Biochem. J. (1981) **197**, 707–714
Printed in Great Britain

Role of carbohydrate as an antigenic determinant of a glycoprotein from rye-grass (*Lolium perenne*) pollen

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(*Received 6 April 1981/Accepted 24 April 1981*)

The carbohydrate component of Glycoprotein 2 (12% carbohydrate) from rye-grass (*Lolium perenne*) pollen has saccharide sequences that contribute to its antigenicity. Radioimmunoassay inhibition tests show that the antiserum to this glycoprotein cross-reacts with a number of other plant glycoproteins. In contrast, antiserum to another glycoprotein from rye-grass pollen, Glycoprotein 1 (5% carbohydrate), does not cross-react with any of the test glycoconjugates. Treatment of glycoproteins with sodium metaperiodate (0.02 M, 4°C, 6 h, in the dark) causes the loss of their ability to cross-react antigenically with Glycoprotein 2, and a loss of capacity to bind ^{125}I-labelled concanavalin A. The cross-reactivity of this plant glycoprotein with other glycoconjugates imposes limitations on the interpretation of ultrastructural studies aimed at localizing a particular glycoprotein to a cellular site by using fluorescent or ferritin-labelled antisera. A radioimmunoassay inhibition technique for quantitative determination of the amounts of antigens in plants is also described.

The purification and partial characterization of two glycoproteins from rye-grass (*Lolium perenne*) pollen was described in the preceding paper (Howlett & Clarke, 1981*a*). These glycoproteins are quite distinct in terms of their charge, size, amino acid and monosaccharide composition, and allergenic activity. Both are effective antigens in rabbits. In the present paper we provide evidence that the two glycoproteins have immunological cross-reactivity that is due, at least in part, to common antigenic carbohydrate components.

Materials and methods

Microtitre trays were purchased from Cooke Laboratories, Alexandria VA, U.S.A. Iodogen was obtained from Pierce, IL, U.S.A. Na^{125}I was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Protein A was purchased from Pharmacia Ltd., Uppsala, Sweden. D-Galactose, L-fucose, D-glucose, bovine serum albumin (catalogue no. A7638), ovalbumin, polyvinylpyrrolidone 40 and bovine submaxillary-gland mucin (type 1) were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. L-Arabinose, *N*-acetyl-D-glucosamine, D-xylose, horseradish peroxidase (A grade), glucose oxidase (fungal, A grade) and Miracloth were from Calbiochem, Los Angeles, CA, U.S.A. The disaccharide 3-*O*-α-D-galactopyranosyl-D-arabinose was from Pfanstiehl laboratories, Waukegan, IL, U.S.A. Concanavalin A and human transferrin were from Miles–Yeda Ltd., Elkhart, IN, U.S.A. Pollens [*Lolium perenne* (rye grass), *Lolium multiflorum* (Italian rye), *Secale cereale* (rye), *Phleum pratense* (timothy grass), *Dactylis glomerata* (Bermuda grass) *Cynodon dactylon* (cocksfoot)], were obtained from Greer Laboratories, Lenoir, NC, U.S.A. Pollen of *Gladiolus gandavensis* (gladiolus) was collected from locally grown plants. Sodium metaperiodate, Nonidet P40 and D-mannose were obtained from BDH, Poole, Dorset, U.K. α_1-Acid glycoprotein isolated from rat serum was supplied by Professor G. Schreiber, Department of Biochemistry, University of Melbourne, Parkville, Vic., Australia. Fetuin was kindly provided by Dr. C. Ward (Division of Protein Chemistry, C.S.I.R.O., Parkville, Vic., Australia). *Lolium multiflorum* endosperm cultures grown under conditions described by Smith & Stone (1973), were a gift from Professor B. A. Stone (Department of Biochemistry, La Trobe University, Bundoora, Vic., Australia). *Psophocarpus tetragonolobus* (winged bean) leaf callus cells were grown in liquid-suspension culture as described elsewhere (Meimeth *et al.*, 1981). Arabinogalactan-protein from *Gladiolus* style secretions was prepared as

Abbreviations used: IgG, immunoglobulin G; IgE, immunoglobulin E.

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previously described (Gleeson & Clarke, 1979). Glycoproteins 1 and 2 from *Lolium perenne* pollen and their specific antisera were prepared as described in the preceding paper (Howlett & Clarke, 1981*a*).

Solid-phase radioimmunoassays

These were performed as described in the preceding paper (Howlett & Clarke, 1981*a*).

Radioimmunoassay inhibition studies

These were performed as described in the preceding paper (Howlett & Clarke, 1981*a*).

Removal of sialic acid from glycoproteins

Sialic acid was removed from glycoproteins by hydrolysis with 0.05 M-H_2SO_4 at 80°C for 1h (Kieda *et al.*, 1978). The desialylated glycoproteins were dialysed exhaustively against water and freeze-dried.

