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CHARACTERISTIC DIFFERENCES IN MONOSACCHARIDE COMPOSITION OF GLYCOCONJUGATES FROM OPPOSITE MATING TYPES OF *Chlamydomonas eugametos*

GERRIT J. GERWIG, JOHANNIS P. KAMERLING, JOHANNES F. G. VLIEGENTHART,
Department of Bio-Organic Chemistry, State University of Utrecht, Utrecht (The Netherlands)

WIEGER L. HOMAN, PIET VAN EGMOND, AND HERMAN VAN DEN ENDE
Department of Plant Physiology, University of Amsterdam, Amsterdam (The Netherlands)

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ABSTRACT

The monosaccharide composition of the isoagglutinins derived from the flagellar membranes of the opposite mating types of *Chlamydomonas eugametos* (mt^+ and mt^-) differ strikingly. Besides the common occurrence of L-arabinose, L-rhamnose, D-xylose, D-mannose, D-galactose, L-galactose, D-glucose, and 2-acetamido-2-deoxy-D-glucose, the mt^+ isoagglutinin carbohydrate chains contain gulose, 4-*O*-methylxylose, a 2-*O*-methylpentose, and 3-*O*-methyl-D-galactose, whereas those of the mt^- isoagglutinin contain 6-*O*-methyl-D-mannose and 3-*O*-methyl-L-glucose. Monosaccharide constituents were analysed by g.l.c. and g.l.c.–m.s. of trimethylsilylated methyl glycosides and alditol acetates. For the assignment of the absolute configurations using g.l.c., trimethylsilylated (−)-2-butyl glycosides were analysed. In view of the absolute specificity of the agglutination process displayed by the isoagglutinins of the sexually different mating types, the carbohydrate differences might be significant for the sexual interaction.

INTRODUCTION

An essential stage in the sexual intercourse of the green alga *Chlamydomonas* is the intimate flagellar association of mating cell-pairs[1]. It brings the partner cells in close contact and initiates cellular activities mandatory for gametic fusion, for example, activation of the fertilisation tubule and cell-wall shedding[2,3]. The interaction is restricted to gamete flagella of different mating types (mt^+ and mt^-) of one species. The mating process is extremely rapid and efficient, as is evident from the massive clumping of cells (agglutination), within seconds, after mixing mt^+ and mt^- gametes. The ability to interact disappears rapidly after cell fusion.

For *C. eugametos*, it has been demonstrated that glycoconjugates of high molecular weight are major constituents of the flagellar membrane[4]. Several findings suggest that the carbohydrate chains of these glycoconjugates are involved

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in the agglutination reaction[5,6], *e.g.*, treatment of flagella with periodate completely abolishes agglutinability in both mating types and destroys mating-type, specific, antigenic sites[7].

From the medium of gamete cultures, membrane vesicles can be isolated which have a structure less complicated than that of the flagella, but which exhibit similar adhesive properties. Mixing vesicles with gametes of the opposite mating type results in the isoagglutination of these gametes. The vesicles (isoagglutinins) are allegedly derived from the flagellar membrane[8]. Gel-electrophoresis patterns of flagella and isoagglutinins of the same mating type, as visualised by staining with the periodate–Schiff reagent, show only minor differences. Isoagglutinins have the advantage that they are enriched in carbohydrate-containing material having a monosaccharide profile similar to that of the corresponding flagella. Preparations of whole flagella contain a large excess of axonemal proteins, which disturb sugar analysis procedures to some extent.

In order to obtain more insight into the biological role of the carbohydrate chains of the flagellar membrane glycoconjugates, the structure elucidation of these chains is of interest. We now report on the constituent monosaccharides present in the mt^+ and mt^- isoagglutinins of *C. eugametos*.

RESULTS AND DISCUSSION

Pure isoagglutinin particles of both mating types of *C. eugametos* ($mt^+$and mt^-) were obtained from corresponding cell suspensions *via* various centrifugation and filtration steps (see Experimental)[1,9]. Generally, the carbohydrate compositions of these isoagglutinins were determined by g.l.c. and g.l.c.–m.s. of their derivatised methanolysates or hydrolysates. Methyl glycosides were analysed as the corresponding trimethylsilylated derivatives[10–12], and the monosaccharides as the alditol acetates[13,14].

