Carbohydrate Research, 83 (1980) 187–192
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Comparison of the structures of the major components of the stigma and style secretions of *Gladiolus*: the arabino-3,6-galactans

PAUL A. GLEESON*† AND ADRIENNE E. CLARKE*

School of Botany, University of Melbourne, and CSIRO Division of Protein Chemistry†, Parkville, Vic. 3052 (Australia)*

(Received July 9th, 1979; accepted for publication, September 4th, 1979)

Arabinogalactans and arabinogalactan–proteins are widely distributed[1] in plant tissues, gums, and mucilages. They are major components of the gums produced from many species of *Acacia*[2]. Our interest is in the secretions of the female sexual tissues of flowering plants which might be involved in the capture and recognition of compatible pollen and nurture of the pollen tube during its growth through the style, prior to fertilisation.

We have used *Gladiolus* (family Iridaceae) as an experimental system, as flowers are readily available commercially throughout the year. When the pistil is mature, the cells lining the style canal secrete a mucilage that fills the hollow canal through which the pollen tubes grow in their passage to the ovary. This mucilage contains an arabinogalactan–protein as a major component[3]. We now report that the surface secretion of the stigma contains a closely related, but distinct, arabinogalactan as a major component.

The surface secretion of *Gladiolus* is water-soluble and can be washed from the surface of mature stigmas[4]. Electrophoresis revealed that the secretion contained a complex mixture of proteins, glycoproteins, and glycolipids, and that galactose, arabinose, and glucose were the major monosaccharides present[5], in the proportions 5.4:2.0:1.0. These data, together with the observation that the secretion bound to tridacnin (a galactose-binding lectin from the giant clam *Tridacna maxima*[6]) and the β-D-glucosyl, artificial carbohydrate-antigen[5] (a glycosyl phenylazo-dye that interacts with plant arabinogalactans and arabinogalactan–proteins[7–9]), suggested the presence of an arabinogalactan or an arabinogalactan–protein.

Washing the surface of mature stigmas of receptive *Gladiolus* flowers with Tris–saline buffer[4] removed material that gave single precipitin-bands in double-diffusion tests in agar with the β-D-glucosyl, artificial carbohydrate-antigen and with tridacnin. This observation suggested the use of affinity chromatography on insolubilised tridacnin[10] for isolation. The stigma extract, dissolved in 0.15M NaCl containing 0.01M $CaCl_2$, was applied to tridacnin–Sepharose 4B. The material that

0008-6215/80/0000–0000/$ 02.25,

TABLE I

METHYLATION ANALYSES OF THE STIGMA AND STYLE ARABINOGALACTANS OF *Gladiolus*

Tentative identification of components[a]	T[b]	*Linkage type*	*Linkage composition (mole %)*[c]		
			Gladiolus		Lilium longiflorum[e]
			Stigma[d] *arabinogalactan*	*Style arabinogalactan–protein*	*Stigma-exudate fraction*
2,3,4-Tri-*O*-methyl-L-rhamnitol		L-Rha*p*-(1→	0	0	7
2,3,5-Tri-*O*-methyl-L-arabinitol	0.43	L-Ara*f*-(1→	17	13	32
2,3,4,6-Tetra-*O*-methyl-D-glucitol	1.01	D-Glc*p*-(1→	7	trace	0
2,3,4,6-Tetra-*O*-methyl-D-galactitol	1.19	D-Gal*p*-(1→	16	29	11
2,4,6-Tri-*O*-methyl-D-galactitol	2.04	→3)-D-Gal*p*-(1→	13 } 76	14 } 88	10 } 56
2,3,4-Tri-*O*-methyl-D-galactitol	2.89	→6)-D-Gal*p*-(1→	6	6	5
2,4-Di-*O*-methyl-D-galactitol	5.1	→3)→6)-D-Gal*p*-(1→	41	39	30