Periodate oxidation of glycoproteins

The glycoprotein samples (200μg) were incubated with 0.02 M-sodium metaperiodate at 4°C in the dark for 6h; the oxidized glycoproteins were then dialysed exhaustively against phosphate-buffered saline (0.01 M-sodium phosphate buffer/0.15 M-NaCl, pH 7.0), and stored at −20°C.

^{125}I-labelled Concanavalin A binding of periodate-treated glycoproteins

The capacity of native and periodate-treated glycoproteins to bind ^{125}I-labelled concanavalin A was measured by using the lectin-binding assay described by Howlett & Clarke (1981*a*,*b*).

Preparation of extracts of grass pollens

Pollen samples (*Lolium perenne, Lolium multiflorum, Dactylis glomerata, Phleum pratense, Secale cereale, Cynodon dactylon* and *Gladiolus gandavensis*) (100mg) were separately suspended in phosphate-buffered saline (1ml) and mixed by repeated inversion for 10min at room temperature. The pollen suspensions were centrifuged (10000*g*, $r_{av.}$ 4cm, 2min, Microfuge, Beckman Instrument Co.), the supernatants collected and stored at −20°C. The protein concentration of the supernatants was determined by the microbiuret technique of Goa (1953), with bovine serum albumin as a standard.

Preparations of stem, leaf and inflorescence extracts of L. perenne

Field-grown *L. perenne* plants were collected just before flowering and washed by dipping in water. The leaves were cut from the stem and the immature inflorescences individually removed 24h before anthesis. Samples (400mg) (leaves, stems, inflorescences) were cut into small pieces with scissors and ground periodically during 10min in a mortar and pestle with polyvinylpyrrolidone 40 (400mg) in 10ml of water at 4°C. The resulting suspensions were filtered through Miracloth; the filtrates were centrifuged (10000*g*, $r_{av.}$ 4cm, 1min) and the supernatants dialysed exhaustively against phosphate-buffered saline and treated as described for the pollen extracts.

Preparation of extracts from cultured callus cells

Lolium multiflorum endosperm suspension cultures were grown to stationary phase in a medium containing glucose as carbon source. *Psophocarpus tetragonolobus* suspension-cultured cells were harvested during the exponential phase. Cells (2ml packed cell volume in 10ml of culture medium) were harvested by centrifugation (4000*g*, $r_{av.}$ 10cm, 2min at room temperature) and the supernatant (culture filtrate) retained. The cells were washed five times in phosphate-buffered saline (10ml) and disrupted by homogenization at 4°C in an all-glass hand-held homogenizer in a minimum volume of water. After a 5min period of homogenization, few intact cells could be detected microscopically. The disrupted cells were centrifuged (5000*g*, $r_{av.}$ 10cm, 10min) and the supernatants (cytoplasmic preparations) stored at −20°C. The cell debris was washed three times with phosphate-buffered saline then incubated in 0.5% Nonidet P40 in phosphate-buffered saline for 1h at 20°C. The mixtures were centrifuged (10000*g*, $r_{av.}$ 4cm, 10min) and the supernatants were exhaustively dialysed against phosphate-buffered saline. The protein contents of culture filtrates, cytoplasmic preparations and membrane preparations were determined (Goa, 1953).

Assay of amounts of Glycoprotein 1 in tissues of L. perenne, L. multiflorum endosperm, P. tetragonolobus leaf callus cells and pollens of monocotyledonous plants

The assay is based on the radioimmunoassay inhibition experiments described above. The wells of a microtitre tray were coated with Glycoprotein 1 (40μl of a 50μg/ml solution). Dilutions of this glycoprotein, samples of pollen extracts, *L. perenne* tissues, *L. multiflorum* endosperm extracts or *P. tetragonolobus* leaf-callus extracts were serially diluted in a constant dilution (1:360) of anti-(Glycoprotein 1) antiserum in 1% bovine serum albumin in phosphate-buffered saline. Portions of these inhibitor–antiserum samples (75μl) were added to the Glycoprotein 1-coated wells. The wells were washed and ^{125}I-labelled Protein A was added as described for the radioimmunoassay. There was a linear relationship between the amount of Glycoprotein 1 as an inhibitor and the amount of ^{125}I-labelled Protein A bound over the concentration range 1–200μg of Glycoprotein 1/ml.

Results

The following studies were all performed with antisera from single rabbits. Antisera from other rabbits produced by using the same immunization schedules behaved similarly in all the tests described.

