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Amino
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Amino acids in chitin – glucan complex of *Aspergillus niger*

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Summary. Deproteinated *A. niger* biomass contains several covalently bound amino acids. The most abundant are arginine, serine, and proline in molar ratio of 3:2:2. One order of magnitude less is the amount of valine, phenylalanine, leucine and glycine. On deacetylation and separation of chitosan from glucan, the main three amino acids remain bound predominantly to chitosan, whereas the hydrophobic amino acids accompany mainly glucan. The presence of arginine could be the cause of stronger basicity of fungal chitosan compared to polyglucosamine.

Keywords: Amino acids – *Aspergillus niger* – Chitosan – Glucan

Introduction

Composition of fungal cell walls was studied by numerous authors. Various contents of polysaccharides, proteins and lipids were found in different strains (Bartnicki-Garcia, 1968). Although a major portion of the cell wall polysaccharides can be solubilized by treatment with hot water, phenol or alkali, a substantial proportion remains insoluble even in alkali (Johnston, 1965). The alkali-insoluble cell wall residue of ascomycetes and basidiomycetes consists mainly of (1-3)-β-D/(1-6)-β-D-glucan and chitin. Chitin is thought to be present as microfibrils physically embedded in a β-glucan matrix (Rosenberger, 1976; Sietsma and Wessels, 1981).

Chitin, poly-β-(1-4)-N-acetyl-D-glucosamin, a cellulose-like biopolymer is among the most abundant organic compounds on earth (Knorr, 1984). It has an unusual combination of properties, including toughness, bioactivity and biodegradability, which makes it an attractive specialty material. Free amino groups contribute to Chitosan (deacetylated chitin) polycationic, chelating and film-forming properties (Austin et al., 1981).

Amino acids were commonly found in the alkali-insoluble chitin-glucan complex of fungi and were generally thought to be derived from peptidoglycans (Rosenberger, 1976). Kisser et al. (1980) reported that predominant amino acids of the whole *A. niger* biomass were: aspartate, serine, threonine, glutamate, proline, glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine, tyrosine, lysine, arginine and histidine.

Amino acids bound to the chitin-glucan complex of the *A. niger* cell wall are reported in present paper.

Material and methods

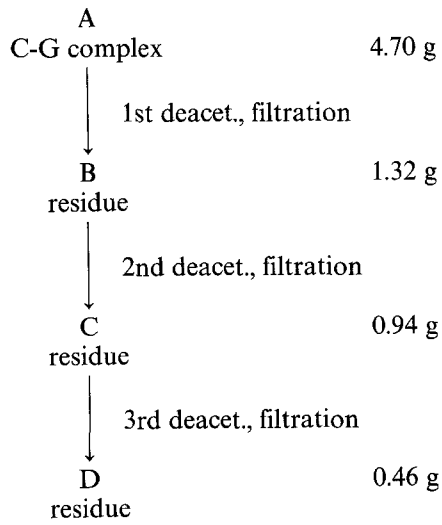
Aspergillus niger biomass was obtained as byproduct from surface production of citric acid in TOK, Ilirska Bistrica, Slovenia.

Isolation of chitin – glucan complex

Chitin-glucan (C-H) complex was isolated by a modified Stojanov et al. (1986) procedure. *A. niger* mycelium was exhaustively extracted with 0.2M NaOH and water at 50°C, alternatively. The treatment was monitored by determination of soluble proteins in supernatant with standard Biuret method (Coakley and James, 1978).

