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Incorporation of Cadmium into Proteins in a Cadmium Tolerant Fungi

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ABSTRACT

Aspergillus carbonarius and a strain of *Penicillium*, a cadmium tolerant fungi, are able to metabolize cadmium chloride up to 2% (w/v). Their amino acids analysis on cadmium free and cadmium chloride containing media indicated certain disorders in their metabolic activities. Cystathionine was only detected in both fungi in the presence of cadmium chloride. However, cadmium was incorporated into several types of low and high molecular weight proteins. The amino acids hydrolyzates of a cadmium containing protein are characterized by the presence of high levels of sulfur amino acids; cysteine and methionine. Ethylasparagine was detected in the hydrolyzate of that cadmium containing protein as well.

Index Entries: Cadmium-tolerant fungi; cadmium proteins; *Aspergillus carbonarius*; strain of *Penicillium*; Cd/protein ratio.

INTRODUCTION

Microorganisms seem able to alter their metabolic activities in order to compensate for the presence of toxic compounds in their external environment. Cadmium was found to be associated with low molecular weight protein in horse kidney (*1*). This protein was later termed metallothionein because of its metal and high sulfur content (*2,3*). Metallothio-

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nein has since been shown to be present in human renal cortex (4), human liver (5), and several animal tissues (5–9).

Metallothionein has been described as a low molecular weight protein with an extremely high content of cysteine, which comprises about 30% of its amino acids (10,11).

Accommodation and fungal tolerance to cadmium effect can possibly be evaluated with identification of cellular micro- and macromolecules. The deleterious influence of cadmium on the metabolic activities would certainly affect the biosynthetic abilities of certain important compounds such as amino acids and proteins. So, in this work the influence of cadmium on amino acids biosynthesis as well as its possible incorporation into proteins are concerned.

MATERIALS AND METHODS

Organisms

A previously identified cadmium tolerant fungi from Sinai soil at Egypt; *Aspergillus carbonarius* and a strain of *Penicillium* were used for the study (12).

Preparation of Fungal Cell Free Extract

Aspergillus carbonarius and *Penicillium sp.* were cultivated on 50 mL Harrold's media supplemented with different concentrations of cadmium chloride (w/v): 0.1, 0.5, 1.5, and 2% as well as cadmium free media. Triplicates for each treatment were considered. The cultures were incubated at 28°C for 7 d.

The harvested mycelia for each treatment were homogenized with an approximately equal volume of 70% ethanol using a MSE homogenizer. The slurry was centrifuged at 6000 rpm for 10 min then dried in a vacuumed desiccator.

Amino Acids Separation

Amino Acids were separated chromatographically on 20 × 20 cm glass plates of thin layer cellulose using two-dimentional separation technique (13).

Identification of amino acids were carried out according to the previously described methods (14).

Determination of Protein

Protein was determined quantitatively with the Folin-phenol reagent using bovine serum albumin as a standard protein (15).

Disc Electrophoresis

Polyacrylamide gel electrophoresis was carried out using a Pharmacia Power Supply EP 400/500 and a Pharmacia gel electrophoresis GE 2/4 (*15*). Ten × 0.8 cm glass tubes were used for the preparation of the gel rods.

Gel Filtration of the Fungal Protein Extract

The concentrate of 45 g fungal extract, 5 mL, was applied on the top of a Pharmacia column (2.6 × 70 cm) packed with Sephadex G 150-120 (Sigma) and allowed to pass into the gel by running the column with tris HCl buffer, pH 7.1. After discarding the void volume, 15 fractions of 5 mL each were collected.

Protein as well as cadmium content of each fraction were determined. Cadmium was determined using Dithiol Loral solution reagents (*17*). Cadmium chloride was used as a standard cadmium containing compound.

RESULTS

Influence of Cadmium on the Fungal Amino Acids Pools

Amino Acids analysis of *A. carbonarius* cultivated on both Cd free and Cd. containing media (Table 1) indicated some alterations in the metabolic pathways. Aspartic acid, glycine, citrulline, hydroxyproline, and γ-amino-butyric acid were not detected in the presence of cadmium. However, other members of their specific pathways were detected. So, on conclusive action of cadmium could be derived from such pattern. Cystathionine and γ-glutamyl derivative of an amino acid were only detected in the presence of cadmium.

On the other hand in the amino acids pool of the *Penicillium sp.* (Table 2) cysteine, phenylalanine, hydroxyproline, and γ-amino butyric acid were not detected in the fungus grown on cadmium containing media. However, other members of their specific pathways were detected. Alternatively cystathionine, glycine, and citrulline were detected only in the presence of cadmium.

Cystathionine has been proved to be synthesized in *Penicillium chrysogenum* and *Candida humicola* only in the presence of selenium in high concentrations (*18*). Glycine is known to play an important part in detoxificiation of foreign compounds in mammals by a mechanism of conjugation with certain toxic compounds (*19*).