[a]Based on g.l.c. data only for the *Gladiolus* arabinogalactans. [b]Relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [c]Calculated by using the molar response factors given by Sweet *et al.*[17]. [d]There was an additional peak (*T* 10.2) which accounted for 16% of the total peak area. The nature and origin of this component has not been established. [e]Data from Aspinall and Rosell[13], recalculated excluding the glucuronic acid residues. Two other, minor, galactosyl components were also identified, namely, 2,6-di-*O*-methyl-D-galactitol (2%) and 2-*O*-methyl-D-galactitol (4%).

bound to the column was eluted with calcium-free 0.15M NaCl. This material bound to the β-D-glucosyl, artificial carbohydrate-antigen and gave a single precipitin-band against tridacnin. Electrophoresis of this material on cellulose acetate at pH 8.8 showed only one, positively charged, diffuse band after staining with the β-D-glucosyl, artificial carbohydrate-antigen, suggesting a single, major component. The material contained galactose (76.1%), arabinose (20%), and glucose (4%); *cf.* the style arabinogalactan–protein, which contained galactose (85.8%) and arabinose (14.2%), together with traces of glucose and rhamnose.

The yield of the stigma-surface arabinogalactan varied over the range 30–50% by weight of the total surface-secretion, corresponding to <1 μg per flower. The arabinogalactan was methylated by the Hakomori[11] procedure and the methylation-analysis data are shown in Table I, together with those for the style arabinogalactan–protein. The analyses were similar. All of the arabinose was present as terminal arabinofuranosyl residues, and the glucose residues were also in terminal positions. The galactose was mainly 1,3,6-linked, with smaller amounts of (1→3)-linked, (1→6)-linked, and terminal residues. Thus, the molecule is highly branched. The two molecules differ in the terminal residues, the stigma arabinogalactan having a higher proportion of arabinose and glucose and the style having a higher proportion of galactose. The possibility that these differences resulted from enzymic modification of the arabinogalactan during the extraction and purification procedures was minimised by discarding any pistil contaminated with pollen, which is known to release glycosidases on hydration[12], and by performing all of the extraction and isolation steps at 4°.

The methylation-analysis data of the style arabinogalactan–protein, together with the results of mild, acid and enzymic hydrolysis, is consistent with a (1→3)-linked galactan having branches (some of which carry terminal arabinofuranosyl groups) linked[3] through O-6. The methylation-analysis data reported here for the stigma arabinogalactan are also compatible with this model.

Also included in Table I are figures calculated from the data of Aspinall and Rosell[13] on the composition of a fraction of the surface exudate of stigmas of another monocotyledon, *Lilium longiflorum* (*var.* Ace), from a different family (Liliaceae). The number of molecular species present in this fraction was not established, although the methylation analyses indicated that a major component was an arabino-3,6-galactan. We have confirmed this finding; freshly collected, stigma exudate from *L. longiflorum* formed a single precipitin-band with the β-D-glucosyl, artificial carbohydrate-antigen. This complex, formed between the carbohydrate antigen and the exudate, contained galactose, arabinose, and rhamnose in the proportions 11:5:1 (since the carbohydrate antigen contains glucose, the proportion of glucose derived from the stigma exudate could not be assessed). This ratio for neutral sugars is similar to that given by Aspinall and Rosell[13] for the stigma-exudate fraction of *Lilium*. Arabinogalactans that interact with the β-D-glucosyl, artificial carbohydrate-antigen are structurally similar[8]. The interaction of the *Lilium* and *Gladiolus* arabinogalactans with this antigen indicates that they belong to the same

group of macromolecules. This conclusion is borne out by comparison of the methylation-analysis data in Table I. Although the total amount of the internal galactosyl residues is somewhat lower in the *Lilium* than in the *Gladiolus* arabinogalactans, the molar ratios are approximately the same (2:1:6.5) for (1→3)-, (1→6)-, and 1,3,6-linked galactosyl residues. There are major differences in the proportions of terminal residues. The *Lilium* arabinogalactan has a higher proportion of arabinose (32%), and a significant content (7%) of rhamnose which was not detected in the *Gladiolus* arabinogalactans. The *Lilium* arabinogalactan also has a lower proportion of terminal galactose and no terminal glucose, and contains glucuronic acid (11%) both as terminal units and as (1→4)-linked residues[13]. The uronic acid content of the arabinogalactan–protein of *Gladiolus* style is low[3] (0.9%).

