Comparative study of procedures for histological detection of lectin binding by use of *Griffonia simplicifolia* agglutinin I and gastrointestinal mucosa of the rat

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Summary

The histological localisation of α-D-galactopyranosyl residues in glycoconjugates of rat stomach and duodenal mucosae was studied by use of *Griffonia simplicifolia* agglutinin I, i.e. the isolectin mixture (A+B) and the isolectin B_4 (B_4). Cryostat sections which were either unfixed or acetone fixed and paraffin sections from both ethanol-acetic acid and formaldehyde fixed tissue blocks were compared. Cellular details were better preserved in paraffin than in cryostat sections. Reactivity of cells binding GS I was less sensitive after formaldehyde than after ethanol-acetic acid fixation inasmuch as higher concentrations of lectins were needed. This drawback could be overcome by trypsinisation of the sections. The binding pattern of GS I (A+B) corresponded with that of GS I (B_4) in either cryostat or paraffin sections. GS I was detected in the cytoplasm of parietal cells and in Brunner's gland cells. In duodenal crypts and villi, lectin was bound to supranuclear regions in the cytoplasm of columnar and goblet cells. The staining efficiency of fluorescein (FITC), horseradish peroxidase (HRP) and colloidal gold particle (CGP) labels in both direct and indirect lectin stainings was compared. Under all experimental conditions, indirect methods required lower concentrations of lectins than direct ones; indirect procedures increased sensitivity about 5-10 fold. CGP labels were always of highest sensitivity when gold particles were further developed by a silver precipitation me-thod. HRP was not as efficient in lectin localisation as CGP, but cytochemical staining was more convenient in routine work. Direct FITC labellings proved to be of lowest sensitivity.

Introduction

Lectins possess a high affinity and a narrow range of specificity for defined sugar residues and are thus a powerful tool for the study of carbohydrate moieties in biomolecules (Lis and Sharon 1973; Goldstein and Hayes 1978; Barondes 1981; Uhlenbruck 1981). Apart from their use in serological assays for qualitative and quantitative analytical informations, lectins are today the most specific molecular probes for the histological localisation of glycoconjugate glycosylation (Nicolson and Singer 1971; Etzler and Branstrator 1974; Freeman et al. 1980; Stoward et al. 1980; Boland et al. 1982; Sato and Spicer 1982; Suzuki et al. 1982).

In a similar way to immunohistological techniques, lectins which are either conjugated with markers such as fluorescein, enzymes etc. or are unconjugated can be employed in "direct"

and "indirect" staining procedures. In the latter techniques it is necessary that a defined antibody system is labelled with a suitable marker instead of the lectin itself; soluble enzyme-antienzyme complexes (e.g. peroxidase-antiperoxidases) are suitable as well (Borisch et al. 1983; Schwechheimer et al. 1983). Clear-cut recommendations for reliable, optimal and highly sensitive staining schedules are still lacking. It is suggested that the principles described for immunohistology also hold true for lectin histology: otherwise, false negative and false positive results would have to be expected (Kuhlmann 1984).

One important point is certainly tissue sampling, on which there are controversial views. Whether cryostat or paraffin sections should be preferred is difficult to decide because interpretation of staining results can be conflicting in either case (Spicer and Schulte 1982; West et al. 1982; Powell 1983; Rittman et al. 1983; Spicer et al. 1983). Moreover, a comparison of different marker systems available has not been fully exploited at yet. Also, no quantitative studies were performed to examine the effect of tissue sampling on subsequent lectin binding and to compare direct and indirect labellings with different marker molecules.

Our studies on the nature of gastrointestinal glycoconjugates involved a panel of different lectins and a variety of histological preparations from which detailed results are described and concern the binding of *Griffonia simplicifolia* (GS) agglutinin I (with specificity for N-acetyl-D-galactosamine and D-galactose residues) to stomach and duodenal mucosae of the normal rat. The procedures studied included (a) cryostat sectioning followed by fixation or no fixation; (b) fixation in aldehyde or in organic solvents and embedment in paraffin; (c) staining of tissue sections by direct and indirect methods. In the latter case, staining efficiency of fluorescein isothiocyanate (FITC), horseradish peroxidase (HRP) and colloidal gold particle (CGP) labels in both direct and indirect procedures was compared. The influence of treatment of sections by trypsin, hydrogen peroxide and periodic acid prior to incubation in lectins was also examined.

Materials and methods

Lectins and lectin conjugates. Lectins from *Griffonia simplicifolia,* their specificities and inhibitors are summarized in Table 1. FITC, HRP and CGP (10-15 nm) conjugates as well as nonconjugated isolectins of controlled purity were obtained from E-Y Laboratories (San Mateo, USA).