Possibly, cystathionine is synthesized as a result of certain disorders in the metabolic activities. Glycine likely plays a similar role in microorganisms as in mammals.

Table 1
Influence of Cadmium on the Biosynthesis of Amino Acids in *A. carbonarius* Grown on Harrold's Liquid Media Provided with Different Concentrations of Cadmium Chloride for 7 d at 28°C[a]

	$Cd\ Cl_2$ concentration, %w/v	
Amino acids	0	0.5
Cysteic acid	M	M
Cysteine	T	S
Cystine	T	W
Cystathionine	O	W
Aspartic acid	M	O
Glutamic acid	M	W
Asparagine	M	M
Glutamine	T	M
Glycine	W	O
Serine	M	W
∞-amino adipic acid	S	M
Threonine	W	M
Tyrosine	M	M
Citrulline	M	O
Hydroxy proline	M	O
Valine	M	M
Norvaline	M	M
γ-aminobutyric acid	W	O
Leucine/isoleucine	M	M
Arginine	W	M
Methionine sulfone	M	M
γ-glutamyl derivative of an amino acid	O	M

[a]Data are expressed according to the intensity of amino acids reactions with ninhydrin as; S, strong; M, medium; W, weak; T, traces; and O, not detected.

Incorporation of Cadmium into Proteins of A. carbonarius *and* Penicillium sp.

Electrophoretogram of fungal proteins showed the presence of several protein bands of irregular distribution (Fig. 1). Generally, no drastic action of cadmium on the qualitative protein biosynthesis was observed. However, it is not clear whether the absorbed cadmium is incorporated into proteins or any other cellular metabolites or just precipitated as a foreign compounds within the fungal cells.

The estimated cadmium content of one cm long gel rod (Table 3) showed that relatively high quantities of cadmium were detected over a wide range of protein bands. The highest quantities of cadmium contents were mostly estimated in the protein extract of *A. carbonarius* grown at 0.5% $Cd\ Dl_2$. Although metallothionein is known to be of low molecular weights, the highest cadmium contents were associated with a high molecular weight proteins. Similar pattern was observed in the *Penicillium sp.* as well.

Table 2
Influence of Cadmium on the Biosynthesis of Amino Acids in *Penicillium sp.* Grown on Harrold's Liquid Media Provided with Different Concentrations of Cd Cl_2 for 7 d at 28°C[a]

	Cd Cl_2 concentration, % w/v	
Amino acids	0	0.1
Cysteic acid	S	W
Cysteine	S	M
Cystine	W	O
Aspartic acid	S	M
Glutamic acid	S	A
∞-amino adipic acid	A	W
Serine	W	M
Methyl-glutamic acid	A	S
Asparagine	W	M
Glutamine	M	M
Threonine	W	W
Tyrosine	M	M
Valine	M	M
Leucine/isoleucine	M	M
Phenyl alanine	M	O
Hydroxy proline	M	O
γ-amino butyric acid	M	O
Cystathionine	O	M
Glycine	O	M
Citrulline	O	W

[a]Amino acids concentrations were judged according to their color intensity as: A, abundant; S, strong; M, moderate; W, weak; and O, not detected.

For further clarifications, protein extracts of *A. carbonarius* was fractionated using a gel filtration columns (Table 4). Obviously, cadmium is mostly distributed over the fractionated proteins. No conclusive pattern of cadmium distribution with protein fractions in respect to their molecular weights could be derived. It is distributed with both high and low molecular weight proteins. Although Cd/protein ratios in the different gel filtration fractions are very low, the occurrence of cadmium over the majority of the separated protein bands on gel electrophoresis as well as on mostly the gel filtration fractions may indicate its actual incorpoation into proteins forming cadmium protein complexes.

Amino Acids Composition of Protein Hydrolyzate of the Seventh Fraction of A. carbonarius *Extract*

The detected amino acids in that hydrolzate were aspartic acid, asparagine, glutamic acid, glutamine, alanine, serine, glycine, threonine, tyrosine, valine, leucine, methionine (sulfoxides), cysteine, and ethylasparagine.