Establishing conditions for the radioimmunoassay

1. Saturation binding of Glycoproteins 1 and 2 to polyvinyl microtitre tray plates. Binding curves for different concentrations of Glycoprotein 1 and 2 coated on the plates, made to react with an excess of homologous antiserum (1:40 dilution) and detected wtih ^{125}I-labelled Protein A, are shown in Fig. 1. Saturation of binding capacity of the plate is achieved by using solutions of glycoproteins at a concentration greater than 20μg/ml. All subsequent experiments were performed by using 50μg/ml solutions of glycoproteins for coating the plates. In this and other experiments the Glycoprotein 1–anti-(Glycoprotein 1) serum complex bound more Protein A than did the Glycoprotein 2–anti-(Glycoprotein 2) serum complex. In all experiments, there was less than 5% variation between replicate determinations.

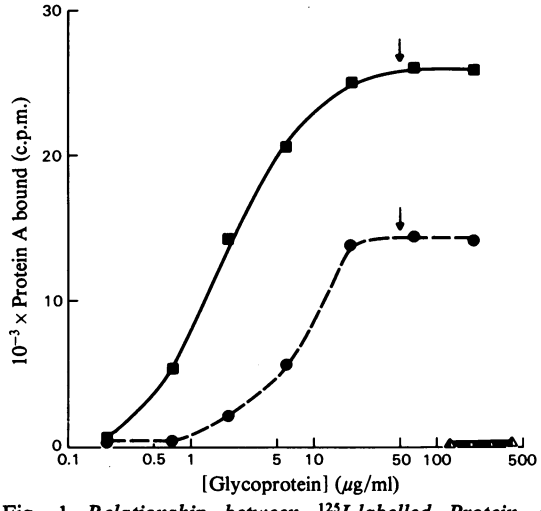

Fig. 1. *Relationship between ^{125}I-labelled Protein A binding and concentration of antigen coated to well*
Solutions of Glycoprotein 1 (■), Glycoprotein 2 (●) or bovine serum albumin (▲) (40μl) at the concentrations indicated, were added to wells of a microtitre tray. A 1:40 dilution of their homologous antiserum was added (75μl). ——, Anti-(Glycoprotein 1); – – – –, Anti-(Glycoprotein 2). Total ^{125}I-labelled Protein A added was 42000c.p.m. Each point represents the mean of three determinations. The arrows indicate the concentrations of Glycoproteins 1 and 2 used for coating in subsequent experiments.

2. Relationship between antiserum dilutions and bound Protein A. Plates were coated with Glycoprotein 1 or 2 at 50μg/ml, and incubated with various dilutions of antisera, before addition of ^{125}I-labelled Protein A. Four antisera were used, anti-(Glycoprotein 1), anti-(Glycoprotein 2), anti-(*Lolium perenne* pollen extract) and pre-immune serum. Typical sigmoid curves were obtained, although complete saturation was not achieved at the highest concentration of antisera tested (Fig. 2). Dilutions of 1:360 (below saturation) were used for inhibition experiments. The curves obtained with anti-(Glycoprotein 1) and anti-(Glycoprotein 2) sera and their homologous antigens paralleled those obtained by using antisera raised to unfractionated pollen extract of *Lolium perenne* and the same antigens.

3. Relationship between concentration of Protein A and amount of Protein A bound. The experiments require complete saturation of IgG–antigen complexes with Protein A; the Protein A concentration (0.6μg/ml) that was used in similar systems (Romani *et al.*, 1980) was chosen: for each experiment, an assay was set up with this concentration and twice this concentration. In each case there was no significant diference in the amounts of Protein A bound to the wells.

Antigenic cross-reactivity of Glycoproteins 1 and 2

There was extensive antigenic cross-reactivity between the two glycoproteins, which was only detected when one of the antisera was used. Thus antiserum to Glycoprotein 2 bound to wells coated with Glycoprotein 1 at all dilutions tested, to an extent of up to 70% of the homologous interaction (Fig. 2*a*). However, antiserum to Glycoprotein 1 bound poorly to wells coated with Glycoprotein 2 (Fig. 2*b*). Pre-immune serum bound to a 'background' extent to wells coated with either Glycoprotein 1 or 2.

This cross-reactivity was confirmed by inhibition experiments (Figs. 3*a* and 3*b*). Experiments were designed where wells coated with Glycoprotein 1 were made to react with antiserum to either Glycoprotein 1 or 2 that had been preincubated with various dilutions of either glycoprotein. When Glycoprotein 1 was used as an inhibitor, the homologous reaction [Glycoprotein 1–anti-(Glycoprotein 1)] was effectively abolished. On the other hand, Glycoprotein 2 was a relatively poor inhibitor, giving only 15% inhibition at 200μg/ml. As mentioned above, anti-(Glycoprotein 2) serum bound effectively to Glycoprotein 1-coated wells, and both glycoproteins effectively abolished this interaction when used as inhibitors (Fig. 3*a*).

