Isolation and partial characterization of rat duodenal-gland (Brunner's-gland) mucus glycoprotein

Henk L. SMITS, Peter J. M. VAN KERKHOF and Mebius F. KRAMER
*Department of Histology and Cell Biology, State University of Utrecht, School of Medicine,
Nic. Beetsstraat 22, 3511 HG Utrecht, The Netherlands*

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A mucus glycoprotein was isolated from the duodenal glands of the rat and purified by repeated density-gradient centrifugation. The characterized glycoprotein is unique to the mucous cells of the duodenal glands and is not present in parts of the small intestine devoid of these glands. The chemical composition of the purified glycoprotein is characteristic for glycoproteins of the mucin-type. Its protein content is relatively high and amounts to 35% by weight. No neuraminic acid and little sulphate (2%) is present. Evidence is presented that the native glycoprotein is built up from subunits held together via disulphide bridges in a non-glycosylated region of the protein core.

Mucus glycoproteins of the small intestine have been isolated and characterized from rat (Forstner *et al.*, 1973), man (Jabbal *et al.*, 1976) and pig (Mantle & Allen, 1981), as well as from various other parts of the gastro-intestinal tract, but up to now no attention has been paid to the isolation and characterization of mucus glycoprotein of the duodenal glands. We have now isolated a mucus glycoprotein of the rat duodenal glands and investigated its biochemical properties.

Materials and methods

Preparation of duodenal-gland homogenate fraction

Male Wistar rats (200–250 g body wt.) were kept without food for 24 h and killed by decapitation. The proximal part (1.5 cm) of the intestine, containing the duodenal glands, was removed and washed in ice-cold saline (0.9% NaCl). The tissue was frozen at –25°C on microscope slides with the mucosal face upwards and the mucosa was removed by scraping the frozen tissue with a razor blade. Microscopic examination of the scraped tissue revealed that the villi and the major part of the crypts were removed and that only the muscularis externa and the submucosal layer including the duodenal glands were left. The following procedures were conducted at 4°C. Duodenal-gland preparations were collected in 10 vol. of 1 mM-EDTA and homogenized first with a Polytron P CU-2 tissue homogenizer and subsequently with a tight-fitting glass/Teflon Potter–Elvehjem homogenizer. The homogenate was centrifuged at 1700 g for 20 min to remove nuclei, cell debris and undissolved mucus. The pellet was suspended in 5 vol. of 1 mM-EDTA, again homo-

genized and centrifuged. The supernatants were combined and freeze-dried (water-soluble homogenate fraction). The pellet was resuspended in 1 mM-EDTA/0.5 M-2-mercaptoethanol and kept at 37°C for 30 min, after which it was centrifuged. The supernatant was freeze-dried (reduced homogenate fraction). Occasionally, homogenate fractions of the mucosa and the submucosa were prepared from more distal parts of the small intestine not containing the duodenal glands. These fractions were obtained by extraction with 2-mercaptoethanol.

Purification of duodenal-gland mucus glycoprotein

Duodenal-gland mucus glycoprotein was purified by equilibrium centrifugation on density gradients (Creeth & Denborough, 1970; Starkey *et al.*, 1974) of the freeze-dried water-soluble homogenate fraction, as previously described (Spee-Brand *et al.*, 1980). After an initial run on a CsCl density gradient, the glycoprotein was finally purified by a second run on a CsBr or a CsCl density gradient. CsCl and CsBr (Suprapur grade) were from Merck.

Gel filtration

Purified glycoprotein was applied to a Sepharose 2B column (1.6 cm × 90 cm) or a Sepharose CL-4B column (1.6 cm × 90 cm) and eluted with 10 mM-Tris/HCl buffer, pH 7.6, containing 1 mM-EDTA and 1 mM-NaCl. When reduced glycoprotein was chromatographed, 10 mM-dithiothreitol was added to the buffer. Fractions of volume 2 ml were collected.

Proteolytic digestion of purified glycoprotein

Trypsin digestion was performed essentially as described by Scawen & Allen (1977). Reduced

glycoprotein (1 mg/ml) was dissolved in 0.1 M- NH_4HCO_3 , pH 8.3, containing 3 mM- NaN_3 . Diphenylcarbamoyl chloride-treated trypsin from bovine pancreas (type XI; Sigma Chemical Co.) was added to a concentration of 0.15% (w/w) and the mixture was incubated at 37°C for 5 days. After 2 days 0.15% (w/w) trypsin was added again. For amino acid analysis trypsin-digested glycoprotein was additionally purified on a CsCl density gradient.