Anti-lectin immune sera. Immune sera against the isolectins were prepared by hyperimmunization of rabbits. The IgG fraction was isolated by chromatography of the immune sera over a DEAE ion exchange column followed by affmity chromatography on Protein A - Sepharose (Goudswaard et al. 1978).

Table 1. Lectins used in the study

Lectins	Abbreviation	Specificity, inhibitor
Griffonia simplicifolia I tetrameric isolectin mixture (A_4, A_3B, A_2B_2, AB_3, B_4)	GS I (A + B)	α-D-GalNAc α-D-Gal
Griffonia simplicifolia I tetrameric isolectin (B_4)	GS I (B_4)	α-D-Gal

Antibody conjugates. In the case of indirect localisation, specific HRP and CGP labelled antibodies were employed. For the indirect peroxidase labelled antibody method, we used sheep anti-rabbit IgG antibodies. These were isolated from crude hyperimmune sera by specific immunoadsorbents and covalently conjugated with HRP. Specific antibody-HRP conjugates were then purified by gelfiltration (Sephacryl S-200) and affinity chromatography (Sepharose 4B-Concanavalin A); all procedures have been described (Kuhlmann et al. 1974; Lanner et al. 1978; Kuhlmann 1984). In the indirect CGP technique, sheep anti-rabbit IgG antibodies adsorbed with 10-15 nm CGP were prepared (Horisberger and Rosset 1977); one CGP labelled goat anti-rabbit IgG preparation was provided by Dr. A. Chu from E-Y Laboratories (San Mateo, USA).

Additional biochemicals. D-Galactose was purchased from Merck (Darmstadt, FRG) and N-acetyl-D-galactosamine from C. Roth (Karlsruhe, FRG). Trypsin (ca. 4 U/mg) was obtained from Serva-Labor (Heidelberg, FRG) and *Vibrio cholerae* neuraminidase from Behringwerke (Marburg, FRG). All other chemicals were from Merck (Darmstadt, FRG).

Tissue specimens. Stomach and duodenum were resected from normal male rats of the inbred strain BD X aged 4-6 months and fasted overnight. Both corpus and duodenal mucosa specimens were either fixed in 8-10% formaldehyde for 12-24 h at room temperature, washed, dehydrated and embedded in paraffin according to classical procedures in histopathology, or specimens were fixed in 99% ethanol-1% acetic acid for 12-18 h at 0-4° C, dehydrated in absolute ethanol, cleared in benzene and embedded in paraffin (Kuhlmann 1975; Wurster et al. 1978). In parallel, unfixed tissues were snap frozen in liquid nitrogen cooled isopentane. 5-7 µm thick frozen sections were cut and air-dried. From the latter, slides were also fixed in 100% acetone for 10 min at 0° C and air-dried again. Rehydrated sections were processed for lectin staining.

Paraffin blocks from ethanol-acetic acid fixed specimens were cut at 5-7 µm thickness and mounted on slides cleaned with acetone. In the case of formaldehyde flxation, sections from paraffin blocks were mounted on specially conditioned slides (dipped in 1 % bovine serum albumin, dried at 90° C, placed overnight into 2% glutaraldehyde in distilled water at 4° C, washed for 1 h in multiple changes of distilled water and finally air-dried). Sections were deparaffinated in xylene and passed from absolute ethanol into phosphate-buffered saline (0.01 M phosphate buffer pH 7.2 plus 0.15 M NaCl; PBS). Rehydrated sections were then submitted to lectin staining. Because trypsin digestion is reported to enhance immunoreactivity in the sections (Curran and Gregory 1977; Mepham et al. 1979), the effect of such enzyme digestion was also examined: prior to incubation in lectins, sections were placed into freshly prepared 0.05 or 0.1% trypsin solution in 0.05 M Tris-HCl pH 7.6 plus 0.1% $CaCl_2$ for 5 or 10 min at 37° C followed by rinsings in 1% gelatin/PBS.

In the case of HRP labelling, endogenous peroxidases were inhibited first. Slides were immersed in 1% hydrogen peroxide/PBS in a Coplin jar and treated for 1 h at room temperature under occasional shaking; slides were then rinsed with PBS (Kuhlmann 1975). This step was not necessary when FITC or CGP stainings were performed.