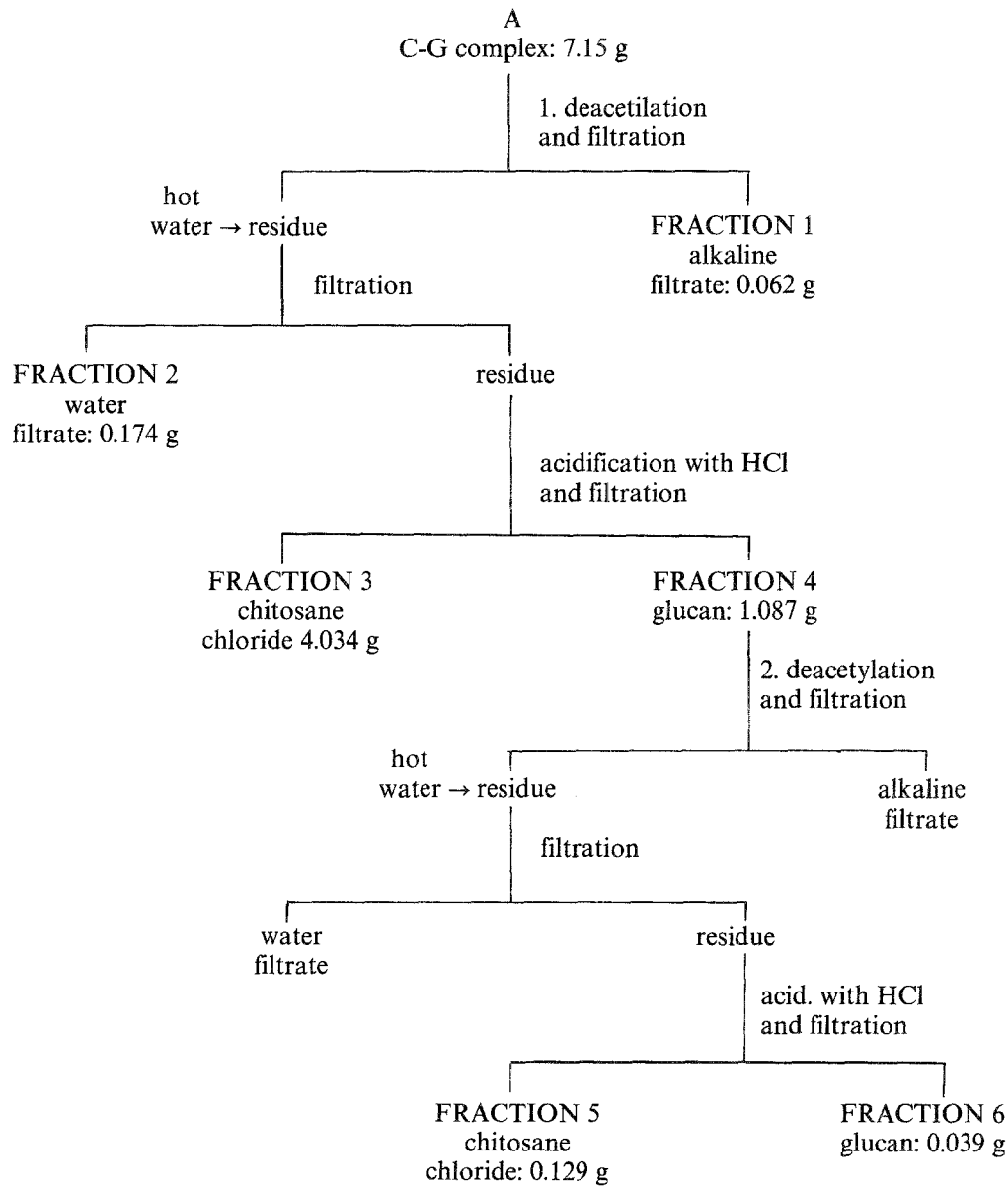
Chitin-glucan complex fractionation before and after deacetylation

In the first series of experiments one part of chitin-glucan complex was analysed for amino acids while the other part was deacetylated three times with fresh 40% NaOH at its boiling point for 20 min (Mima et al., 1983), Scheme 1. After each step the complex was liophylized and tested for remaining amino acids.