Reduction and carboxymethylation of glycoprotein

For reduction and carboxymethylation of the purified glycoprotein the method of Press *et al.* (1966) was used. Non-reduced glycoprotein was dissolved (1 mg/ml) in a solution of 6 M-guanidinium chloride in 0.5 M-Tris/HCl buffer, pH 8.5. After addition of 2-mercaptoethanol to a concentration of 0.5 M, the sample was reduced for 24 h at 4°C. Carboxymethylation was achieved by adding an equivalent volume of 1.5 M-iodoacetamide in 0.5 M-Tris/HCl buffer, pH 8.5, and keeping the solution in the dark for 24 h at 25°C. The pH was maintained at 8.5 by addition of 1.0 M-NaOH. Subsequently the samples were precipitated with ethanol. To this end, to 1 ml of sample 0.1 ml of 2 M-potassium acetate buffer, pH 5.0, and 7.5 ml of cold 96% (v/v) ethanol was added and precipitation was allowed to take place overnight. The precipitates were collected by centrifugation, washed with ethanol and freeze-dried.

Carboxymethylation with iodo[^3H]acetic acid was performed by incubating 0.5 ml of a solution of reduced glycoprotein (1 mg/ml) in 1 M-Tris/HCl buffer, pH 8.2, containing 0.1 M-dithiothreitol (Calbiochem) with 500 μCi of iodo[^3H]acetic acid (sp. radioactivity 212.5 mCi/mmol; The Radiochemical Centre) for 2 h at 37°C in the dark. Subsequently, after the mixture had been cooled on ice, a 1000-fold excess of solid iodoacetic acid was added and the material was precipitated with ethanol.

Miscellaneous

Ultracentrifugation studies, gel electrophoresis on 2%-(w/w)-polyacrylamide/0.5%-(w/w)-agarose gels and on 5% or 5–15%-(w/w)-polyacrylamide/sodium dodecyl sulphate gels, analytical procedures and haemagglutination inhibition tests were performed as described previously (Spee-Brand *et al.*, 1980). Blood group H was tested with 'anti-H' lectin from *Ulex europaeus* (Sigma) with a highest concentration of 3.4 $\mu\text{g/ml}$.

Results

Isolation of duodenal-gland mucus glycoproteins

A typical distribution on a CsCl density gradient of hexose- and protein-containing material of the water-soluble duodenal-gland homogenate fraction is

shown in Fig. 1(a). Most of the hexose-containing material was present in a peak with 1.37 g/ml density (fraction a). Fraction a was separated from protein-rich material of lower density, and from another hexose-rich fraction (fraction b) with 1.49 g/ml density. Fraction a was absent from homogenates of

Fig. 1. Fractionation of duodenal fractions on density gradients

(a) Water-soluble homogenate fraction of duodenal glands (CsCl density gradient): O, hexose; ●, protein. (b) Homogenate fraction of small-intestinal mucosa (Δ , hexose; $\blacktriangle$, protein) and submucosa ($\square$, hexose; $\blacksquare$, protein) not including duodenal glands (CsCl density gradients). (c) Re-centrifugation of glycoprotein fraction a (a) (CsBr density gradient). The starting density of the gradients was 1.42 g/ml. For full experimental details see the text.

both more-distal intestinal mucosa and submucosa not containing duodenal glands (Fig. 1*b*). Fraction *b* (Fig. 1*a*) presumably originates from a contamination by goblet-cell mucin, because it is the main glycoprotein present in the homogenate fraction of the more-distal intestinal mucosa not containing duodenal glands (fraction *b*; Fig. 1*b*). Fraction *b* (Fig. 1*a*) and fraction *b* (Fig. 1*b*) showed the same electrophoretic mobility, but their mobility differed from fraction *a* (Fig. 1*a*), as shown in Fig. 2. In view of its high hexose/protein ratio and its absence from intestinal regions devoid of duodenal glands, fraction *a* is considered to contain glycoprotein derived of the duodenal glands. Its tissue-specific origin has been confirmed by immunocytochemistry.

A final purification was obtained by a second run on a CsBr density gradient of fraction *a* (Fig. 1*c*).

