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BINDING OF A PLANT GLYCOPROTEIN TO STAPHYLOCOCCAL PROTEIN A

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Abstract—An antigenic glycoprotein (Antigen P) isolated from mature styles of *Prunus avium* L, the sweet cherry, binds Staphylococcal Protein A with an apparent affinity constant $4.5 \times 10^7\ M^{-1}$, which is of the same order as that for the Protein A—Immunoglobulin G (IgG) interaction Another antigenic glycoprotein (Antigen S), also isolated from *P avium* styles, binds poorly to Protein A The binding of Antigen P to Protein A invalidates radioimmunoassays of style glycoproteins which depend on the use of Protein A as a probe for bound IgG

INTRODUCTION

The interaction between antigens and rabbit antibodies is commonly monitored by using *Staphylococcus aureus* (Cowan strain 1) cells or Staphylococcal Protein A to measure the amount of rabbit IgG bound If, for example, a specific antiserum is raised to an isolated antigen, the levels of this antigen in a tissue extract can be measured by radioimmunoassay with [^{125}I]Protein A or by immunoprecipitation with whole *Staphylococcus aureus* cells [1, 2]

We attempted to use this approach to quantitate the levels of an antigenic glycoprotein (Antigen S) isolated from styles of a particular cultivar (cv Lambert S_3S_4) of *Prunus avium* L the sweet cherry [3–5], in the styles of other cultivars. The reason for this work is that the glycoprotein under consideration which corresponds to a particular self-incompatibility group, is a potent inhibitor of *in vitro* pollen tube growth and is likely to be involved in the control of fertilization [4, 5]

The Antigen S preparation contains two closely related glycoproteins and is a minor component in a buffer extract of styles (Table 1), the main components are a high MW (> 90000) poorly antigenic glycoprotein and a low MW (< 15000) uronicacid-containing component, which is also only weakly antigenic [4] As well as these major components, there is another minor glycoprotein (Antigen P) which is strongly antigenic in rabbits It is present in the styles of all members of the genus *Prunus*, in contrast to Antigen S which is apparently restricted to styles of *Prunus avium* cultivars [4]

Our attempts to quantitate the levels of Antigen S in style extracts by radioimmunoassay using Protein A were unsuccessful Here we present evidence that the lack of success is due to the property of Antigen P (present in all style extracts of *Prunus* species) to bind Protein A with a high affinity, thus invalidating the radioimmunoassay

RESULTS

Binding of Protein A to IgG and Prunus avium *style glycoproteins*

Polyvinyl chloride plates were coated with either rabbit IgG, Antigen P, Antigen S, or BSA (40 μl, 50 μg/ml in PBS) (The composition of PBS is given in the Experimental) The plates were washed with PBS, incubated with BSA, washed and drained as described in the Experimental Serial dilutions (1 10), of [^{125}I]Protein A (40 μl containing 2×10^6 cpm, 50 μg/ml) were then applied to the wells, incubated at 4° overnight, washed with 1% BSA ($3 \times 160\ \mu$l), water ($6 \times 200\ \mu$l) and the wells cut and counted The results are shown in Fig 1

The most important observation is that at concentrations of Protein A, required for maximum binding to the IgG on the plate (13 2 μg/ml), there is significant binding of Protein A to Antigen P, while the binding of Protein A to Antigen S or BSA is only slightly above background levels

Comparison of apparent association constants (K_{ass}) of Antigen P–Protein A binding and IgG–Protein A binding

These experiments were performed similarly to that described above, except that more points were measured in the narrow (non-saturating) range of Protein A concentrations indicated in Fig 1 In replicate experiments the apparent K_{ass} for each interaction varied by less than 5%

Antigen P–Protein A binding Plates were coated with Antigen P (40 μl, 50 μg/ml in PBS), washed with PBS, incubated with BSA and washed again as described in the Experimental The amount of Antigen P bound to each well was 0 042 μg (0 0019 nmol) and the same amount was bound when Antigen P was added at 200 μg/ml The unoccupied sites were blocked with BSA (1% in PBS) and dilutions of [^{125}I]

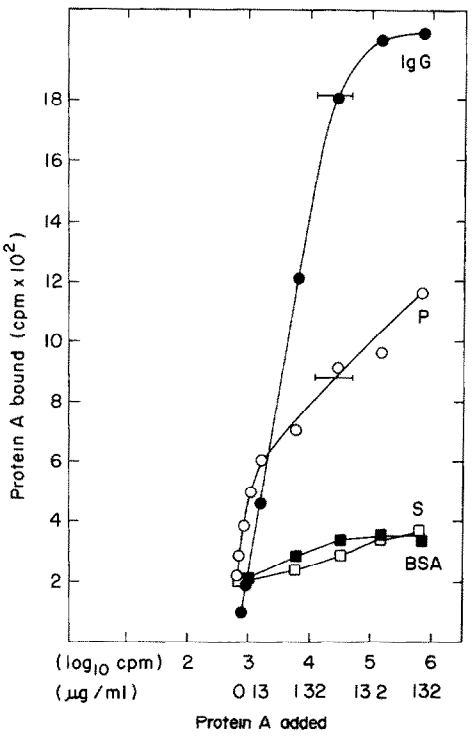

Fig. 1 Binding of Protein A to wells of a microtitre plate coated with bovine serum albumin, immunoglobulin G or the antigenic glycoproteins isolated from *Prunus avium* styles, Antigen S and Antigen P

