains only three different amino acids, cysteine, glutamate and glycine in a ratio between 2:2:1 and 3:3:1 (Table I). The compound does not undergo Edman degradation, even after treatment with TFA for 90 minutes at 55°C to remove any acetyl groups that may be present. Furthermore, treatment with *Staphylococcus aureus* V8, known to cleave at the carboxyl side of glutamate residues, does not generate polypeptide fragments which are sensitive to Edman degradation. These results suggest that the N-terminal amino acid is glutamate and that all glutamate residues are linked to adjacent amino acids via the γ carboxyl group. ^{13}C NMR of α, γ - ^{13}C -glutamate labelled complex is being used to confirm the presence of peptide linkages through the γ carboxyl group of glutamate. The apparent molecular weight under denaturing conditions is higher than that of glutathione, although cadmium binds to glutathione with a stoichiometry of 1:1, 1:2, or 1:3 at pH 7 (19). Moreover, the amino acid composition and metal affinities are inconsistent with cadmium glutathione. Furthermore, oxidized apopeptide from *D. innoxia* is not a substrate for glutathione reductase. This phytochelatin may therefore be structurally similar to a cadmium peptide from *Schizosaccharomyces pombe* (20,21) which contains three linkages through the carboxyl of glutamate, one d-form cysteine residue and the following amino acid sequence;

The presence of linkages through the γ carboxyl of glutamate indicates that synthesis of both the peptide from S. pombe and D. innoxia occur via biosynthetic pathways, possibly using glutathione and/or γ glutamylcysteine as precursors, and it is probable that neither is encoded by a structural gene.

The copper/cadmium complex (Fig. 1, peak 2) has an amino acid composition similar, in some respects, to mammalian and microbial metallothioneins, but contains more glutamine/glutamate than these. This could result from contamination by glutathione, γ glutamyl-cysteine or the peptide found in peaks 3, 4, and 5 (Fig. 1). Classification of this compound as a metallothionein would require the demonstration of characteristic cysteine-x-cysteine sequences (where x is an amino acid other than cysteine, usually lysine, arginine or serine) (22). The amino acid sequence of this compound has not yet been determined.

Whether metallothioneins occur in higher plants remains an unanswered question. However, the majority of the cadmium in cadmium resistant D. innoxia cells is bound to a small metal induced peptide which is clearly not metallothionein.

Acknowledgement

This work was performed under the auspices of the U.S. Department of Energy, NJR was supported by the Natural Environment Research Council of Great Britain. The authors gratefully acknowledge the assistance of Clifford Unkefer, Thomas Whaley and Jane Doolen.

References

1. Karin, M. (1985) *Cell* 41, 9-10.
2. Bartolf, M., Brennan, E., and Price, C.A. (1980) *Plant Physiol.* 66, 438-441.
3. Wagner, G.T. and Trotter, M.M. (1982) *Plant Physiol.* 69, 804-809.
4. Casterline, J.L., Jr. and Barnett, N.M. (1982) *Plant Physiol.* 69, 1004-1007.
5. Lolkema, P.C., Donker, M.H., Schouten, A.J., and Ernst, W.H.O. (1984) *Planta* 162, 174-179.
6. Rauser, W.E. (1983) *Z. Pflanzenphysiol.* 112, 69-77.
7. Rauser, W.E. (1984) *Plant Sci. Lett.* 33, 239-247.
8. Rauser, W.E. (1984) *Plant Physiol.* 74, 1025-1029.
9. Rauser, W.E. (1984) *J. Plant Physiol.* 116, 253-260.
10. Jackson, P.J., Roth, E.J., McClure, P.R., and Naranjo, C.M. (1984) *Plant Physiol.* 75, 914-918.
11. Jackson, P.J., Naranjo, C.M., McClure, P.R., and Roth, E.J. (1984) In: "UCLA Symposium on Molecular and Cellular Biology", J.L. Key and T. Kosage eds., A.R. Liss, Inc., New York, pp. 145-160.
12. Robinson, N.J. (1984) Ph.D. thesis, University of Liverpool.
13. Bennetzen, J.L. and Adams, T.L. (1984) *Plant Cell Rep.* 3, 258-261.
14. Wagner, G.J. (1984) *Plant Physiol.* 75, 67.
15. Rauser, W.E. and Curvetto, N.R. (1980) *Nature* 287, 563-564.
16. Rauser, W.E., Hartmann, H.J., and Weser, U. (1983) *FERS Lett.* 164, 102-104.

17. Ryden, L. and Deutsch, H.F. (1978) J. Biol. Chem. 253, 519-524.
18. Riordan, J.R. and Richards, V.R. (1980) J. Biol. Chem. 255, 5380-5383.
19. Christie, N.T. and Costa, M. (1984) Biol. Trace Elem. Res. 6, 139-158.
20. Murasuge, A., Wada, C., and Hayashi, Y. (1981) J. Biochem. 90, 1561-1564.
21. Kondo, N., Isobe, M., Imai, K., Goto, T., Murasugi, A., and Hayashi, Y. (1982) Tetrahedron Lett. 24, 925-928.
22. Nordberg, M. and Kojima, Y. (1979) In: "Metallothionein", J.H.R. Kagi and M. Nordberg eds., Birkhauser Verlag, Boston, pp. 41-116.

Nigel J. Robinson, Ph.D., Genetics Group, M886, Los Alamos National Laboratory, Los Alamos, NM 87545, USA.