Similar experiments were set up with wells coated with Glycoprotein 2. Glycoprotein 2 effectively abolished the Glycoprotein 2–anti-(Glycoprotein 2)

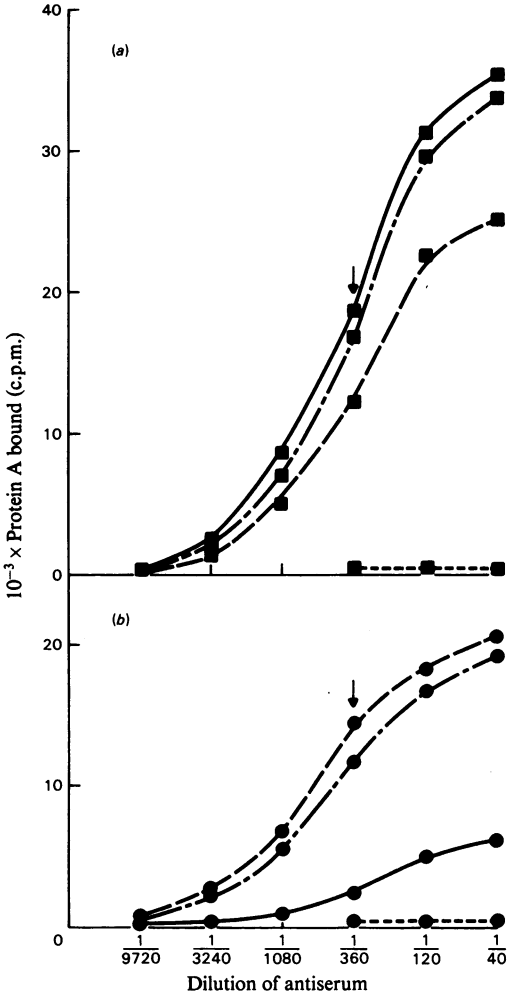

Fig. 2. *Relationship between binding of ^{125}I-labelled Protein A and antiserum concentration*

Wells of a microtitre tray were coated with either (*a*) Glycoprotein 1 (■) of (*b*) Glycoprotein 2 (●) (40 μl); 50 μg/ml in phosphate-buffered saline). Antisera to pollen extracts (———), Glycoprotein 1 (——), Glycoprotein 2 (— —), or pre-immune rabbit serum (----) were threefold serially diluted in 1% bovine serum albumin in phosphate-buffered saline and 75 μl added to each well. ^{125}I-labelled Protein A (75 μl containing 50000 c.p.m.) was delivered to each well. Each point represents the mean of two determinations. The arrows indicate dilutions of antisera used in subsequent inhibition experiments.

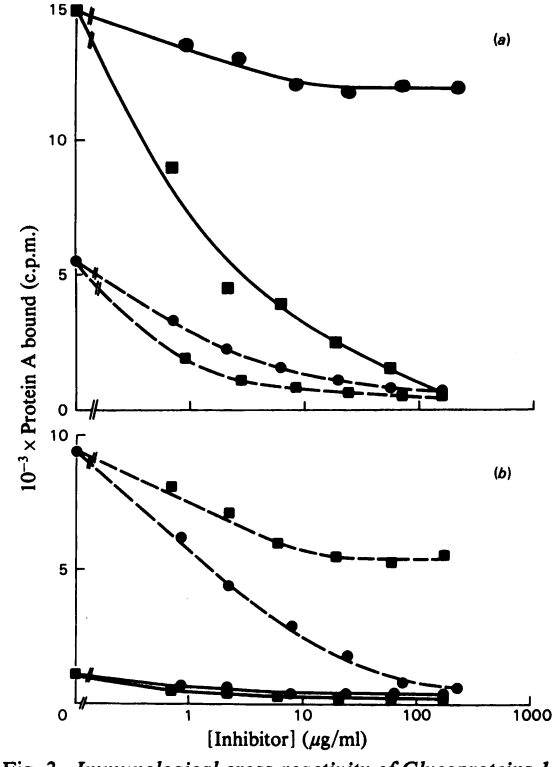

Fig. 3. *Immunological cross-reactivity of Glycoproteins 1 and 2*

Wells of a microtitre tray were coated with either (*a*) Glycoprotein 1 or (*b*) Glycoprotein 2 (40 μl of a 50 μg/ml solution). Antiserum to either Glycoprotein 1 (——) or Glycoprotein 2 (----) (1:360 dilution, in 1% bovine serum albumin in phosphate-buffered saline) was preincubated for 2 h in various concentrations of either Glycoprotein 1 (■) or Glycoprotein 2 (●) and then added to the wells. The total ^{125}I-labelled Protein A added was 20000 c.p.m. Each point represents the mean of two determinations.

serum interaction. Glycoprotein 1 was also a potent inhibitor, giving a maximum of 40% inhibition above 20 μg/ml. Anti-(Glycoprotein 1) antiserum bound poorly to the Glycoprotein 2-coated wells, and this binding was completely abolished by either glycoprotein (Fig. 3*b*).