Table 1 Properties of Antigens S and P isolated from *Prunus avium* style extracts (from refs [4, 5])

	Antigen P	Antigen S
MW		
Immunoprecipitation	32000	37000
SDS-PAGE (10% gels)		39000
Airfuge	22000	17000
% carbohydrate (as glucose)	17 2	16 3
Isoelectric point	<4 5	>9 5

Protein A (initial concentration 40 μl containing $3\,8 \times 10^5$ cpm, 50 μg/ml) added over the concentration range. After overnight incubation the plates were washed and individual wells cut and counted.

The data and calculations are shown in Table 2. The Scatchard [6] plot of the data is shown in Fig. 2, the slope and hence the apparent $K_{ass} = 4\,5 \times 10^7\ M^{-1}$

The apparent number of binding sites was calculated from the ratio of the amount of Protein A bound to the amount of Antigen P present on each well when (bound/free) = 0

Table 2 Binding of Protein A to Antigen P

Protein A added			Protein A bound		
cpm	ng	pmol	cpm	ng	pmol
130600	130	3 25	1470	1 46	0 037
100500	100	2 70	1440	1 43	0 036
90400	90	2 25	1360	1 35	0 034
69300	69	1 73	1310	1 23	0 031
54200	54	1 34	1210	1 20	0 030
35200	35	0 88	929	0 93	0.023

Calculation of binding constant data

Protein A bound (pmol)	Protein A* bound ($M \times 10^{-10}$)	Protein A free (pmol)	Bound / Free	K_{ass} (M^{-1})
0 037	9 25	3 21	0 0115	
0 036	9 00	2.66	0 0135	$4\,5 \times 10^7$
0 034	8 50	2 22	0 0153	
0 031	7 75	1 70	0 0182	
0 030	7 50	1 31	0 0229	
0 023	5.75	0.86	0.0267	

Plates coated with Antigen P (50 μg/ml), 0 042 μg bound to each well [^{125}]Protein A sp act $1\,005 \times 10^6$ cpm/μg

*Concentration of Protein A bound (M) calculated from amount bound (nmol) in 40 μl sample [Protein A] bound = $\frac{(\text{nmol bound})}{40} \times 10^{-3}$ M

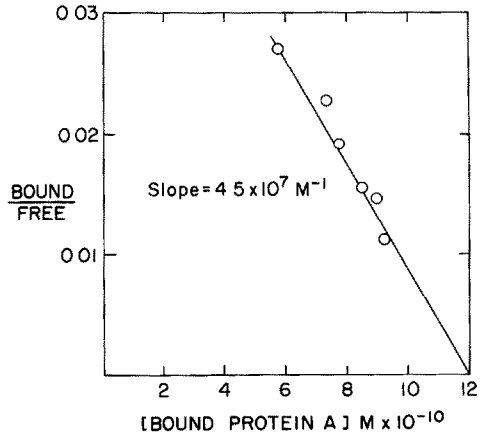

Fig 2 Scatchard plot of Antigen P–Protein A binding data Data from Table 2

IgG–Protein A binding Plates were coated with IgG (40 μl, 50 μg/ml); the amount of IgG bound to each well was 0 32 μg (0 008 nmol) and the same amount was bound when IgG was added at 100 μg/ml. The unoccupied sites were blocked with

Protein A bound = 12×10^{-10} M and 40 μl would theoretically contain $48\,0 \times 10^{-6}$ nmol;

$$\text{Antigen P present} = \frac{0\,042 \times 1000}{22000}\ \text{nmol}/40\ \mu\text{l} = 1.9 \times 10^{-3}\ \text{nmol},$$

$$\text{Apparent number of Protein A binding sites per mol of Antigen P} = \frac{0.048}{1.9} = 0\,025$$

BSA (1% in PBS) and [^{125}I]Protein A added over the concentration range 0 21–4 2 μ/ml After overnight incubation, the plates were washed and individual wells cut and counted

The data and calculations are shown in Table 3 The Scatchard [6] plot of the data is shown in Fig. 3, the slope of the line and hence the apparent $K_{ass} = 1\,8 \times 10^{7}\ M^{-1}$