Scheme 1. Repeated deacetylation of chitin-glucan complex

In the second series of experiments the chitin-glucan complex was deacetylated as above and separated into different fractions, Scheme 2. Fraction 1 was the filtrate obtained after treatment of chitin-glucan complex with 40% NaOH. The precipitate was washed with water and the water solution was fraction 2. The remaining precipitate was acidified with HCl and filtered. Fraction 3 was the filtrate containing chitosane chloride. Fraction 4 was the precipitate remaining after removal of chitosane chloride. The latter precipitate was deacetylated again and the treatment was repeated. Fraction 5 was the filtrate containing chitosane chloride, whereas fraction 6 was the precipitate. Each fraction was concentrated, lyophylized and analysed for amino acids.



Scheme 2. Deacetylation with separation of components of the chitin-glucan complex

Hydrolysis of chitin-glucan complex

10 mg of lyophylized sample was hydrolysed with 2 ml 6 M HCl for 48 hours at 110°C in nitrogen atmosphere.

Amino acids analysis

Amino acids analysis was performed according to Pečavar et al. (1991) by HPLC of 9-fluorenylmethyl chloroformate derivatives.

Results and discussion

The results of protein determination in supernatants indicate that successive extractions of biomass with alkali and hot water successfully remove soluble proteins from other cell wall components of *Aspergillus niger* biomass, Table 1.

Scheme 1 represents the course of the first series of experiments, deacetylation of the chitin-glucan complex without separation of its components. The contents of amino acids in chitin-glucan complex is presented in Table 2. Arginine, serine, and proline are the most abundant cell wall bound amino acids in molar proportion of approx. 3:2:2.

Table 1. The proteins contents in supernatants remaining after successive deproteination of *A. niger* biomass. Values are given as mg of proteins/l

	Alkali	Water
Water wash		1275
1st deproteination	688	475
2nd deproteination	812	<20
3rd deproteination	<20	/
4th deproteination	<20	<20
5th deproteination	<20	<20

After repeated deacetylation of the chitin-glucan complex arginine, serine, and proline remained predominant cell wall bound amino acids in almost the same molar ratio as before deacetylation. Besides the main amino acids arginine, serine, and proline in the cell wall of *Aspergillus niger* there is also significant the amount of valine, phenylalanine, leucine, and glycine.

Sietsma and Wessels (1979) reported that predominant amino acids in chitin-glucan complex of *Schizophyllum commune* were lysine, citrulline and glutamic acid whereas in crustacean chitin aspartic acid, serine, and glycine predominated (Brine and Austin, 1981). Whether these differences are a consequence of either differences in phylogenetic position or they are connected with the need to bind anionic cell wall constituents or Ca^{2+} ions to the chitin-glucan complex, respectively, can not be concluded from these few data. The amount of covalently bound amino acids was 10,7% of total chitin-glucan complex of *S. commune*, (Sietsma and Wessels, 1981), and 3,3% of chitin-glucan complex of *A. niger* (Table 2B).

Table 2. A The amino acids composition of chitin-glucan complex before and after repeated deacetylation. **B** Amino acids content in insoluble residues

A

Amino acids	A Chitin-glucan μmol/g	B 1st deacet. μmol/g	C 2nd deacet. μmol/g	D 3rd deacet. μmol/g
His	/	0.50	0.43	0.35
Arg	89.10	100.15	126.23	111.71
Ser	60.40	69.92	90.54	79.03
Asp	0.25	0.74	/	/
Glu	0.29	0.21	0.17	0.17
Thr	2.59	1.29	0.46	0.46
Gly	4.10	7.77	6.30	6.45
Ala	2.84	4.57	1.36	0.74
Pro	61.72	53.31	75.38	72.23
Met	0.30	0.15	/	/
Val	7.51	16.90	13.33	11.64
Phe	4.86	9.66	7.33	5.13
Ile	1.51	2.94	1.85	2.35
Leu	2.60	4.11	4.44	8.05
Lys	2.71	1.96	1.88	2.03
Tyr	1.94	0.67	1.64	0.67
Total	242.71	274.83	331.33	300.99

B

Sample	% Amino acids
A – chitin-glucane complex	3.29
B – residue after 1st deacet.	3.27
C – residue after 2nd deacet.	4.51
D – residue after 3rd deacet.	4.08

Scheme 2 shows the course of the second series of experiments, where after the first deacetylation, the chitosane fraction is separated from the glucan fraction and the latter is deacetylated and separated again.