Antigenic cross-reactivity of defined glycoproteins with Glycoprotein 1 and 2

A series of monosaccharides and glycoproteins of known composition were examined for their ability to inhibit both homologous interactions. For the Glycoprotein 1–anti-(Glycoprotein 1) interaction, only Glycoprotein 1 and periodate-oxidized Glycoprotein 1 were effective inhibitors (Fig. 4*a*, Table 1). Glycoprotein 2 was a poor inhibitor, and the glycoproteins glucose oxidase, ovalbumin, horseradish peroxidase, α_1-acid glycoprotein, asialo-

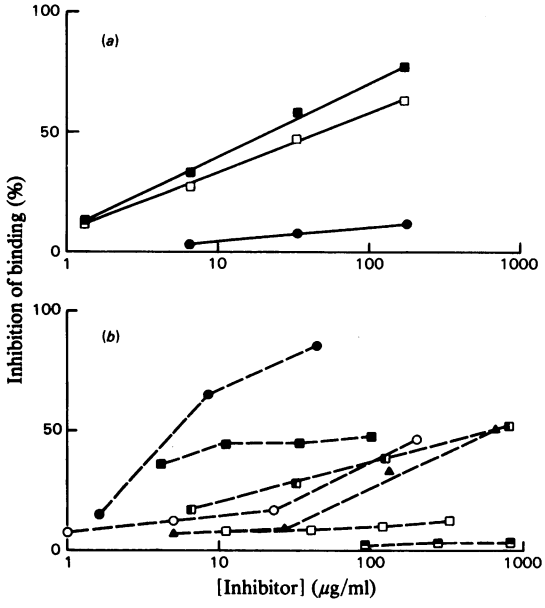

Fig. 4. *Inhibition of homologous antigen–antiserum interactions by glycoconjugates*

(*a*) Glycoprotein 1–anti-(Glycoprotein 1) serum interaction; (*b*) Glycoprotein 2–anti-(Glycoprotein 2) serum interaction. Wells of a microtitre tray were coated with Glycoprotein 1 (*a*) or 2 (*b*) (40μl of a 50μg/ml solution in phosphate-buffered saline). Homologous antisera (1:360 dilution in 1% bovine serum albumin in phosphate-buffered saline) were preincubated in various concentrations of Glycoprotein 1 (■), periodate-oxidized Glycoprotein 1 (□), Glycoprotein 2 (●), periodate-oxidized Glycoprotein 2 (○), arabinogalactan-protein (▲), horseradish peroxidase (◧) or periodate-oxidized horseradish peroxidase (◨). These solutions (75μl) were then added to the wells. The total ^{125}I-labelled Protein A added was 15000c.p.m. Glycoprotein 1-coated wells bound the homologous antiserum in the absence of inhibitors to give 7000c.p.m. Glycoprotein 2-coated wells bound the homologous antiserum in the absence of inhibitors to give 5000c.p.m. Each point represents the mean of two determinations. Other glycoproteins tested as inhibitors were glucose oxidase, α_1-acid glycoprotein, ovalbumin, asialomucin, asialotransferrin and asialofetuin. None of these were effective as inhibitors at concentrations up to 1mg/ml.

fetuin, asialomucin and asialotransferrin did not give significant inhibition. None of the monosaccharides tested (glucose, *N*-acetylglucosamine, mannose, fucose, arabinose, xylose and galactose) or the disaccharide galactosyl-β1→3-arabinose gave any inhibition at concentrations up to 0.2M. These experiments indicate that the carbohydrate component, which is periodate-vulnerable, does not apparently contribute significantly to the antigenic determinants of Glycoprotein 1.

Table 1. *Comparison of glycoproteins as inhibitors of the Glycoprotein 2–anti-(Glycoprotein 2) serum binding*

Data were obtained from Fig. 4. Other glycoproteins tested (glucose oxidase, rat α_1-acid glycoprotein, ovalbumin, desialylated fetuin, desialylated human transferrin, desialylated bovine submaxillary mucin) were not effective inhibitors at concentrations up to 1 mg/ml.

Inhibitor	Concentration required for 50% inhibition (μg/ml)
Glycoprotein 2	5
Periodate-oxidized Glycoprotein 2	280
Glycoprotein 1	>100
Periodate-oxidized Glycoprotein 1	>300*
Horseradish peroxidase	600
Periodate-oxidized horseradish peroxidase	>600*
Arabinogalactan-protein	600

* Less than 10% inhibition at this concentration.