Direct and indirect histological lectin stainings. The incubation schedules are summarized in Table 2. In order to establish the limits of detection of cellular glycoconjugates by use of the different methods, concentrations of isolectins (first step reagents) and labelled antibodies (third step reagents) were from 1 to 50,000 ng/ml. In a first set of experiments, serial dilutions were examined. Then, exact concentrations in decreasing steps of 100 ng/ml (down to working concentration of 100 ng/ml) and 10 ng/ml (down to working concentration of 10 ng/ml) were employed, respectively. When the working concentration dropped below 10 ng/ml, then decreasing concentrations in steps of 1 ng/ml were used. All experiments were repeated three times and the lowest concentration which still permitted histochemical detections was finally tabulated.

Table 2. List of incubation schedules for the localisation of glycoconjugates with Griffonia simplicifolia isolectins GS I (A+B) and GS I (B$_4$)

Step	Direct method			Indirect method	
1	GS I[a] – FITC GS I – HRP GS I – CGP concentration from 1 to 50,000 ng/ml[b]			GS I unlabelled GS I unlabelled concentration from 1 to 50,000 ng/ml	
2	–	–	–	anti-lectin anti-lectin immunoglobulin fraction: 5 µg/ml	
3	–	–	–	anti-rabbit IgG anti-rabbit IgG HRP labelled CGP labelled antibody concentration from 1 to 50,000 ng/ml	
4	fluorescence	DAB/H$_2$O$_2$[c]	silver[d]	DAB/H$_2$O$_2$	silver

[a] GS I (A+B) and GS I (B$_4$), respectively
[b] Lectin concentration expressed as ng protein/ml
[c] 3,3′-Diaminobenzidine and H$_2$O$_2$-substrate mixture
[d] Gold particles revealed by a silver precipitation method

In indirect techniques, the immunoglobulin concentrations of anti-lectin immune sera (second step reagents) were established by preliminary experiments and finally used in a constant way: 5 µg/ml proved optimal for this purpose. During all lectin and antibody incubations, constant volumes were applied to the sections.

Incubation times of both labelled and unlabelled lectins were always 18 h at 4° C. This schedule was chosen because previous experiments have demonstrated that such prolonged incubation times of the first step reagents considerably enhance the sensitivity of both immunohistological and lectin stainings (Kuhlmann 1981; Kuhlmann et al. 1983; Wurster et al. 1983). In the case of indirect procedures, second and third step antibodies/conjugates were always incubated for 30 min at room temperature. Unreacted lectins and antibodies were washed off by three successive washings (5 min each in PBS for FITC and HRP labellings; 10 min each for CGP labellings).

Peroxidase activity was revealed by incubation in 3,3'-diaminobenzidine and H$_2$O$_2$ (Graham and Karnovsky 1966). Then, slides were dehydrated and mounted in resinous medium under coverglass. Gold particles in CGP labelled preparations were subsequently visualized by a silver precipitation reaction (Danscher 1981; Holgate et al. 1983); slides were mounted in glycerol-gelatin. FITC stained specimens were mounted in glycerol/PBS (9 vol / 1 vol) and

examined under a fluorescence microscope equipped with a HBO 50 lamp, epi-illumination, 490 nm primary filter and 515 nm barrier filter.

Controls. Specificity of lectin binding was checked on serial sections by incubation as above in labelled or unlabelled lectins which have been supplemented with either of the respective inhibitors: 0.2 M N-acetyl-D-galactosamine and 0.2 M D-galactose for GS I (A + B); 0.2 M D-galactose for GS I (B$_4$). Other saccharides such as fucose, glucose, mannose, N-acetyl-D-glucosamine were also used together with the lectins. In the indirect techniques, these measures were followed by the corresponding antibodies. Moreover, second step antibodies were replaced by non-immune normal rabbit serum; HRP and CGP labelled sandwich antibodies (third step) were also applied to sections without previous lectin and anti-lectin antibody incubations. Each procedure with the exception of the FITC experiments was finally followed by enzyme substrate and silver development, respectively.

The effect of hydrogen peroxide (1% H$_2$O$_2$ for 1 h in order to abolish endogenous peroxidases in HRP experiments) on subsequent lectin binding was examined in FITC and CGP labelling experiments. In further controls, rehydrated sections were submitted to (a) periodate oxidation with 1% periodic acid in distilled water for 10 min at room temperature (Stoward et al. 1980; Suzuki et al. 1982); (b) reduction with 0.1% sodium borohydride in 1% Na$_2$HPO$_4$ for 30 min at room temperature (Lillie and Pizzolato 1972); oxidation with periodic acid followed by reduction with sodium borohydride as above (Culling et al. 1981); (d) digestion with 100 units/ml neuraminidase in 0.05 M acetate buffer pH 5.5 containing 0.15 M NaCl and 0.09 M calcium chloride for 24 h at 37° C (Culling et al. 1974). Then, lectin binding to sections was studied by the indirect incubation schedule.

