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Cadmium Organo - Chelators in Aspergllus Fumigatus and Penicillium Chrysogenum

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ABSTRACT

Aspergillus fumigatus and Penicillium chrysogenum were cultivated on Dox medium amended with 0.1%(w/v) cadimum sulphate. The presence of cadmium in the growth medium induces the biosynthesis of several low molecular weight metalothionein chelators as well as several short chain cadmium chelatins in O.chrysogenum. A. fumigatus was able to synthesis everal low molecular weight cadmium organo-chelators. Their amino acids composition indicate the presence of restricted number of amino acids. The general characteristics feature is the detection of high levels of cysteine, cystine, glycine, asparagine, serine, methionine and leucine The results confirmid our previous thought that fungi detoxify the action of heavy metals by synthesizing high levels of low molecular weight peptides, chelators and chelatins.

Index Entries : A. fumigatus, P. chrysogenum, heavy metal chelatins, amino acids, chelators cadmium chelatins

INTRODUCTION

It is evident that fungi can withstand levels of heavy metals far in excess of those tolerated by higher plants. Even in uncontaminated habitat fungi tend to have greater internal levels of heavy metals than angiosperms (Hinneri, 1975).

Several heavy metal-binding compounds have been isolated,e.g.two cadmium-binding peptides have been isolated from the fission yeast *Schizosaccharomyces pombe* and from higher plant as well (Salt *et al.*, 1989).

Razak (1989) reported out the biosynthesis of several cadmium-binding proteins in *Aspergillus carbonarius* and a strain of *penicillium.*

The biosynthesis of tellurium, selenium and copper organobinding chelators were induced in fungi in the presence of their heavy metal correspondances. Those organobinding compounds were identified as non enzymatic metalloproteins, metallothioneins, short chains of peptides (chelators) and very short chains of peptides chelatins (Razak *et al.*, 1990 a and b; El-Meleigy., 1991).More recently, Abbass and Razak(1991) reported on the biosynthesis of cadmium, selenium and tellurium-binding low molecular weight proteins and metallothionein in *A. terreus.*

The organochelators, in fungi have been extensively investigated in this laboratory in order to establish a new mode of detoxification mechanism of toxic heavy metals in fungi.

The present investigation is the latest report in series of research work aiming at establishing a new mode of detoxification mechanism in heavy metals tolerant fungi.

MATERIALS AND METHODS

Organisms

Previously identified cadmium-toterant fungi, *Aspergillus fumigatus and Penicilium chrysogenum* were used (Ramadan *et al*, 1989). The fungi were cultivated on Dox medium supplemented with 0.1% (w.v) cadmium sulphate. The P^H was adjusted at 6.5 and incubated at 28± 2 °C for 7 days.

The harvested mycelia of each fungus were homogenized with an approximately equal volume of 70 % (v / v) ethanol using a MSE homogenizer followed by a morter. The slurry was centrifuged at 8000 rpm for 10 min. The supernatant was collected and concentrated using a vaccumed dessicator.

Gel Filtration

The concentrates of *A. fumigatus* and *P. chrysogenum* were applied separately on the top of pharmacia column (2.0x70) cm and 1.5x30 cm) packed with sephadex G_{25} fine (pharmacia) and allowed to pass into the gel by running the column with potassium phosphate buffer of pH 7.1, after discarding the void volume, 30 fractions of 5 ml each from the first colum while 48 fractions of 2 ml each from the second were collected, cadmium as well as the protein content of each fraction were determined.

To determine the approximate molecular weight of the

isolated peptides,a mixture of several standard proteins as well as dyes of known molecular weights were mixed together and fractionated on both columns under the same conditions. The used proteins are : Thyroglobulin,669000; Ferritin, 440000; Catalase, 232000; Lactate dehydrogenase 140000; Bovine serum albumin 67000; Myoglobulin, 181800; Ribonuclase, 137000; Chym-otrypsinogen A, 25000, Ovalbumin, 45000 and Dextrane, 2000000. The used dyes are : Methylthymol blue, 844.76; Methyl blue, 799.80, Light green, 792.86; Bromophenol blue, 669.96; Eosin 409.93 and Methyl orang, 327.34.

Amino acid separation

Amino acids were separated chromatographically on 20x20 cm glass plates with layer cellulose using the two dimensional separation technique, phenol / water : ammonia(200:1,v/v) in the first and n- butanol:acetic acid : water(90:10:29, v / v) in the second direction following the previously reported methods as described in a previous report (Ramadan *et al.*, 1989). The molecular weight of different fractions were constructed from standard curves of the different known molecular weight compounds fractionated under the same conditions

Determination of cadmium

Cadmium content was performed by an Atomic Absorption spectrophotometry (Per kin Elmers, model 2380 at central research

laboratory, Faculty of Science, El-Menoufyia Universty, Shebin El-Komm, Egypt).

Determination of protein

Protein was determined quantitively with the Folin phenol reagent according to the method of Lowry *et al*,(1951) bovine serum albumin was used as standard protein.