For the Glycoprotein 2–anti-(Glycoprotein 2) interaction, Glycoprotein 2 was an effective inhibitor (5μg/ml required for 50% inhibition), but periodate-oxidized Glycoprotein 2 was a much less effective inhibitor (280μg/ml required for 50% inhibition) (Fig. 4*b*, Table 1). Glycoprotein 1 was also an inhibitor, as well as two of the other glycoconjugates tested: horseradish peroxidase and *Gladiolus* style arabinogalactan-protein. However, oxidized Glycoprotein 1 and oxidized horseradish peroxidase were ineffective inhibitors. Arabinogalactan-protein, after oxidation, became extremely insoluble and could not be tested as an inhibitor in this assay. The 'high-mannose' glycoproteins glucose oxidase, ovalbumin and α_1-acid glycoprotein were not effective inhibitors, neither were the desialylated glycoproteins asialofetuin, asialomucin or asialotransferrin, or any of the sugars tested. These experiments indicate that, in contrast with Glycoprotein 1, the carbohydrate component of Glycoprotein 2 contains antigenic determinants.

Effect of periodate treatment on Glycoproteins 1 and 2

A change in the nature of the carbohydrate component by periodate treatment was indicated by the change in the capacity of Glycoprotein 2 to bind concanavalin A (Table 2). Native Glycoprotein 2 bound significant amounts of concanavalin A; this binding was effectively abolished by periodate

Table 2. *Concanavalin A binding of periodate-treated and native glycoproteins*

Wells of a mitrotitre tray were coated with native or periodate-treated glycoproteins (40 μl of a 200 μg/ml solution in 1 mM-$MgCl_2$/1 mM-$MnCl_2$/1 mM-$CaCl_2$/0.1 M-sodium acetate/0.5 M-NaCl, pH 6.0). Unbound sites were saturated with 1% bovine serum albumin in phosphate-buffered saline. Portions of ^{125}I-labelled concanavalin A (50 μl of a 0.62 μg/ml solution in the above buffer containing 1% bovine serum albumin) were then added to the coated wells. After 6 h at 20°C, wells were washed and counted for radioactivity. Each value is the mean of three determinations. Glycoproteins listed as periodate-treated were prepared as follows. Glycoproteins (200 μg in 200 μl of phosphate-buffered saline) were incubated with an equal volume of 0.04 M-sodium metaperiodate at 4°C in the dark for 6 h. The periodate-treated glycoproteins were dialysed exhaustively against phosphate-buffered saline.

Glycoprotein	^{125}I-labelled concanavalin A bound (ng)
Glycoprotein 1	<0.05
Periodate-treated Glycoprotein 1	<0.05
Glycoprotein 2	5.02
Periodate-treated Glycoprotein 2	<0.06
Ovalbumin	13.10
Periodate-treated ovalbumin	<0.05
Horseradish peroxidase	6.50
Periodate-treated horseradish peroxidase	<0.05

treatment. Native Glycoprotein 1 did not bind significant amounts of concanavalin A, and no change in binding was detected after periodate treatment. Two commercially available glycoproteins, ovalbumin and horseradish peroxidase, behaved similarly to Glycoprotein 2 after periodate treatment with regard to concanavalin A binding.

Glycoprotein 1 content of plant extracts

The amounts of Glycoprotein 1 determined by radioimmunoassay inhibition in extracts of pollen, *L. perenne* tissues and plant cells in culture are shown in Table 3. A range of monocotyledonous-plant pollens were tested. All festucoid-grass pollens tested had detectable amounts of Glycoprotein 1. Pollen samples from the distantly related chloridoid grass *Cynodon dactylon* and from *Gladiolus gandavensis* (Iridaceae) contained no detectable Glycoprotein 1.

Stem extracts of *L. perenne* contained significant amounts and leaf extracts contained lower, but detectable, amounts of Glycoprotein 1. Whole

Table 3. *Determination of Glycoprotein 1 content of pollen, L. perenne tissues, L. multiflorum endosperm and P. tetragonolobus leaf callus by radioimmunoassay inhibition*

Glycoprotein 1 was determined by radioimmunoassay inhibition. Wells of a microtitre tray were coated by Glycoprotein 1 (40 μl of a 50 μg/ml solution in phosphate-buffered saline). Antiserum to Glycoprotein 1 (1:360 dilution in 1% bovine serum albumin in phosphate-buffered saline) was preincubated for 2 h at room temperature in various concentrations of Glycoprotein 1 (standard or plant extracts). Portions of these solutions (75 μl) were added to the glycoprotein-coated wells. ^{125}I-labelled Protein A was added (20000 c.p.m./well). Each determination is the mean of three replicates. Values for Glycoprotein 1 content of the plant extracts were read from a standard curve. Protein contents of each plant extract were determined by the microbiuret technique of Goa (1953).