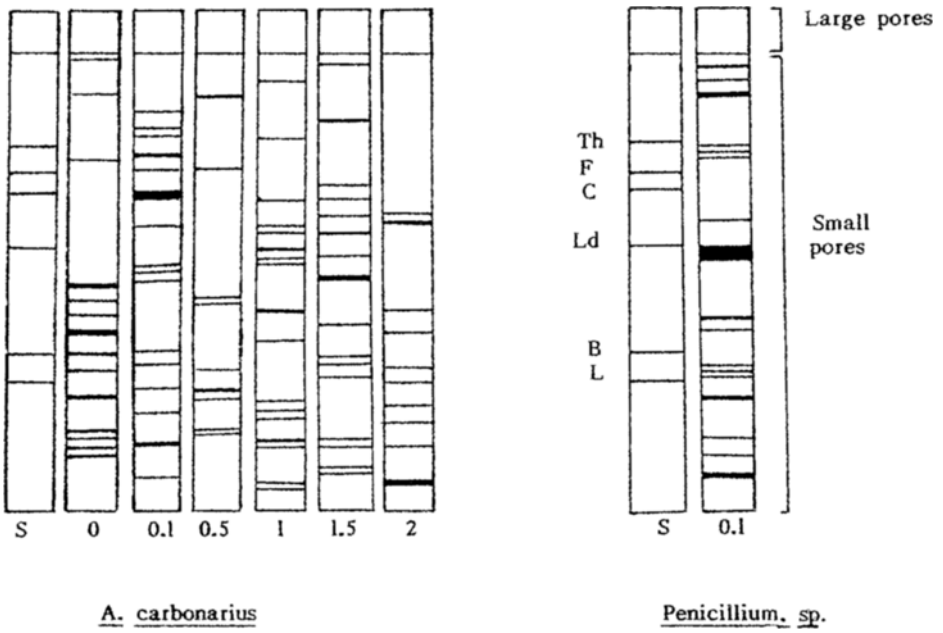

Fig. 1. Patterns of fungal protein extreacts on polyacrylamide gel. S, standard proteins; Th, thyroglobulin (669000); F, ferritin (240000); C, catalase (232000); Ld, lactate dehydrogenase (140000); B, bovine serum albumin (67000); and L, lipase (38000). The indicated numbers; 0, 0.1, 0.5, 1.5, and 2 represent Cd Cl_2 concentrations (% w/v).

Methionine and cysteine were detected in relatively high quantities. Moreover, the detection of ethylasparagine is unusual.

The presence of cysteine and methionine has been known for several metallothionein as a major constituents of their amino acid hydrolyzates.

DISCUSSION

Aspergillus carbonarius and the *Penicillium sp.*, which tolerate high levals of cadmium in their environment, have been unable to control cadmium passage into their cells. However, they seem to have the ability to alter their metabolic activities, avoiding the deleterious action of cadmium. The amino acids contents were mostly normal in the presence of elevated levels of cadmium. More likely, the main biosynthetic pathways are well operated; most of the aspartate and glutamate families members of amino acids were detected. No marked differences were obtained for the fungus grown on cadmium free or cadmium containing media. Such results indicate the ability of the fungi to adapt their metabolic activities and accommodate themselves against cadmium toxicity.

The fractionation of the fungal protein on polyacrylamide gel electrophoresis as well as on gel filtrations revealed the actual incorporation of

Table 3
Determination of Cadmium in Segments of Gel Electrophoresis of Fungal Extract Cultivated on Harrold's Liquid Medium Containing Cadmium for 7 d at 20°C[a]

Organism	Segment distance from the origin, cm	$CdCl_2$ concentration, g% w/v				
		0.1	0.5	1	1.5	2
Aspergillus carbonarius	1	0	62	32	42	U
	2	19	56	38	26	U
	3	35	18	20	36	25
	4	33	U	15	0	U
	5	26	35	48	15	30
	6	32	36	42	U	38
	7	56	22	44	38	42
	8	0	32	0	36	32
	9	0	0	0	0	U
Penicillium sp.	1	54	0			
	2	37	0			
	3	27	0			
	4	15	0			
	5	14	0			
	6	17	0			
	7	21	0			
	8	22	0			
	9	45	0			

[a]Data are expressed as µg cadmium per each gel segment, U, unmeasurable quantities.

Table 4
Gel Fractionation of *A. carbonarius* Protein Extract Cultivated on Harrold's Liquid Medium Amended with 0.1% Cd Cl_2, using a Pharmacia Column (2.6 × 70 cm) Packed with Sephadex G150-120 Equilibrated with Tris HCl Buffer, pH 7.1, Protein was Eluted with the Same Buffer[a]

Fraction number	Protein conc., μg/5 mL	Cadmium conc., μg/5 mL	Cd/protein ratio
1	1300	0.33	0.00025
2	U	0	0
3	1020	0.88	0.00086
4	2500	1.55	0.00062
5	1200	2.05	0.001710
6	1060	2.16	0.00204
7	1150	2.45	0.002130
8	1200	2.05	0.001710
9	1020	1.95	0.001910
10	3200	1.42	0.00044
11	2400	1.65	0.00069
12	U	0.52	U
13	U	0.63	U
14	U	0.48	U
15	350	0.84	0.00240

[a]N.B. U, unmeasurable quantity.

cadmium into high and low molecular weight proteins, such wide distribution in respect to the molecular properties of proteins consistent with a copper proteins (*20*).

Metallothionein is known to play a recognized role as an intermediate in the processing of toxic elements by cells (*21*).

Possibly, both organisms seem capable of producing compounds that bind the metal ions by an absorbents or able to incorporate such ions into certain nonenzymic proteins. The detection of such high quantities of cadmium containing proteins could be a mode of metabolic adaptation against cadmium toxicity.

DEDICATION

To the soul of my late wife and colleague, Dr. Shadia E. Ramadan, who particiapted in carrying out this work.

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