For routine histological examination, sections were stained with hematoxylin-eosin.

Results

All observations with the different types of rat stomach and duodenal mucosa cells are summarised in Tables 3 and 4. The binding pattern of GS I (A + B) corresponded with that of GS I (B$_4$) and both were obtained with either cryostat or paraffin methods. The same staining results were achieved with all labelling procedures under the condition that sufficient amounts of the respective reagents were employed.

Influence of specimen preparation
The minimum quantities of isolectins which were necessary to obtain histological detection of their binding to mucosa cells are summarised in Table 4. Experiments with the cryostat method have shown that approximately the same concentrations of lectins must be employed as for sections from formaldehyde and paraffin embedded tissues.

Studies with ethanol-acetic acid and formaldehyde fixed tissues demonstrated that both fixations could be employed in the paraffin technique, but that higher concentrations of lectins were needed for formaldehyde fixed specimens. Trysinisation of rehydrated sections (paraffin method) enhanced the sensitivity of lectin binding.

Direct and indirect staining procedures
Indirect procedures needed lower concentrations than direct ones (Table 4). CGP was of highest sensitivity in both direct and indirect methods under the condition that gold was

developed by a silver precipitation technique. HRP as marker was less sensitive than CGP, but cytochemical staining was more rapidly and easily performed in routine work. From all experiments, direct FITC labellings proved to be of lowest sensitivity. Typical concentrations in routine work were 5 fold to those given in Table 4.

Table 3. Detection of lectin bindings in gastrointestinal mucosa cells

Tissue section	Localisation of *Griffonia simplicifolia* isolectins GS I (A + B)[a]		
	No pre-treatment[b]	Pretreatment with HJO$_4$	Con-trol[c]
Stomach mucosa			
Surface mucus cells	–	(+) cytoplasm	–
Mucus neck cells	–	+ cytoplasm	–
Parietal cells	+ cytoplasm	–	–
Chief zymogenic cells	–	(+) cytoplasm	–
Duodenal mucosa			
Columnar cells	+ supranuclear	mainly – and few (+) supranuclear	–
Brush border	–	–	–
Villous goblet cells	+ and – supranuclear	mainly – and few (+) supranuclear	–
Crypt goblet cells	mainly + and few – supranuclear, granules	+ and – supranuclear, granules	–
Brunner's gland cells	+ cytoplasm	+ cytoplasm	–

[a] Ethanol-acetic acid and formaldehyde fixed specimens gave same results; GS I (A + B) staining pattern was identical with that of GS I (B$_4$); + = positive reaction, – = negative reaction
[b] No pretreatment, sections were passed after rehydration into the lectins
[c] Saccharide added for the control of specificity, see Table 1

Table 4. Minimum amount of lectins necessary for the localisation of their binding to sections from differently processed tissue specimens

Staining method	Ethanol-acetic acid fixation paraffin embedment, microtomy, sections		Formaldehyde fixation paraffin embedment microtomy, sections		No fixation, cryostat sections	
	Rehydrated[a]	Trypsinised	Rehydrated	Trypsinised	air-dried Rehydrated	acetone fixation Rehydrated
Direct – FITC	500[b]	400	1200	900	800	800
Direct – HRP	300	100	300	200	700	700
Direct – CGP	60	20	100	40	–[c]	–
Indirect – HRP	50	5	70	20	–	–
Indirect – CGP	10	2	10	2	–	–

[a] No pretreatments, sections were passed after rehydration into the respective lectins
[b] Lectin concentration expressed as ng protein/ml, see Material and methods for quantitation
[c] Not done

In indirect HRP and CGP procedures, the immunoglobulin concentration of rabbit anti-isolectins could be largely varied. Amounts between 2 and 10 µg/ml did not affect the threshold of detection of lectin binding irrespective of the concentration of isolectins used in the first step incubation. Finally, clear-cut reactions were still observed with HRP conjugated antibodies or CGP labelled antibodies (third step reagents) in concentrations as low as 1 µg/ml and 0.5 µg/ml, respectively. For routine purposes, HRP and CGP labelled antibodies were employed in excess, i.e., 2-5 µg/ml.

Histological localisation

In the stomach, parietal cells stained strongly with GS I (A + B) and GS I (B$_4$). A zonal division was apparent with strongest reactions in upper and lower parts of the gastric glands. Other mucosa cells did not react. While FITC gave almost homogenous stainings, HRP and CGP labelled cells exhibited fine granular appearance (Figs. 1 and 2).