Plant extract	Content of Glycoprotein 1 (μg/mg of protein)
Pollen source	
Lolium perenne	50.00
Lolium multiflorum	44.00
Dactylis glomerata	15.00
Phleum pratense	3.00
Secale cereale	15.00
Cynodon dactylon	<0.03
Gladiolus gandavensis	<0.03
Tissues of *L. perenne*	
Leaf	0.04
Stem	0.61
Inflorescences (immature)	3.04
Lolium multiflorum endosperm	
Culture filtrate	<0.03
Cytoplasmic preparation	<0.03
Membrane preparation	3.2
Psophocarpus tetragonalobus leaf callus	
Culture filtrate	<0.03
Cytoplasmic preparation	<0.03
Membrane preparation	<0.03

inflorescences dissected from the plant before dehiscence of the anthers also contained large amounts of Glycoprotein 1. Neither of the cells in culture secreted Glycoprotein 1 into the medium, but *Lolium multiflorum* cultured endosperm cells contained detectable amounts (3.2 μg/mg of protein) in a membrane fraction.

This solid-phase radioimmunoassay-inhibition method for antigen detection is extremely sensitive; concentrations of 1 μg/ml can be assayed with less than 5% variation between replicates, with small quantities of antisera.

Discussion

In the present study we have demonstrated antigenic cross-reactivity between two different rye-grass pollen glycoproteins. The cross-reactivity was only detected by using antisera to one of the glycoproteins, namely Glycoprotein 2. This antiserum cross-reacted strongly with Glycoprotein 1 and a number of other plant glycoconjugates, whereas antiserum to Glycoprotein 1 cross-reacted only to a minor extent with Glycoprotein 2, and not at all to the other glycoconjugates tested.

The likelihood of this cross-reactivity being due to an impurity of Glycoprotein 2 in the Glycoprotein 1 solutions can be discounted for several reasons. Firstly, the glycoproteins were separated on the basis of differing charge by ion-exchange chromatography and size by gel filtration [Glycoprotein 1, pI = 5.2, 5.4, mol.wt. 33000; Glycoprotein 2, pI > 9, mol.wt. 67000; see the preceding paper [Howlett & Clarke, 1981*a*)], and were apparently homogeneous when examined by both one- and two-dimensional gel electrophoresis. Secondly, if Glycoprotein 1 solutions were contaminated with Glycoprotein 2, then antiserum to Glycoprotein 1 would also contain antibodies to Glycoprotein 2 and hence cross-react with it. That is, cross-reactivity with both antisera would be expected and would not, as is observed, be restricted to anti-(Glycoprotein 2) antiserum. Also, if Glycoprotein 2 were present as an impurity, more effective binding of this glycoprotein to the polyvinylchloride microtitre-tray wells compared with Glycoprotein 1 would be required to account for the large extent of binding of anti-(Glycoprotein 2) serum [Glycoprotein 1 binds anti-(Glycoprotein 2) to the extent of up to 35% of the homologous interaction].

This leads to the possibility that the antigenic cross-reactivity is due to common immunodeterminants on the molecule. Such common determinants could reside in either the carbohydrate or the protein component of the glycoproteins. This first possibility was tested by examining the antigenic cross-reactivity after mild periodate oxidation. Under these conditions, monosaccharide ring structures at vicinal hydroxy groups are cleaved and there is no significant alteration of the polypeptide chain (Simeral *et al.*, 1980). The role of the carbohydrate as a common antigenic determinant is indicated by the abolition of cross-reactivity after periodate treatment of Glycoprotein 1. Supporting evidence for the role of carbohydrate as an antigenic determinant is given by the cross-reactivity of anti-(Glycoprotein 2) serum with other plant glycoconjugates, namely horseradish peroxidase and arabinogalactan-protein, which is also abolished by periodate oxidation. Some idea of the nature of the carbohydrate implicated as antigenic determinants can be deduced from inhibition studies of the Glycoprotein 2–anti-(Glycoprotein 2) serum interaction. Firstly, a saccharide sequence is required, as none of the monosaccharides tested were effective inhibitors, although the plant glycoconjugates horseradish peroxidase and arabinogalactan-protein were effective inhibitors at high concentrations. Secondly, these saccharide sequences include arabinose and galactose, probably in terminal positions, and do not include mannose sequences. The evidence for this is the effective inhibition by arabinogalactan-protein (3% protein, 90% carbohydrate), in which the carbohydrate consists of a 1,3β-galactose backbone, side-branched through C(O)6 to 1,6β-linked galactose residues, some of which bear terminal arabinose residues (Gleeson & Clarke, 1979). The galactose and arabinose residues of the side branches are most accessible and most likely to bind to anti-(Glycoprotein 2) serum. This finding is compatible both with the binding of Glycoprotein 2 to specific antiserum raised to arabinogalactan-protein and with the monosaccharide analysis of Glyprotein 2 [arabinose/galactose, 6:7; see the preceding paper (Howlett & Clarke, 1981*a*)]. The antiserum to Glycoprotein 2 bound horseradish peroxidase, which also contains arabinose but not galactose residues (Clarke & Shannon, 1976). The absence of binding of anti-(Glycoprotein 2) serum to 'mannose-rich' glycoproteins of both fungal and animal origin suggests that the mannose present in Glycoprotein 2 [preceding paper (Howlett & Clarke, 1981*a*)] does not contribute directly to the antigenicity.