Arabinogalactans often occur in association with protein[1]. The arabinogalactan–protein of *Gladiolus* style contains 3% of protein, but the low yield of the stigma arabinogalactan made it impractical to determine the protein content. There was no report of any protein associated with the *Lilium* arabinogalactan[13].

The similarity in the core structure of the *Gladiolus* and *Lilium* arabinogalactans and the differences in the structure of the side branches are characteristic of the arabino-3,6-galactans. For example, the variations in the terminal saccharide sequences of the *Acacia* gums[2,14] may have potential as taxonomic markers[14]. These terminal sequences may also represent markers of identity in individual plants and tissues. Such markers exist on the surface of plant (and animal) cells, but whether arabinogalactans are in this category is not known[15]. There is evidence that arabinogalactans are present on the surface of plant protoplasts[16].

EXPERIMENTAL

Preparation of the surface extract of Gladiolus *stigma.* — Cut flowers of *Gladiolus* were kept in aqueous 0.01% 8-hydroxyquinoline citrate and 1% sucrose. Mature pistils were collected 24 h after flower opening, and the stigmas were washed in 0.05M Tris–HCl, 0.15M NaCl, and mM $CaCl_2$, at pH 7.4 and 4°, by repeated dipping during 15 min; the filtrate was dialysed and freeze-dried (0.2 mg per 100 stigmas).

Precipitation of material from the surface secretion of Lilium longiflorum *stigma with the β-D-glucosyl, artificial carbohydrate-antigen.* — The surface secretion of the stigmas of *L. longiflorum* flowers, purchased locally, was collected as for *Gladiolus* (yield, 2 mg/stigma). To a solution of the surface extract (8.6 mg) in aqueous 1% NaCl (5 ml) was added antigen (20 mg), and the mixture was incubated at room temperature for 1 h. The resulting precipitate (6 mg) was collected and washed as described by Jermyn and Yeow[7]. This precipitate was hydrolysed directly for monosaccharide analysis.

Isolation of the arabinogalactan from Gladiolus *stigma by affinity chromatography on tridacnin–Sepharose 4B.* — Tridacnin was purified and coupled to Sepharose 4B as previously described[10]. A solution of *Gladiolus* stigma extract (8 mg) in 0.15M NaCl containing 0.01M $CaCl_2$ was added[10] to a column (3.5 × 1 cm) of tridacnin–

Sepharose 4B and eluted with the same solvent (18 ml) and then with calcium-free 0.15M saline (14 ml). Subsequent elution with 0.1M lactose did not yield any further material. The capacity to bind to the β-D-glucosyl, artificial carbohydrate-antigen (precipitin bands in double-diffusion tests) was exclusively associated with the material in the calcium-free, saline eluate.

Monosaccharide analysis. — Samples were hydrolysed in 2 ml of 2.5M trifluoroacetic acid at 100° for 2 h in a sealed tube under nitrogen. The acid was then evaporated, the residue was reduced and acetylated[18], and the resulting alditol acetates were subjected to g.l.c. on a column (1.85 m × 4 mm) of 3% of SP2340 on Supelcoport (100–200 mesh).

Methylation analysis. — A sample (2 mg) was dissolved in methyl sulphoxide (4 ml), and methylated three times by the method of Hakomori[11]. The methylsulphinyl anion was prepared as described by Conrad[19]. After methylation, the sample was dialysed exhaustively against water and freeze-dried, and the residue was hydrolysed with formic acid followed by sulphuric acid[20]. The products were reduced and acetylated[18], and the resulting alditol acetates were subjected to g.l.c. on a glass column (1.85 m × 2 mm) of 3% of OV-225 on Chrom WHP (80–100 mesh).

The retention times (T) of the separated components were measured relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. The components were tentatively identified by reference to published[21] T-values and by comparison with reference materials prepared from larch galactan and 3-*O*-β-D-galactopyranosyl-D-arabinose.