The strong reaction of GS I isolectins with Brunner's gland cells was a prominent feature (Fig. 3). No difference was observed between gland cells close to the lumen and those of lobules deep in the duodenal submucosa. The positive reactions were dense and appeared either to be homogenous or slightly granular; positive reactions predominated in apical cell areas (Fig. 3 inset). In crypts and villi, GS I (A + B) and GS I (B$_4$) positive reactions occurred in small areas of the supranuclear cytoplasm of columnar and goblet cells (Fig. 3 inset and Fig. 4). Crypt goblet cells could also contain conspicuous granules with GS I positivity in their cytoplasm. Apart from those stainings, non reactive goblet cells were also observed. No lectin bindings were detected along the brush border. Small amounts of mucus also stained in crypt's lumina and between villi.

Controls

Incubation of histological sections with isolectins in the presence of inhibitory saccharides prevented lectin binding; other saccharides (listed above) did not influence the reactions. No staining was observed when second step antibodies were omitted or replaced by non-immune serum; labelled sandwich antibodies also did not stain. Treatment of sections with H$_2$O$_2$ alone prior to lectin incubation eliminated endogenous peroxidases but did not influence lectin binding. Sodium borohydride reduction and neuraminidase digestion were without effect on subsequent lectin staining. Periodate oxidation changed lectin binding of stomach mucosa inasmuch as parietal cells became negative and hitherto negative mucus neck cells became positive (Fig. 5). Faint cytoplasmic staining could also occur in surface mucus cells and in zymogenic chief cells. Generally, GS I positivity was more pronounced in ethanol-acetic acid than in formaldehyde fixed specimens. In the case of periodate oxidation of sections from duodenal mucosa, GS I binding to Brunner's gland cells was not affected while columnar cells lost most of their reactivity and showed almost negative or only faint labels. Goblet cells still reacted but to a lesser degree than in untreated sections.
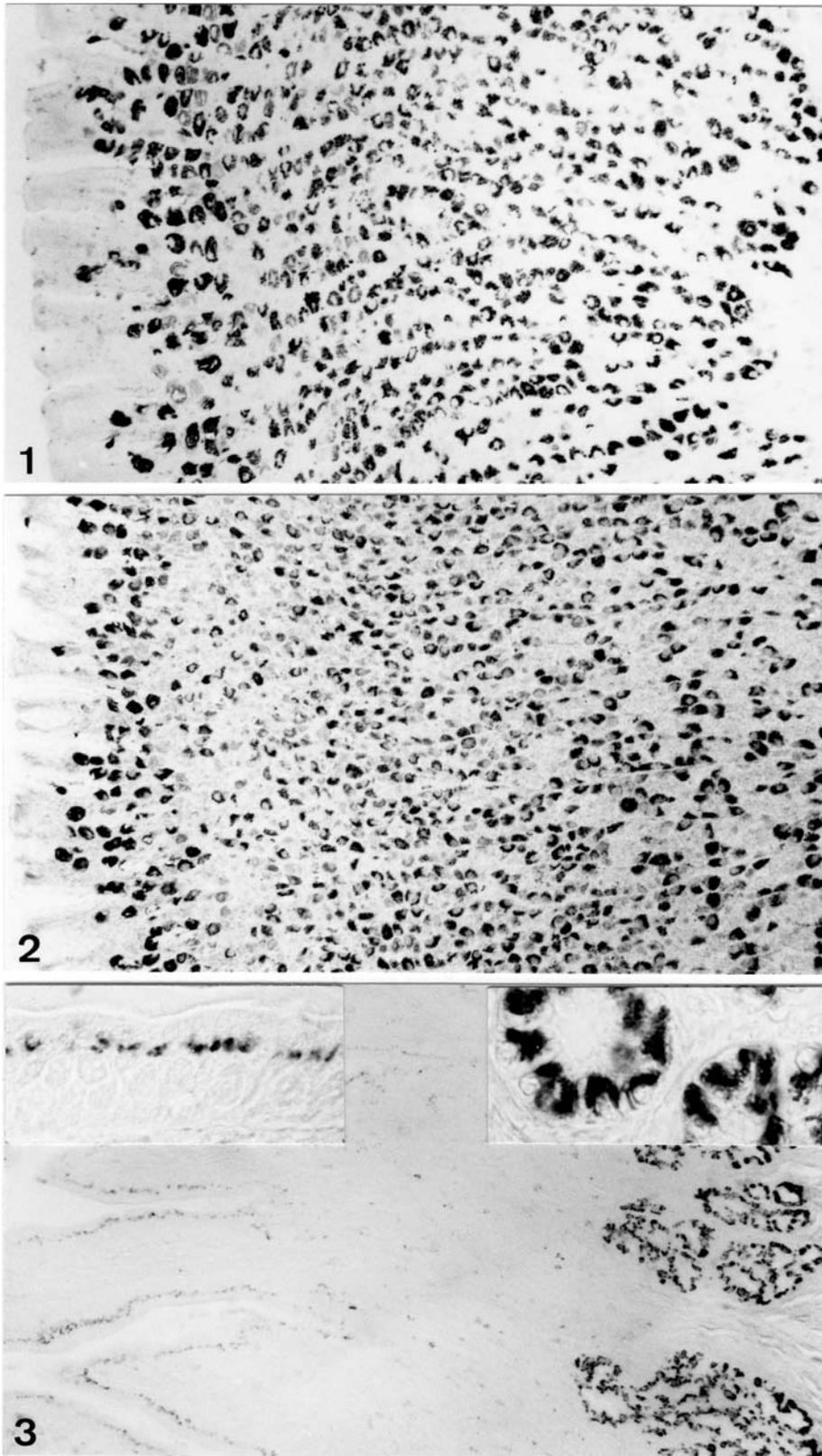
Fig. 1. Gastric body mucosa, formaldehyde fixation and paraffin embedment. GS I (A+B) binding to parietal cells, indirect HRP labelling. Original x 160