RESULTS

The fractionation of a mixture of the different molecular weight dyes under the same separation conditions indicated the approximate molecular weight of the obtained fractions of the fungal proteins. The results (Table1) showed that, proteins of molecular weight over 5000 Da passed htrough the first 10 ml after discarding the void volume.

In *P. chrysogenum* cadmium was found to be mostly associated with all proteins and peptides fractions However, their ratio varied from one fraction to the other although, the highest levels of cadmium were associated with fractions number 6, 7, 9, 10, 11 and 14, of molecular weight over 1600 Da. The highest cd / protein ratio was obtained in fractions number 36, 37, 38, 39, 41 and 42 indicate the high capacity of these very low molecular weight peptides for binding cadmium. Such compounds are more likely serving mainly as cadmium binders, chelatins. The biosynthetic abilities of the fungus to synthesize such wide range

Table (1) : Fractionation pattern of *P. Chrysogenum* cell free extract fractionated on a pharmacia column (1. 5x30 cm) packed with sephadex G_{25}, fine.

Fracion no.	mgm Proteinacious compunds per	PPm of Cd per 2 ml	Cd / protein or peptide ratioy
1	0.736	1.09	1.48
2	1.176	2.23	1.89
3	1.088	3.55	3.26
4	0.968	9.00	9.29
5	0.704	4.37	6.20
6	0.500	7.41	14.82
7	0.572	11.99	20.96
8	0.784	11.10	14.15
9	1.120	9.75	8.76
10	2.208	9.80	4.43
11	2.944	9.55	3.24
12	2.944	9.76	3.31
13	2.576	6.90	2.67
14	0.888	7.40	8.33
15	0.720	7.64	10.61
16	0.776	8.43	10.86
17	0.588	4.18	7.10
18	0.664	6.03	9.08
19	0.404	4.00	9.90
20	0.244	3.32	13.60
21	0.168	1.29	7.67
22	0.160	2.13	13.13
23	0.160	1.62	10.12
24	0.168	1.32	7.85
25	0.184	0.99	5.38
26	0.168	1.04	6.19
27	0.148	0.71	4.79
28	0.124	0.80	6.45
29	0.104	0.89	8.55
30	0.112	0.92	8.21
31	0.060	0.90	15.00
32	0.040	0.17	3.86
33	0.030	1.29	43.00
34	0.075	0.96	12.80
35	0.015	0.46	30.66
36	0.007	1.29	172.00
37	0.015	1.15	76.66
38	0.007	1.04	138.66
39	0.007	0.84	112.00
40	0.000	0.39	0.00
41	0.007	1.04	138.66
42	0.007	0.87	116.00
43	0.015	0.90	60.00
44	0.000	0.10	0.00
45	0.000	0.45	0.00
46	0.000	0.95	0.00
47	0.000	0.92	0.00
48	0.000	0.00	0.00

of cadmium binding low molecular weight peptides and such very low molecular weight peptides of the extraordinary high levels of cadmium supported the fungal growth and thriveness in the presence of such toxic heavy metal. However, it seems unable to channel cadmium incorporation into different compunds.

Amino acids composition of different fractions of *P. crysogenum*

Fraction number 7 reprsented a metallothionein of molecular weight around 1700 Da.Its hydrolysate revealed the presence of cysteine, Cystine,glycine, lysine, serine, hydroxyproleine, asparagine and arginine in addition to several other unknown ninhydrin reacting compounds.

While, fraction 22 represent a metallothionein of molecular weight approx. 1400 Da. Its hydrolysate contained very restricted number of amino acids, cystine, glycine, threonine and methioneine. It seems possible that is some other amino acids in very low concentrations of undetectable quantities.

Although fraction number 41 is a peptide of molecular weight around 500 Da, its hydrolysate contained cysteine, glycine, serine, tyrosine and methioneine.

Interstingly most of the detected amino acids have more than group of lone pair of electrons which enable the amino acids to form complex with the cadmium atom.

Table (2) : Fractionation pattern of A. fumigatus cell free extract fractioned on a pharamacia column (2.5x70cm) packed with G_{25} fine.

Fraction no.	mgm proteinacious per 5ml	PPm of cd per 5ml	Cd / protein orpeptide ratio
1	2.86	0.56	0.195
2	2.58	0.00	0.000
3	0.40	0.00	0.000
4	1.03	0.00	0.000
5	2.58	0.12	0.046
6	7.36	0.00	0.000
7	7.36	0.00	0.000
8	1.94	0.00	0.000
9	2.54	0.37	0.145
10	2.06	0.18	0.087
11	0.75	0.41	0.546
12	0.37	0.32	0.864
13	0.39	0.00	0.000
14	0.50	0.00	0.000
15	0.44	0.00	0.000
16	0.48	0.00	0.000
17	0.46	0.00	0.000
18	0.28	0.00	0.000
19	0.18	0.00	0.000
20	0.06	0.00	0.000
21	0.06	0.00	0.000
22	0.06	0.00	0.000
23	0.11	0.00	0.000
24	0.04	0.00	0.000
25	0.02	0.00	0.000
26	0.02	0.00	0.000
27	0.02	0.00	0.000
28	0.02	0.00	0.000
29	0.02	0.00	0.000
30	0.02	0.00	0.000

On the other hand, the fractionation of *A. fumigatus* cell free extract (Table 2) revealed the presence of only 6 cadmium containing fractions.