The absolute configuration of each monosaccharide constituent was determined by g.l.c. of the trimethylsilylated (−)-2-butyl glycosides, prepared from methanolysates of the glycoconjugates[15,16]. Fig. 1 shows the gas chromatograms of the mixtures of trimethylsilylated methyl glycosides from mt^+ and mt^- isoagglutinins. The results of the various g.l.c. and g.l.c.–m.s. analyses are summarised in Table I.

Comparison of the data obtained for the mt^+ and mt^- isoagglutinin preparations shows a number of striking differences in the monosaccharide compositions, especially with respect to the *O*-methylated sugar patterns. Definite assignments for the mt^+ isoagglutinin demonstrated the occurrence of 4-*O*-methylxylose, a 2-*O*-methylpentose, and 3-*O*-methyl-D-galactose, whereas the constituent *O*-methylated sugars of the mt^- preparation were 6-*O*-methyl-D-mannose and 3-*O*-methyl-L-glucose. The presence of a trace of 6-*O*-methylmannose in the mt^+ preparation could not be excluded (see Fig. 1*A*). The structure determination of the *O*-methylated monosaccharides was accomplished by the specific m.s. data for correspond-

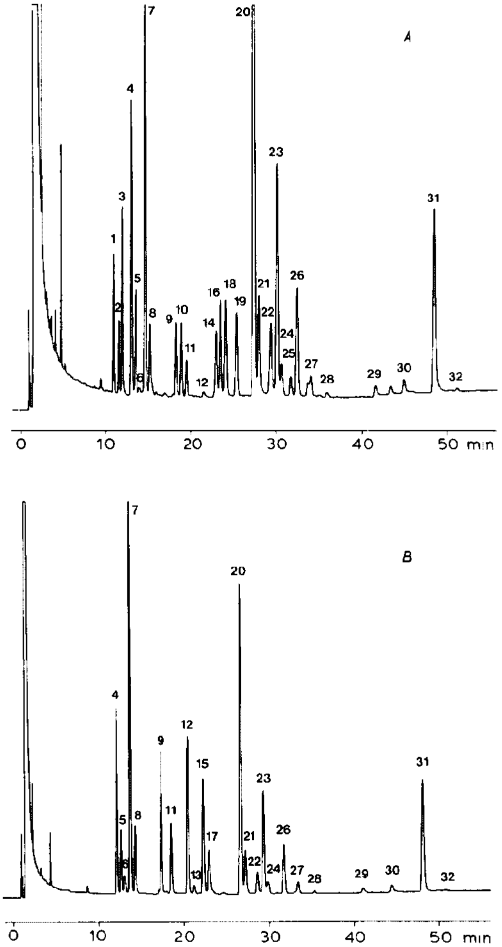

Fig. 1. *A*, SE-30 Capillary gas chromatogram of the mixture of trimethylsilylated methyl glycosides derived from the *mt*$^+$ isoagglutinin; *B*, SE-30 capillary gas chromatogram of the mixture of trimethylsilylated methyl glycosides derived from the *mt*$^-$ isoagglutinin. Key: 1 and 2, 4-*O*-methylxylose; 3, 2-*O*-methylpentose; 4 and 5, arabinose; 6, 9, and 11, xylose; 7 and 8, rhamnose; 10, 18, 19, and 22, gulose; 12 and 13, 6-*O*-methylmannose; 14, 16, and 18, 3-*O*-methylgalactose; 15 and 17, 3-*O*-methylglucose; 20, 22, and 25, mannose; 21, 23, 24, and 26, galactose; 27 and 28, glucose; 29, 30, 31, and 32, 2-acetamido-2-deoxyglucose.