Fig. 2. Same tissue as in Fig. 1., GS I (B$_4$) binding to parietal cells, indirect CGP labelling. Original x 160

Fig. 3. Duodenal mucosa, formaldehyde fixation and paraffin embedment. Low magnification view of GS I (A+B) binding, indirect HRP labelling. Positive reactivity is clearly detected in Brunner's glands and in supranuclear regions of columnar enterocytes of apical crypts and villi. Original x 160.

Inset top left: GS I (B4) binding in supranuclear areas of columnar cells. Original x 540

Inset top right: high magnification view of GS I (B$_4$) binding to Brunner's gland cells. Original x 540
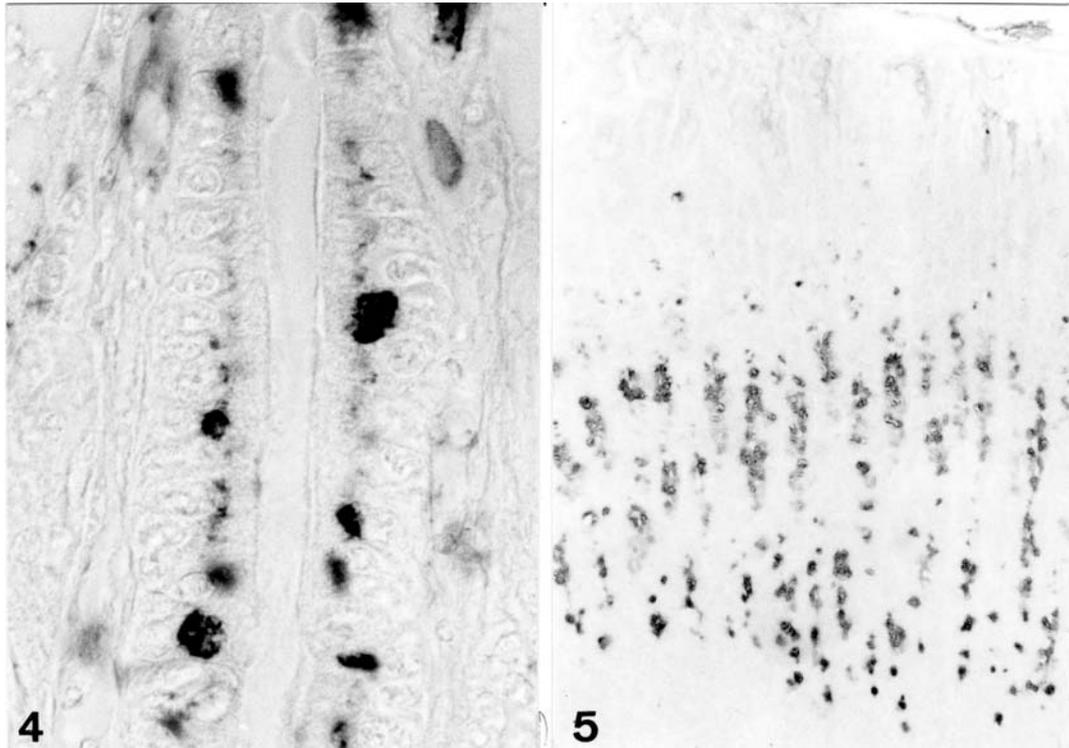
Fig. 4. Crypt of duodenal mucosa, ethanol-acetic acid fixation and paraffin embedment; indirect GS I (A+B) staining with HRP method. Positive reactions are seen in mucus granules of some goblet cells and in supranuclear regions of columnar cells showing variable intensities. Original x 540