Fig. 2. Polyacrylamide-gel electrophoresis of purified glycoprotein

Gel electrophoresis was performed on a 5% polyacrylamide/sodium dodecyl sulphate gel. No stacking gel was used. Lane 1, duodenal-gland mucus glycoprotein (fraction *a* of Fig. 1*c*); lane 2, small-intestinal mucus glycoprotein (fraction *b* of Fig. 1*b*); lane 3, small-intestinal mucus glycoprotein (fraction *b* of Fig. 1*a*); lane 4, duodenal-gland glycoprotein after tryptic digestion (fraction *c*₁ of Fig. 4*c*); lane 5, low-molecular-weight components of duodenal-gland glycoprotein after tryptic digestion (fraction *c*₂ of Fig. 4*c*). Lanes were loaded with 100 μ g of hexose or its digestion product. Lane 5 was loaded with the digestion product of 500 μ g of hexose. For full experimental details see the text.

The glycoprotein obtained after the second centrifugation step is the non-reduced purified glycoprotein as studied throughout the work described in the present paper. The sugar/protein ratio of the glycoprotein was increased from about 1.0 to 1.2 after the second centrifugation step. The buoyant density of the glycoprotein on a CsBr density gradient (1.41 g/ml) was somewhat higher than on a CsCl density gradient (1.37 g/ml). The water-soluble homogenate fraction of the duodenal glands contained almost all (85%) of the duodenal-gland mucus glycoprotein. An additional extraction with 0.5 M-2-mercaptoethanol yielded less than 10% of the glycoprotein, and the remaining glycoprotein could be obtained only by solubilization with 6 M-guanidinium chloride. The amount of glycoprotein obtained from the water-soluble fraction was only 50–75 μ g per rat.

Gel electrophoresis of the twice-centrifuged duodenal-gland glycoprotein shows that a highly purified glycoprotein preparation was obtained. Only one periodate-Schiff positive band could be seen on a 5%-polyacrylamide-gel electrophoretogram (Fig. 2), and no Coomassie Blue-stained bands could be seen on a 15%-polyacrylamide-gel electrophoretogram. On a 5% polyacrylamide gel supplemented with 1% sodium dodecyl sulphate the reduced glycoprotein barely entered the gel, and its electrophoretic mobility was not altered if 8 M-urea, 0.01% dithiothreitol, 0.01% 2-mercaptoethanol or 0.01% Nonidet P-40 was added to the gel. On a 2%-polyacrylamide/0.5%-agarose gel supplemented with 0.1% sodium dodecyl sulphate too, the reduced glycoprotein hardly penetrated into the gel.

To determine whether the intact glycoprotein possesses a multi-glycopeptide structure, non-reduced glycoprotein and reduced glycoprotein were subjected to gel filtration on Sepharose 2B. The non-reduced glycoprotein was eluted in a broad peak just behind the void volume of the column (fraction *a*₁; Fig. 3*a*). Part of the glycoprotein, however, was eluted in a second, more-retarded, peak (fraction *a*₂). The latter material presumably represented the monomeric form of the glycoprotein, as the reduced glycoprotein was eluted in the same volume of the column (Fig. 3*b*). Determination of cysteine residues after alkylation, with or without prior reduction of the glycoprotein (Table 1), shows that reduction is complete under the conditions used.

On gel filtration on Sepharose 4B the non-reduced glycoprotein was excluded, but a small amount of material was included, forming a trailing edge towards the excluded peak (Fig. 4*a*). The included fraction presumably contained some degraded material, and was discarded. After reduction of the completely included fraction, it was again eluted in the void volume of the column (Fig. 4*b*). After tryptic digestion of the reduced glycoprotein, two

Fig. 3. Gel filtration of purified duodenal-gland mucus glycoprotein on Sepharose 2B
(a) Non-reduced glycoprotein; (b) reduced glycoprotein. For full experimental details see the text.

retarded peaks were present on gel filtration on Sepharose 4B (Fig. 4c). Apparently, a small part of the reduced glycoprotein was not digested and remained in the void volume of the column. On gel electrophoresis of the major fraction of the trypsin-digested glycoprotein (fraction c_1) only one periodate-Schiff-positive band was present. This band clearly had a slightly higher electrophoretic mobility than did the non-digested reduced glycoprotein (Fig. 2). The minor fraction (fraction c_2) presumably contained a heterogeneous population of low-molecular-weight glycoprotein fragments, as no (glyco-)protein bands could be detected in this fraction on gel-electrophoresis. In order to determine the nature of this low-molecular-weight fraction more precisely, purified glycoprotein was reduced and cysteine residues were labelled by alkylation with iodo[^3H]acetic acid. The radioactively labelled glycoprotein had a buoyant density of 1.37 g/ml on a CsCl density gradient and was eluted in the void volume of a Sepharose 4B column (Fig. 4d). On digestion with trypsin, radioactivity was mainly eluted in the low-molecular-weight fraction (fraction d_2), thus indicating the presence of polypeptides rich in cysteine in this fraction. As radioactivity in the high-molecular-weight fraction (fraction d_1) was low, this hexose-rich fraction apparently contained only a small amount of cysteine residues.