As shown in Table 3, after first deacetylation predominant amino acids remain mainly in the chitosan fraction. In this fraction remain 50% of primarily present arginine and serine and 40% of proline, respectively, in molar ratio of approx. 8:7:5. Amino acids valine and phenylalanine remain mainly in the glucan fraction, while leucine and glycine are present in both the chitosan and glucan fraction.

As shown in Table 4, after further deacetylation of the former glucan fraction and its separation into two fractions, the predominant amino acids in chitosan 5 as well as in glucan fraction 6 remained arginine and serine. The content of proline strongly decreased compared to arginine and serine. In the remaining

Table 3. Amino acids in fractions of chitin-glucan complex, Scheme 2, after first deacetylation

Amino acids	Fraction 1 Alk. filt. μmol/g	Fraction 2 Water filt. μmol/g	Fraction 3 Chit. Cl μmol/g	Fraction 4 Glucan μmol/g
His	/	0.97	1.23	0.97
Arg	2.07	42.88	78.01	30.48
Ser	3.90	37.30	68.32	27.41
Asp	/	/	0.53	0.45
Glu	1.79	0.60	0.71	0.52
Thr	1.09	0.25	1.09	0.67
Gly	6.93	2.66	3.33	4.13
Ala	3.23	0.79	1.24	2.69
Pro	3.39	29.88	47.43	28.14
Met	/	1.34	1.21	1.01
Val	3.07	4.27	3.59	19.63
Phe	1.03	2.48	4.18	21.55
Ile	1.45	1.29	1.37	2.29
Leu	1.60	2.13	2.21	1.98
Lys	1.16	0.68	2.53	2.53
Tyr	/	/	0.88	1.60
Total	30.74	127.52	217.86	146.05

Table 4. A Amino acids distribution in fractions of chitin-glucan complex, Scheme 2, after second deacetylation. **B** Amino acids content in analysed samples

A

Amino acids	Fraction 5 Chit. Cl μmol/g	Fraction 6 Glucan μmol/g
His	/	/
Arg	19.92	16.25
Ser	20.74	19.69
Asp	/	/
Glu	0.37	0.67
Thr	0.42	0.34
Gly	5.46	2.93
Ala	/	/
Pro	2.87	2.08
Met	/	/
Val	5.29	23.90
Phe	1.27	7.81
Ile	0.46	1.52
Leu	0.61	1.52
Lys	/	1.23
Tyr	/	2.09
Total	57.41	80.03

B

Sample	% Amino acids
Fraction 1 (alkali filtrate)	0.37
Fraction 2 (water filtrate)	1.71
Fraction 3 (chitos. chloride)	2.95
Fraction 4 (glucan)	1.96
Fraction 5 (chitos. chloride)	0.75
Fraction 6 (glucan)	1.06

glucan fraction 6 also significant contents of valine and phenylalanine followed by glycine and tyrosine were determined, whereas in the chitosane fraction the content of glycine and valine was remarkable.

Amino acids arginine, serine, and proline are in chitosan fraction 5 in molar ratio of approx. 7:7:1 and in glucan fraction 6 in molar ratio of approx. 8:10:1.

From the Table 4 follows, that on repeated deacetylation and separation of glucan fraction 4 proline was removed from both fractions 5 and 6 more than arginine and serine. Valine remained largely unremoved.

Muzzarelli (1979) states that the glucan fraction improves the properties of chitosan obtained from the *A. niger* biomass. Li et al. (1990), on the other hand, report that fungal chitosan posesses at pH 7 a positive zeta potential and a marked cationic behaviour not explainable by the properties of poly glucosamine. The presence of covalently bound amino acids, especially of arginine (pK = 12,5), could explain these observations.

Acknowledgement

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