Fig. 5. Gastric mucosa, ethanol-acetic acid fixation and paraffin embedment. Section was treated with periodic acid prior to incubation in GS I (A+B), indirect HRP method. Note staining in mucus neck cells. Original x 160

Discussion

Griffonia simplicifolia agglutinin I consists of five tetrameric α-D-galactopyranosyl-binding isolectins composed of various combinations of two subunits (A and B) with different carbohydrate binding specificities; the A subunit possesses specificity for α-D-GalNAc and α-D-Gal while the B subunit shows a sharp specificity for α-D-Gal residues (Murphy and Goldstein 1977). On the basis of these biochemically known binding specificities, histological lectin labelling experiments allowed visualisation in tissues of glycoconjugates containing these sugar residues. In our studies, GS I (A + B) and GS I (B$_4$) gave almost identical patterns.

In stomach mucosa, the exclusive GS I binding to parietal cells was a phenomenon characteristic for the rat. While rat and guinea pig parietal cells react with GS I isolectins, those of mouse, pig and human (results not described in this paper) do not, thus reflecting species differences. Further studies (and especially ultrastructural work) are needed to determine whether the cytoplasmic staining described above reflect the localisation of cytosolic molecules or reactivity of tubulovesicular membranes.

In the duodenum, Brunner's gland cells were always labelled. Within a given histological section, goblet cells exhibited inhomogenous reactions: positive and negative goblet cells side by side in crypts, and preferentially negative goblet cells in villi; stainings were almost restricted to parts of the cytoplasm. This distribution and in connection with the observation of negative secretory product expressed significant stages of glycosylation of glycoconjugates in cytoplasmic compartments in the course of goblet cell maturation from crypts to villi. Columnar enterocytes in crypts and villi exhibited regularly positive GS I binding in their supranuclear regions, most probably as sign of a defined stage in nascent carbohydrate chains. The binding was confined to the Golgi complex. The clear intracytoplasmic location of specific lectin stain favoured the localisation of mucin or glycoprotein rather than of glycolipid of the plasma membrane.

Controls with selected inhibitory sugars demonstrated that the observed GS I binding depended on a specific lectin-sugar interaction. The latter was not influenced by unrelated saccharides. Sialic acids did not exert shielding effects because neuraminidase digestion of sections could not reveal new GS I bindings to carbohydrate residues in crypt position. In the case of HRP as marker, endogenous tissue peroxidases must be irreversibly inhibited in order not to interfere with stains derived from the lectin labelling experiments. This step was routinely done by 1 % hydrogen peroxide in PBS for 1 h and proved useful for all types of histological immunoperoxidase reactions hitherto performed in our laboratory (Kuhlmann 1975, 1984). While endogenous peroxidases became completely inhibited, subsequent lectin binding was not affected. In any case, the latter possibility must be reconciled. For the inhibition of tissue peroxidases we did not employ schedules which involved periodic acid-borohydride treatments (Isobe et al. 1977; Heyderman 1979); these had deleterious effects on carbohydrates. While sodium borohydride alone did not influence lectin binding, periodic acid considerably impaired subsequent lectin binding. Thus, parietal cells no longer reacted with GS I isolectins, while other cells (in the duodenum) resisted quite well. Oxidation of tissue sections by periodic acid was reported to cleave vicinal glycols in galactopyranoside residues in the terminal position of polysaccharide chains of glycoproteins which could explain the changed lectin binding pattern in our material (Stoward et al. 1980; Suzuki et al. 1982). Furthermore, new staining patterns emerged which were suggested to be due to subterminal position of respective residues which become accessible on periodic acid oxidation (cf. Table 3).

The relationship of galactopyranoside residues seen by GS I binding is still unclear, and it remains to be determined whether binding of GS I is evidence of a defined entity with specific pathophysiological significance. This would require characterisation of the chemical structure. It is thought that digestion of sections with specific glycosidases and subsequent use of lectins will provide more knowledge of sugar sequences in glycoconjugates and, furthermore, prove specificity of lectin binding (Peters and Goldstein 1979).

Experiments with differently prepared tissue specimens and reagents have shown variable quality of lectin localisation. Cryostat sections which were either unfixed or fixed in acetone

always yielded poor morphology, as was evident in all cytochemical reactions. Clear-cut identification of cellular details was much superior in paraffin sections of ethanol-acetic acid or formaldehyde fixed specimens. In the case of formaldehyde fixation, it was necessary to collect paraffin sections onto conditioned slides. Otherwise sections tended to detach easily during the incubation procedures and especially when enzymatic pretreatments were performed. For this purpose, BSA-glutaraldehyde conditioned slides proved useful while acetone cleaning and albumin conditioning alone were insufficient measures.

