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GLYCOPROTEINS FROM *USTILAGO TRITICI*

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Abstract—A complex mixture of glycoproteins has been isolated from *Ustilago tritici* cells obtained from diseased *Triticum vulgaris* plants. Composition analysis and hemagglutination inhibition assay of the purified material shows the presence of an unusual glycoprotein in the fungal spore.

INTRODUCTION

Ustilago tritici causes a commonly known infection of the seeds of *Triticum vulgaris* which is observed as soon as wheat begins to ear. The disease is characterized by a black powdery mass of spores. Antisera against the whole cell preparation and the supernatant cell fractions of *Ustilago maydis*, corn smut, has been reported [1]. The present study was aimed at isolating and identifying an antigenic material, and we report the isolation, composition and nature of a complex mixture of glycoproteins in the water-soluble portion of the cells.

RESULTS AND DISCUSSION

The water extract was dark brown in color and contained carbohydrates and amino acids. Gel filtration of the extract on Sepharose 6B showed the presence of at least two high MW materials containing carbohydrates and proteins. The two components (A and B) contained sugars and amino acids (Table 1) and were present in the ratio of 3:7. Polyacrylamide gel electrophoresis revealed a complex mixture of polymers which gave a positive reaction with periodate-Schiff reagent and Coomassie blue suggesting the presence of glycoproteins. Fraction B, a major fraction from Sepharose 6B, was further fractionated on a column of Sepharose 2B into two fractions, B_1 and B_2. The composition of the two fractions is reported in Table 2. The proportion of carbohydrate to amino acids in the glycoprotein was *ca* 4:1, and the two components together accounted for only 40% of the total wt. It is likely that the brown material, which was present in the H_2O extract and eluted with the glycoproteins on gel filtration from Sepharose 6B and Sepharose 2B, contributed to the remainder of the wt.

Fraction B_2 was eluted as a single component from the DEAE–cellulose column with phosphate buffer. The inhibition studies on Fraction B_2 indicated weak but significant inhibitory activity against *Glycine max* hemagglutinin, concanavalin A, *Triticum vulgaris* hemagglutinin and *Solanum tuberosum* hemagglutinin [2], but no significant activity was observed in the inhibition of agglutination by *Ricinus communis* hemagglutinin. Although hemagglutination inhibition specifities based upon mono- and disaccharides do not always apply to macromolecular structure [3], the results suggest certain structural features. From the known specifities of these hemagglutinins [2] it is possible that this glycoprotein may contain an α-D-glucopyranosyl and/or an α-D-mannopyranosyl residue, a 2-acetamido-2-deoxy-β-D-glucopyranosyl and a non-reducing terminal 2-acetamido-2-deoxy-β-D-galactopyranosyl residue in the

Table 1. Composition of glycoproteins separated on a column of Sepharose 6B

Components	Fraction A	Fraction B
*Amino acids**		
Asp	97	140
Thr	67	95
Ser	119	147
Glu	121	149
Pro	45	65
Gly	155	158
Ala	89	82
Cys 1/2	16	
Val	59	38
Ile	41	20
Leu	70	31
Tyr	11	9
Phe	32	16
Lys	37	25
His	22	9
Arg	19	16
Carbohydrates†		
L-Fucose	1.0	1.0
D-Galactose	13.0	24.5
D-Glucose	4.5	7.0
D-Mannose	1.5	5.0
N-Acetylglucosamine	1.5	2.5
N-Acetylgalactosamine	0.2	0.2

* Residues per 1000 residues.
† Molar ratio relative to L-fucose.

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Table 2. Composition of glycoproteins separated on a column of Sepharose 2B

Components	Fraction B_1	Fraction B_2
*Amino acids**		
Asp	60	123
Thr	66	77
Ser	191	193
Glu	73	91
Pro	56	61
Gly	265	173
Ala	101	91
Val	22	41
Ile	43	35
Leu	115	40
Tyr	2	7
Phe	-	12
Lys	-	12
His	2	22
Arg	4	22
Carbohydrates†		
L-Fucose	1.0	1.0
D-Galactose	13.0	22.0
D-Glucose	5.0	6.0
D-Mannose	2.0	4.0
N-Acetylglucosamine	1.0	1.8
N-Acetylgalactosamine	0.1	0.2

* Residues per 1000 residues.
† Molar ratio relative to L-fucose.

structure, but that it does not have a β-D-galactopyranosyl residue as a terminal sugar.

These results are consistent with the compositional data (Table 2). Inhibitory activity against hemagglutination with concanavalin A is in agreement with the large proportion of mannose and glucose present. The weak inhibition activity against *R. communis* hemagglutinin, despite the larger proportion of galactose in B_2 raises two possibilities. Since galactose represents over 65% of the total carbohydrate residues (Table 2), a high proportion of this component must reside in a terminal position in an active α-configuration or is attached β in an inactive sequence, possibly Gal→Man, which to our knowledge has not been investigated. Both Gal→GlcNAc and Gal→Glc linked β 1,4 would be expected to be active.

The composition of this glycoprotein is unlike those of other glycoproteins of fungal origin, both in secretion and in the cell envelope [4], as it not only contains L-fucose and *N*-acetylhexosamines but also an unusual proportion of D-glucose, D-galactose and D-mannose. In addition, a very small amount of a sugar was detected by GLC with R_t similar to that of L-rhamnose. The function of this glycoprotein in *Ustilago tritici* cells is unclear although it may have some immunogenic significance.

EXPERIMENTAL

Ustilago tritici was collected from diseased plants (*Triticum vulgaris*) and the glycoproteins extracted by dispersing the spores (1 g; previously extracted with Et_2O and EtOH) in H_2O (100 ml) at 4° for 24 hr. The dispersion was centrifuged and the supernatant was lyophilized (10 mg). Gel chromatography was carried out on columns of Sepharose 6B and 2B using 5 mM Tris·HCl (pH 7.3) containing 50 mM NaCl and 50 mM NaPi (pH 7), respectively. Ion-exchange chromatography on DEAE-cellulose was performed with a gradient of 0.1–1 M NaPi (pH 6.8), followed by elution with 0.2 M LiCl. The eluates were examined for carbohydrates using the PhOH-H_2SO_4 reagent [5] and the presence of protein was detected by A at 278 nm. Gel electrophoresis was carried out by the modified procedure of ref. [6]. The gels were prepared by mixing polyacrylamide (2%) and agarose (0.75%). The sample soln, running gel and electrode buffer contained 0.1% SDS. Carbohydrate-containing polymers were detected with the periodate-Schiff reagent and proteins were stained with Coomassie blue. Sugar residues were identified and estimated by GLC [7]. Amino acid analyses were performed on glycoprotein samples, after hydrolysis with 6 M HCl for 20 hr at 105° on an amino acid analyser. *Canavalia ensiformis* hemagglutinin [8], *Ricinus communis* hemagglutinin [9], *Triticum vulgaris* hemagglutinin [10] and *Solanum tuberosum* hemagglutinin were purified by affinity chromatography. *Glycine max* hemagglutinin was used as a crude extract. The titration and inhibition assays were performed with human erythrocytes according to the method of ref. [3]. The cells used for inhibition assays on Concanavalin A and *Glycine max* hemagglutinin were trypsin-treated [11].

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