β-D-Glucosyl, artificial carbohydrate-antigen. — This antigen[22], which was a gift from Dr. M. A. Jermyn (Division of Protein Chemistry, CSIRO, Parkville, Vic., Australia), was prepared by coupling diazotised 4-aminophenyl β-D-glucopyranoside with phloroglucinol[22].

Electrophoresis. — Electrophoresis on cellulose-acetate membranes was carried out in Tris–barbital–sodium barbital buffer (I = 0.05, pH 8.8), using a Beckman Microzone apparatus, for 45 min at 4 mA. The membrane was stained with β-D-glucosyl, artificial carbohydrate-antigen (1 mg/ml in 0.15M NaCl) for 10 min and de-stained with 0.15M NaCl.

ACKNOWLEDGMENTS

We thank Dr. M. A. Jermyn for the gift of β-D-glucosyl, artificial carbohydrate-antigen, and Professor B. A. Stone, Professor R. B. Knox, and Dr. M. A. Jermyn for stimulating discussion. This work was supported, in part, by the Australian Research Grants Committee.

REFERENCES

1 A. E. Clarke, R. L. Anderson, and B. A. Stone, *Phytochemistry*, 18 (1979) 521–540.
2 G. O. Aspinall, *Adv. Carbohydr. Chem. Biochem.*, 24 (1969) 333–379.
3 P. A. Gleeson and A. E. Clarke, *Biochem. J.*, 181 (1979) 607–621.

4 R. B. KNOX, A. E. CLARKE, S. HARRISON, P. SMITH, AND J. J. MARCHALONIS, *Proc. Natl. Acad. Sci. USA*, 73 (1976) 2788–2792.
5 A. E. CLARKE, P. GLEESON, S. HARRISON, AND R. B. KNOX, *Proc. Natl. Acad. Sci. USA*, 76 (1979) 3358–3362.
6 B. A. BALDO, W. H. SAWYER, R. V. STICK, AND G. UHLENBRUCK, *Biochem. J.*, 175 (1978) 467–477.
7 M. A. JERMYN AND M. YEOW, *Aust. J. Plant Physiol.*, 2 (1975) 501–531.
8 R. L. ANDERSON, A. E. CLARKE, M. A. JERMYN, R. B. KNOX, AND B. A. STONE, *Aust. J. Plant Physiol.*, 4 (1977) 143–158.
9 A. E. CLARKE, P. A. GLEESON, M. A. JERMYN, AND R. B. KNOX, *Aust. J. Plant Physiol.*, 5 (1978) 707–722.
10 P. A. GLEESON, M. A. JERMYN, AND A. E. CLARKE, *Anal. Biochem.*, 92 (1979) 41–45.
11 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
12 A. E. CLARKE, S. HARRISON, AND P. GLEESON, *Abstr. Pap. Am. Chem. Soc. Meet.*, 177 (1979) CARB-17.
13 G. O. ASPINALL AND K. G. ROSELL, *Phytochemistry*, 17 (1978) 919–922.
14 D. M. W. ANDERSON AND I. C. M. DEA, *Phytochemistry*, 8 (1969) 167–176.
15 J. W. RAFF, J. F. HUTCHINSON, R. B. KNOX, AND A. E. CLARKE, *Differentiation*, 12 (1979) 179–186.
16 P. J. LARKIN, *J. Cell Sci.*, 26 (1977) 31–46.
17 D. P. SWEET, R. H. SHAPIRO, AND P. ALBERSHEIM, *Carbohydr. Res.*, 40 (1975) 217–225.
18 P. ALBERSHEIM, D. J. NEVINS, P. D. ENGLISH, AND A. KARR, *Carbohydr. Res.*, 5 (1967) 340–345.
19 H. E. CONRAD, *Methods Carbohydr. Chem.*, 6 (1972) 361–367.
20 B. LINDBERG, J. LONNGREN, J. L. THOMPSON, AND W. NIMMICH, *Carbohydr. Res.*, 25 (1972) 49–57.
21 J. LONNGREN AND A. PILOTTI, *Acta Chem. Scand.*, 25 (1971) 1144–1145.
22 J. YARIV, M. M. RAPPORT, AND L. GRAF, *Biochem. J.*, 85 (1962) 383–388.