In view of controversial results obtained with cryostat sections and those from paraffin embedded tissues one could expect loss and masking of lectin binding sites in the course of tissue fixation, dehydration and paraffin embedment (Holthöfer et al. 1982; Spicer and Schulte 1982; West etal. 1982; Powell 1983; Rittman and Mackenzie 1983; Spicer et al. 1983). Hence, it should be recommended that both cryostat and paraffin sections be used for comparison at the beginning of experimentation. In our model, carbohydrate portions in glycoconjugates survived well and excellent localisation of cellular lectin bindings was achieved with paraffin sections from both ethanol-acetic acid and formaldehyde fixed specimens. Yet, more precisely, histological stainings were stronger in rehydrated sections after fixation in ethanol-acetic acid than in formaldehyde. From a quantitative point of view, reactivity ofcarbohydrate residues must be altered differently in formaldehyde fixation than in ethanol-acetic acid fixation inasmuch as higher concentrations of lectins were needed to observe clear-cut localisation of their binding to the cells.

This difference in reactivity was readily overcome when sections from formaldehyde fixed blocks were submitted to trypsinisation. It is well known from immunohistological studies that fixation plays an important role in masking biomolecules (details on reversibility and irreversibility see review by Kuhlmann (1984)) and partial reconstitution was obtained by treatment of rehydrated sections with proteolytic enzymes (Huang et al. 1976; Curran and Gregory 1977; Denk et al. 1977; Mepham et al. 1979). In the case of carbohydrate localisation by lectins this principle also proved useful: sensitivity of lectin binding was comparable to that of rehydrated paraffin sections from ethanol-acetic acid fixed specimens. Moreover, the nonspecific background reaction became significantly reduced. Trypsin digestion of sections from ethanol-acetic acid fixed specimens also increased sensitivity of lectin binding. For this purpose, however, a low enzyme concentration together with a short digestion time was chosen, since otherwise sections became rapidly digested.

From all the above observations we deduced that formaldehyde and ethanol-acetic acid fixation in connection with paraffin embedment are useful for the study of GS I binding to rat gastrointestinal mucosa cells. Fixation procedures, dehydration and embedment might be critical in other models and need experimental adaption. In the case of ethanol-acetic acid fixation, it must be emphasized that good preservation of morphology was only obtained reliably when tissue blocks (about 5 mm in thickness) were immersed in ice-cold fixative which was replaced by fresh solutions at least twice within the first 5-10 min of fixation and followed by occasional and gentle shaking. Later on, fixative should be renewed according to its appearance.

Apart from tissue preparation, efficiency of lectin binding to cells depended on the selected incubation schedule, i.e. the choice of a direct or an indirect detection method, the cytochemical stain (marker system) and the purity of reagents. In this connection, the observations previously made with immunohistological techniques also proved to apply to lectin staining. As a matter of fact, the application of highly purified reagents in appropriate concentrations was essential. Incubations with first step reagents (lectins either labelled or not) were always

carried out overnight because this measure already allowed much lower concentrations of lectins to be used than those usually employed in published lectin staining. In those cases, short incubation times (up to 1 h at 4° C or at room temperature) with high concentration of lectin in the order of several µg/ml were performed (Stoward et al. 1980; Holthöfer et al. 1982; West etal. 1982; Rittman and Mackenzie 1983). However, we deduced from our experiments that such large amounts were not necessary when well-purified lectins and conjugates were used. With respect to the sensitivity of lectin binding, incubation times of first step reagents appeared to be critical while variations of subsequent incubation steps (indirect method) were less important. Optimal amounts of unlabelled anti-lectin antibodies and labelled sandwich antibodies were determined in preliminary assays and were always used in subsequent experiments.

Comparable to immunohistology, indirect staining procedures were of higher sensitivity than direct procedures. Thus, only low concentrations were needed in indirect stainings. Moreover, unlabelled lectins were cheaper to purchase than the labelled ones, and, very importantly, unlabelled lectins proved to be more stable especially under prolonged storage. Colloidal gold particles which were further developed by a silver precipitation method were the most sensitive markers of lectin binding. HRP labellings were not as sensitive as CGP. This observation was comparable to that described recently in PAP immunostaining (Holgate et al. 1983). However, we preferred HRP as marker in our routine work because of its rapid cytochemical performance with clear-cut stainings free of background. In contrast, CGP followed by silver precipitation was cumbersome due to the preparation of silver developer and light microscopic controls of the developing process in the dark; fine granular, non-specific precipitations could also occur.

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