The apparent number of binding sites was calculated from the ratio of the amount (nmol) of Protein A bound to the amount of IgG present on each well when (bound/free) = 0

Protein A bound = $8\,5 \times 10^{-9}$ M and 40 μl would theoretically contain 340×10^{-6} nmol,

$$\text{IgG bound} = 0\,32\ \mu\text{g} = \frac{0\,32 \times 1000}{40000}\ \text{nmol}/40\ \mu\text{l} = 8 \times 10^{-3}\ \text{nmol},$$

$$\text{Apparent number of Protein A binding sites per mol of IgG} = \frac{0\,340}{8} = 0\,042$$

Table 3 Binding of Protein A to IgG

Protein A added			Protein A bound		
cpm	ηg	pmol	cpm	ng	pmol
167000	167	4 2	8823	8 75	0 219
149000	148	3 5	8386	8 35	0 209
122000	121	3 0	8156	8 12	0 203
100000	100	2 5	7290	7 25	0 181
84000	084	2 1	6649	6 62	0 166

Calculation of binding constant data

Protein A bound (pmol)	Protein A* bound (M × 10^{-9})	Protein A free (pmol)	Bound / Free	K_{ass} (M^{-1})
0 219	5 47	3 98	0 055	
0 209	5 22	3 29	0 060	
0 203	5 08	2 80	0 072	$1\,8 \times 10^{7}$
0 181	4 53	2 32	0 078	
0 166	4 15	1 93	0 080	

Plates coated with IgG (50 μg/ml), 0 32 μg bound to each well [^{125}I]Protein A sp act $1\,004 \times 10^{6}$ cpm/μg

*Concentration of Protein A bound (M) calculated from amount bound (nmol) in 40 μl samples [Protein A] bound = $\frac{(\text{nmol bound})}{40} \times 10^{-9} \times 10^{-6}$ M

Inhibition of Protein A–Antigen P interaction by preincubation of Protein A with IgG

Wells of plates were coated with Antigen P (40 μl, 50 μg/ml in PBS), washed, incubated with BSA and washed again as previously described. [^{125}I]Protein A (100 μl, 10 μg/ml) was preincubated with IgG (100 μl of three-fold serial dilutions of IgG starting at 17 μg/ml) for 3 hr at room temperature Portions of this mixture (40 μl) were added to wells of the Antigen P-coated plate and incubated for 3 hr at room temperature The wells were then washed, cut and counted

The results are shown in Fig 4 Preincubation of Protein A with IgG causes an effective inhibition of Protein A–Antigen P binding A maximum inhibition of 24% was obtained when 0 34 μg IgG was present in a 40 μl aliquot which contained 0 2 μg [^{125}I]Protein A

DISCUSSION

The results show that Protein A binds a plant glycoprotein, Antigen P, with an apparent K_{ass} of the same order as that for the Protein A–IgG interaction when measured by a solid state assay This is an unexpected finding as Protein A is generally used as a specific probe for IgG and specific detection of IgG by Protein A is the basis of many radioimmunoassays

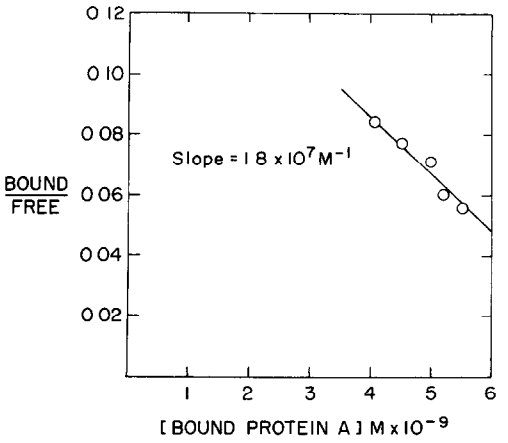

Fig 3 Scatchard plot of IgG–Protein A binding data Data from Table 3

The binding is demonstrated directly by insolubilizing Antigen P and IgG separately on wells of the microtitre plate and adding increasing concentrations of [^{125}I]Protein A (Figs 1–3). The finding that preincubation of Protein A with IgG causes an inhibition of Protein A–Antigen P binding (Fig 4) suggests that the sites on the Protein A molecule involved in IgG binding may be structurally similar or spatially related to those involved in the Antigen P binding

The microtitre tray technique used in this study is now widely used for measuring antigen–antibody interactions [1] and also for glycoconjugate–lectin [7] interactions As it is a solid-state assay, the apparent association constants will differ from those obtained for the same interactions in solution, although the apparent association constants for lectin–glycocon-