There have been very few studies on the role of saccharides of plant glycoconjugates as antigens, compared with the extensive studies on animal and bacterial glycoconjugates. The plant proteoglycan arabinogalactan-protein has terminal arabinose and galactose residues as the major antigenic determinants (Gleeson & Clarke 1980) and xylose has been implicated as part of the antigenic determinant of the pineapple stem proteinase bromelain (Ishihara *et al.*, 1979). Both xylose and arabinose are monosaccharides rarely found in animal glycoconjugates, and it is possible that the 'foreignness' of these saccharides to rabbits confers a particular effectiveness on them as immunogens.

The lack of affinity of the anti-(Glycoprotein 2) serum for monosaccharides is not surprising, in view of the fact that the binding sites of other anti-glycosyl antibodies are known to extend over a number of saccharide units, for example, a hexasaccharide in the dextran–anti-dextran interaction (Kabat, 1966), a tetrasaccharide in the antigenic determinants of human blood-group antigens A and B (Lloyd *et al.*, 1966) and a disaccharide in a keratan sulphate-protein core of bovine nasal cartilage (Christner *et al.*, 1980).

In contrast with the Glycoprotein 1–anti-(Glycoprotein 2 serum) interaction, no detectable interaction occurred between Glycoprotein 2 and anti-(Glycoprotein 1) serum. This could be interpreted in terms of the low carbohydrate content of Glycoprotein 1, which may not contribute a major portion of the antigenic determinants of this molecule. Similar low extents of cross-reactivity were noted for two legume storage glycoproteins, vicilin and legumin (Craig *et al.*, 1980), each of which has a low carbohydrate content (legumin, 1%; vicilin, 0.3%; Basha & Beevers, 1976).

The conclusions rest on the premise that the periodate treatment does not significantly alter the polypeptide chain of the glycoproteins. The modification of tyrosine, tryptophan, methionine and *N*-terminal serine residues in several glycoproteins by periodate treatment has been reported by workers using more drastic conditions than those of the present study (for a review, see Geoghegen *et al.*, 1980). The finding, that the interaction of Glycoprotein 1 with its homologous antiserum is inhibited equally well by both native and periodate-treated Glycoprotein 1, suggests that periodate treatment did not markedly alter the protein of Glycoprotein 1. However, periodate treatment undoubtedly did affect the carbohydrate, since binding of concanavalin A was abolished by periodate oxidation.

From the data presented in Fig. 3(*b*), at least 40% of the antigen-binding sites of anti-(Glycoprotein 2) serum can be occupied by the carbohydrate component of Glycoprotein 1. Thus, in general terms, 40% of the cross-reactivity can be accounted for in terms of a common carbohydrate component.

Ideally the approach of using sequential saccharide- and linkage-specific enzyme modification would be used to further define the contribution of carbohydrate to the antigenic cross-reactivity. The small quantities of purified pollen glycoproteins available precluded this approach.

The demonstration that quite distinct glycoproteins may cross-react significantly on the basis of small proportions of common saccharides imposes limitations on the interpretation of experiments involving whole tissue slices or extracts and specific antisera. For example, in the present study, amounts of Glycoprotein 2 in plant extracts could not be assayed by radioimmunoassay, because of the cross-reactivity of anti-(Glycoprotein 2) serum with other plant glycoconjugates. By contrast, amounts of Glycoproteins 1 could be determined, as the specific antiserum does not cross-react with other glycoconjugates; amounts of Glycoprotein 1 in pollen extracts were high, but smaller amounts were detected in other tissues and in a callus cell-membrane preparation.

Analogies can be drawn between this glycoprotein and the plant lectins: both occur in higher concentrations in a particular plant tissue and in lower concentrations in other tissues (Talbot & Etzler, 1978; Pueppke *et al.*, 1978), and both induce specific effects in animal cells (Glycoprotein 1: release of IgE from sensitized mast cells; lectins: mitotic changes in B-lymphocytes); but for neither can any function within the plant be ascribed.

We are grateful to Dr. P. E. Todd and Professor S. J. Leach for constructive discussions and critical evaluation of the manuscript, and to Professor R. B. Knox for his continuing interest. This work was supported by a grant from the Australian Research Grants Committee.

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