The first fraction contained high molecular weight proteins, so it will not be considered.

Fraction number 5 contained a metallothionein of molecular weight approx.1600 Da and the rest of fractions,4 fractions contained peptides of molecular weight in the range of 799-844 Da.

Such pattern may indicates the inability of the fungus to synthesize organochelators compounds or propably the fungus succeeded to channelize the passage of cadmium and consequently its incorporation into active secondary metabolites.Nevertheless, the low molecular weight peptide chelated more cadmium than the higher molecular weights.It was found that the lower the melecular weight the higer cadmium chelating capacity presumably, the fungus seemed able to descriminate between cadmium as toxix element and the other compounds. So,it synthesized several peptides of an extraordinary high capacity of binding cadmium.

Amino acids composition of different fractions of *A. fumigatus* cell free extract.

Two fractions are considered for the amino acids composition of their peptides content.

Fraction number 5 contained metallothionein of molecular

weight approx.1600 Da, its amino acid composition contained cysteine, cystine, glycine, serine, proline, methionine and leucine. While fraction number 11 is a peptide of molecular weight approx.799, it is a cadmium chelation peptide.Its amino acids composition revealed the presence of cysteine, cystine, asparagine, glycine, serine, methionine and leucine.

The general feature in the amino acids composition of those cadmium chelators is the presence of cysteine, cystine glycine, serine, those compounds are known to have lone pair of electrons which enable them to chelate cadmium.

DISCUSSION

With regard to the previous reports concerning detoxification mechanisms in fungi (Razak and Ramadan,1984; Ragab et al., 1989; Razak *et al.*,1988; Ramadan *et al.*, 1989; Razak *et al.*,1990, Razak *et al* ;1990 a & b ; Abbass and Razak 1991). the present report is the final of a long series investigating the role of low molecular weight proteins and peptides in detoxifying the harmful action of toxic heavy metals.

It have been concluded that the deleterous action of selenium, tellurium and copper were avoided in fungi by several mechanisms, one of them is the biosynthesis of metal chelators, metallothionein, chelators and chelatins (Razak *et al.*, 1990 a, b; El-Meleigy ,1991). The results in this report are consistent with

those achevied in the same laboratory that tolerant-fungi are able to synthesize heavy metals chelators, chelatins in high quantities, while non tolerant are not able to do so. Nevertheless, the variation in amino acids compsition of metals chelators may indicate that fungi synthesize non enzymic high molecular weight proteins, low molecular weight proteins (metallothionein), peptids (Chelators) and very short chains peptides of very restricted number of amino acids composition (chelatins) at different levels and structure depending on the fungus itself and the heavy metal as well. such results are detracted from the previously held views that the tolerance of heavy metals is mainly dependent on the organism itself.

Conclusively, it is become evident that heavy metals tolerant-fungi seem able to synthesize high levels of very low molecular weight peptides(Chelators and chelatins)acting mainly for the binding of heavy metals.

Such nechanism is evident and certainly considered new mode of detoxification mechanisms.

Further consideration in bacteria and higher plants are in progress at the labortory as well.

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كلابات كادميوم عضوية فى فطرتى اسبرجيللس فيوميجاتس وبنسليوم كريسوجينم

الملخص العربى

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قسم النبات - كلية العلوم بنات وكلية العلوم بنين

جامعة الأزهر - مدينة نصر - القاهرة ت

تم تنمية فطرتى أسبرجيللس فيوميجاتس وبنسليوم كريسوجينم على وسط غذائى ملائم (دوكس) يحتوى على عنصر الكادميوم (٠١ر٠٪ كبريتات الكادميوم) وقد حفز الكادميوم الفطرتين لتخليق العديد من الميتالوثيونين ذات الأوزان الجزيئية الصغيرة وكذلك الببتيدات ذات السلاسل القصيرة وقد إستطاعت فطرة أسبرجيللس فيوميجاتس تخليق العديد من كلابات الكادميوم العضوية ذات الأوزان الجزيئية الصغيرة ؛ هذا وقد أحتوت تلك المركبات على نسب من الكادميوم وأظهرت النتائج تواجد عدد محدود من الأحماض الأمينية فى تراكيب تلك الميتالوثيونين بنسب عالية وهى السيستين والسيستئين والجليسين والأسبراجين والسيرين والميثونين والليوسين..

وهذة النتائج تثبت أن الفطرتين تقاوما التأثير السام للعناصر الثقيلة بتخليق محتوى عال من الببتيدات ذات الأوزان الجزيئية الصغيرة وكذلك الكلابات.

وإفترضت النتائج تخليق تلك المركبات بواسطة الفطرتين بغرض التخلص من سمية الكادميوم .