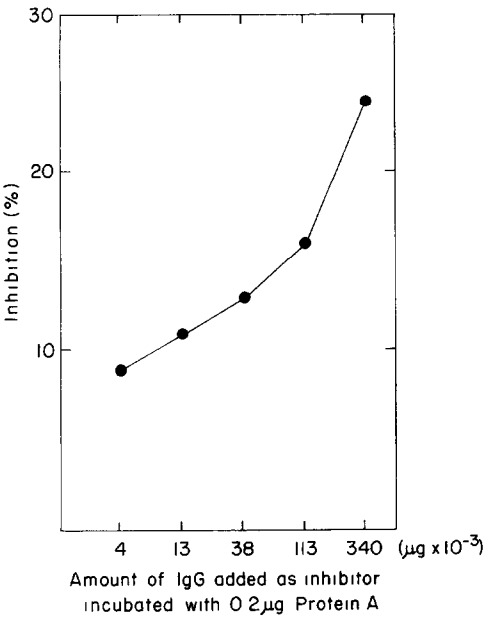

Fig 4 Inhibition of Protein A–Antigen P interaction by preincubation of Protein A with IgG

jugate interactions determined by a similar method are of the same order as those obtained by equilibrium dialysis [7] The multiple washes of the Protein A complex bound to the polyvinyl chloride wells would cause some dissociation, nevertheless, the important finding presented here is that the apparent association constants of the two interactions measured by this method are of the same order, so that significant interference of the anticipated IgG–Protein A binding will occur in the presence of appropriate concentrations of the glycoprotein Antigen P The finding that the apparent number of binding sites calculated from data obtained by this solid state method, is less than unity, indicates the limitations of the method Similar figures of less than unity for the number of binding sites were obtained by Kennedy and Barnes [8] using another solid state assay

In the style extract examined there are several glycoproteins, of which one (Antigen P) but not another (Antigen S) binds IgG As Antigen P is present in all styles from members of the genus *Prunus*, it is not possible to estimate levels of the other antigenic glycoprotein (Antigen S) by radioimmunoassay involving the use of Protein A Whether this is an isolated case, or whether other plant glycoproteins may also bind Protein A is not known, however this single case alerts us to the possibility of this type of interference in other immunoassays of antigens in plant extracts Previously we encountered another difficulty in applying immunological techniques to assays of plant glycoproteins Antisera to two isolated glycoproteins which are quite distinct with respect to MW, isoelectric point and amino acid composition, cross-react significantly by virtue of the presence of minor common saccharide components [8] Thus, although immunological techniques have great potential in the analysis of plant glycoconjugates, there are some hazards to their successful application

EXPERIMENTAL

Microtitre trays were from Cooke Laboratories VA, U S A Iodogen was from Pierce IL, U S A $Na^{125}I$ was from Amersham Protein A was from Pharmacia Rabbit anti-goat IgG was from Cappel Laboratories Bovine serum albumin (Cat No A4503) was from Sigma

Antigenic style glycoproteins (Antigens S and P) were isolated from buffer extracts of mature styles of *Prunus avium* cv Lambert (S_3S_4) by gel and ion exchange chromatography as previously described [4, 5] A summary of their major properties is given in Table 1 The Antigen S preparation contains two components, closely related in MW, immunological and electrophoretic properties [4, 5]

^{125}I labelling was by the Iodogen technique [9] Binding expts were performed on flexible polyvinyl chloride microtitre plates by a modification of the method of ref [10] Essentially, wells of the plate are coated with the test glycoprotein or protein (antigen) (40 μl, 50 μg/ml in PBS*) and incubated at room temp for 4 hr Unadsorbed material is removed with a Pasteur pipette fitted with a flexible plastic tip and the plates washed ×6 with PBS (160 μl) Any unoccupied antigen binding sites on the wells are then blocked by incubating the wells with BSA (1% in PBS, 160 μl) for 30 min at room temp The wells are then washed with 1% BSA in PBS (3 × 160 μl) and the plate drained on a tissue In a radioimmunoassay, bound antigen is then detected by incubating the wells with specific antiserum, the bound IgG being detected by a subsequent incubation with [^{125}I]Protein A In the expts reported here, the [^{125}I]Protein A was incubated directly with the glycoprotein-coated plates after they had been treated with BSA and washed as described The incubation was overnight at 4°, the plates were washed with 1% BSA in PBS (3 × 160 μl), then with H_2O (6 × 200 μl) and the individual wells cut and counted in an autogamma counter (LKB)

The amounts of individual proteins or glycoproteins bound to the wells were established by incubating wells with 40 μl of a soln of ^{125}I-labelled test material, containing either 50 or 200 μg/ml in PBS (sp act 4840 cpm/μg for Antigen P, 2190 cpm/μg for IgG) The plates were incubated at room temp for 4 hr, unadsorbed material being removed with a Pasteur pipette, the plates washed (6 × 160 μl) with PBS, cut and counted

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