ing trimethylsilylated, methylated methyl glycosides[11,17–21] and acetylated, methylated alditols[14,22]. Because of the inherent insensitivity of electron-impact m.s. for the configurations of pentose and hexose derivatives, co-chromatography with several reference *O*-methylated sugars was also carried out. For the proposed *O*-methylated hexoses, suitable reference compounds were available. The *xylo* configuration of the 4-*O*-methylpentose was assigned by comparison of the g.l.c. and m.s. data for the mixture of alditol acetates prepared from an *mt*$^+$ hydrolysate and reference 2-*O*-methylxylitol. In the same way, the *xylo* configuration was excluded

TABLE I

DATA FOR *mt*$^+$ AND *mt*$^-$ ISOAGGLUTININ MONOSACCHARIDE CONSTITUENTS

Monosaccharide	T^a	T^b	*Absolute configuration*^{c}	*Molar ratio*^{d} mt$^+$ *Isoagglutinin*		*Molar ratio*^{a} mt$^-$ *Isoagglutinin*	
				Methanolysis	*Hydrolysis*	*Methanolysis*	*Hydrolysis*
Arabinose	0.31; 0.33	0.65	L	2.0	1.9	2.7	2.5
Rhamnose	0.35; 0.37	0.53	L	2.5	2.1	6.4	5.3
Xylose	0.34; 0.45; 0.48	0.71	D	0.7	0.6	3.1	2.5
Mannose	0.68; 0.73; 0.79	1.00	D	5.0	5.0	5.0	5.0
Gulose^e	0.46; 0.60; 0.63; 0.73	1.05	n.d.^{h}	1.3	4.5	—	—
Galactose^f	0.69; 0.75; 0.76; 0.81	1.05	D and L	3.8		4.0	4.1
Glucose	0.85; 0.90	1.05	D	0.2		0.3	
2-Acetamido-2-deoxyglucose^g	1.04; 1.12; 1.22; 1.28	n.d.	D	2.5	n.d.	3.2	n.d.
4-*O*-Methylxylose	0.27; 0.28	0.55	n.d.	1.0	1.1	—	—
2-*O*-Methylpentose	0.29	0.43	n.d.	0.9	1.1	—	—
6-*O*-Methylmannose	0.53; 0.54	0.77	D	—	—	2.4	2.1
3-*O*-Methylgalactose	0.57; 0.58; 0.60	0.98	D	1.8	1.4	—	—
3-*O*-Methylglucose	0.57; 0.58	0.93	L	—	—	2.0	2.2

[a] G.l.c. retention times of the corresponding trimethylsilylated methyl glycosides relative to Me$_3$Si-mannitol on an SE-30 glass-capillary column (temperature programme, 135→220° at 1°/min). [b] G.l.c. retention times of the corresponding alditol acetates relative to mannitol hexa-acetate on an SP-1000 glass-capillary column (temperature programme, 165→220° at 1°/min). [c] Determination by g.l.c. on an SE-30 glass-capillary column using the corresponding trimethylsilylated (−)-2-butyl glycosides (temperature programme, 135→220° at 1°/min). [d] Methanolysis values were calculated by using molar adjustment factors; for *O*-methylated monosaccharides, the molar adjustment factors of the corresponding unmethylated sugars were taken into account. Hydrolysis values represent uncorrected peak areas. In both cases, the molar ratio of mannose was arbitrarily taken to be 5.0. [e] The *T*-value 0.46 represents 1,6-anhydro-β-gulopyranose. [f] Present as D- and L-galactose; *mt*$^+$ ratio 63:37, *mt*$^-$ ratio 52:48. [g] Not determined by the alditol acetate method. [h] Not determined because of the formation of high yields of 1,6-anhydrogulose.

for the 2-*O*-methylpentose. In view of the occurrence of the unmethylated pentoses xylose and arabinose, it is tempting to propose an *arabino* configuration for the 2-*O*-methylpentose. Finally, it has to be noted that, although glucose occurs in the D configuration, 3-*O*-methylglucose has the L configuration.

Besides the differences in the *O*-methylated sugar patterns mentioned above, gulose was clearly detected in the mt^+ isoagglutinin preparation. The occurrence of a trace of this monosaccharide in the mt^- isoagglutinin could not be excluded (see Fig. 1*B*). The mannose:galactose:glucose molar ratios for the mt^+ and mt^- preparations are identical. Galactose occurs in both enantiomeric forms. The ratio of D- and L-galactose was 63:37 for mt^+ and 52:48 for mt^-. Other polysaccharides have also been shown to contain both enantiomeric forms of galactose[23].

Thus, the sexually different mt^+ and mt^- isoagglutinins, derived from the corresponding flagellar membranes of *C. eugametos*, each have a specific monosaccharide composition. The two different patterns of *O*-methylated sugars and the absence or presence of gulose are particularly interesting. In connection with these observations, it should be mentioned that the amino acid and lipid compositions of mt^+ and mt^- isoagglutinins are identical (unpublished results). In view of the absolute specificity of the agglutination process displayed by the isoagglutinins of the sexually different mating types, the observed carbohydrate differences might be significant for the complex process of sexual interaction.

EXPERIMENTAL

Cell cultivation and preparation of mt$^+$ *and* mt$^-$ *isoagglutinins of* Chlamydomonas eugametos. — The culture of *C. eugametos* cells [stock no. 11-5/9 (mt^+) and 11-5/10 (mt^-) of the Sammlung von Algenkulturen, Göttingen (Germany), or UTEX 9 (mt^+) and UTEX 10 (mt^-) of the Culture Collection of Algae at the University of Texas, Austin (U.S.A.)] has been described[1]. Two-week-old cultures were flooded with sterile distilled water. After 18 h, the cell suspensions were collected and the cells removed by centrifugation at 1,000*g* for 10 min. The cell-free medium was centrifuged at 10,000*g* for 20 min, and the supernatant solution was filtered through a 1.2-μm Millipore filter to remove cell debris. The filtrate was concentrated in an Amicon CH4 concentrator fitted with a Diaflow hollow-fiber membrane cartridge H1P10 (retention 10^3 daltons), followed by centrifugation at 100,000*g* for 1 h. The pellet was subjected to CsCl-cushion centrifugation[9] (twice) to remove cell-wall material, and then dialysed against 10mM Tris-HCl buffer (pH 7.8) and lyophilised, to yield the pure isoagglutinin particles.

Analysis procedures. — Methanolysis (M methanolic HCl) of 2-mg samples was carried out essentially as described earlier[11,12]. Before neutralisation with Ag_2CO_3, the solutions were extracted three times with hexane. After *N*-reacetylation, part of the material was trimethylsilylated, and the resulting mixture of derivatised methyl glycosides was analysed by g.l.c. and g.l.c.–m.s. Another part was subjected to (−)-2-butanolysis with M (−)-2-butanolic HCl, *N*-reacetylation, and trimethylsilylation, and then analysed by g.l.c.[15,16]. Hydrolysis (0.25M H_2SO_4) of 2-mg samples and subsequent derivatisations to alditol acetates were performed as reported[14]. For the reduction of the monosaccharide mixture, sodium borodeuteride was used. Capillary g.l.c. of trimethylsilylated methyl and (−)-2-butyl glycosides on SE-30 (column dimensions, 25 m × 0.35 mm; oven-temperature programme, 135→220° at 1°/min) and of alditol acetates on SP-1000 (column dimensions, 25 m × 0.26 mm; oven-temperature programme, 165→220° at 1°/min) on a Varian Aerograph 2740 were carried out as described earlier[21].

G.l.c.–m.s was performed with a combined Hewlett-Packard 5710A gas chromatograph/Jeol JMS-D300 mass spectrometer/Jeol JMA-2000 mass-data analysis system. Mass spectra (70 eV) were recorded using an ion-source temperature of 170°, an accelerating voltage of 3 kV, and an ionising current of 300 μA. For trimethylsilylated methyl glycosides, a glass column (2 m × 2 mm) packed with 3% of OV-1 on Chromosorb W HP (80–100 mesh) was used; for alditol acetates, 3% of OV-225 was used.

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