On sedimentation-velocity analysis, the non-reduced purified glycoprotein sedimented over a broad range, sharp at the high-molecular-weight side, but with significant trailing to the low-molecular-weight sizes. A mean $s_{25,w}^0$ value of 6.13S was calculated, but this value may be underestimated. After reduction and carboxymethylation of the

Fig. 4. Gel filtration of trypsin-digested duodenal-gland mucus glycoprotein on Sepharose 4B
(a) Non-reduced glycoprotein; (b) reduced glycoprotein; (c) trypsin-digested reduced glycoprotein; (d) reduced glycoprotein labelled with iodo[^3H]acetic acid (—) and trypsin-digested carboxy[^3H]methylated glycoprotein (----). V_0 , Void volume; I, elution position of carboxy[^3H]methylated cysteine. For full experimental details see the text.

glycoprotein, two polydisperse components were found on sedimentation-velocity analysis. The major component sedimented with an $s_{25,w}^0$ value of 1.35S and the minor one at 8.46S. As judged by their absorbance at 280nm, these components were present at a ratio of 7:1. In spite of the small difference in sedimentation coefficient between the non-reduced glycoprotein and the minor component of the reduced glycoprotein, it is assumed that the latter represents some non-reduced glycoprotein in this fraction. The heterogeneity of the non-reduced glycoprotein may be due to the presence of glycoprotein in various degrees of multi-glycopeptide formation. The amounts of material obtained after tryptic digestion of the glycoprotein were too small for accurate sedimentation-velocity analysis.

Chemical composition of the purified duodenal-gland mucus glycoprotein

The chemical composition of the purified glycoprotein is shown in Tables 1 and 2. The sugar and

Table 1. *Chemical composition of duodenal-gland mucus glycoprotein*

The protein value was calculated by adding up the amounts of individual amino acids. The results are expressed as percentages by weight of freeze-dried material.

	Composition (% by wt.)
Galactose	14.8
Fucose	5.0
N-Acetylglucosamine	22.2
N-Acetylgalactosamine	13.7
Total sugar	55.7
Protein	35.0
Sulphate	2.0
Total recovery	92.8
Molar proportions	
Gal:Fuc:GlcNAc:GalNAc = 1.0:0.4:1.3:0.8	
GalNAc:(Thr + Ser) = 0.9:1.0	

Table 2. *Amino acid composition of duodenal-gland mucus glycoprotein*

The samples were S-carboxymethylated in 6M-guanidinium hydrochloride by the method of Press *et al.* (1966). Reduction was performed with 0.5M-2-mercaptoethanol. Tryptic digestion was performed as described by Scawen & Allen (1975). Abbreviations: CmCys, S-carboxymethylcysteine; N.D., not determined.

	Amino acid composition (residues/100 residues)	
	Before tryptic digestion	After tryptic digestion
Asp	4.3	3.4
Thr	13.9	17.9
Ser	11.6	15.0
Glu	7.2	5.8
Pro	8.9	10.3
Gly	5.0	3.7
Ala	4.1	5.6
Val	4.7	3.3
Met	0.7	0.9
Ile	19.2	16.9
Leu	8.7	7.2
Tyr	1.1	0.9
Phe	2.2	2.0
Lys	1.9	1.6
His	2.5	3.2
Arg	2.1	1.9
Cys		
No reduction	2.2	0.3
Reduction	—	N.D.
CmCys		
No reduction	—	—
Reduction	2.2	N.D.

amino acid compositions are characteristic for glycoproteins of the mucin type (Gottschalk, 1972; Horowitz, 1977). Besides the sugars indicated, traces of mannose were present too, as in other purified gastro-intestinal mucus glycoproteins (Carlson, 1968; Scawen & Allen, 1975; Mantle & Allen, 1981). Neither ribose nor deoxyribose was present, showing the absence of DNA from the purified glycoprotein preparation. Xylose, glucose and neuraminic acid were also absent. Threonine and serine together account for over 25% of the total amino acids.

On tryptic digestion, cysteine was completely lost from the intact glycoprotein, and an increase of the molar proportion of serine plus threonine was found (25.5 to 32.9%), although the molar proportions of the other amino acids were not altered significantly. The molar ratio of sugar to protein increased from 1.2 to 1.4 on digestion with trypsin, but no significant change in carbohydrate composition was observed.

Investigation of blood-group activity of the glycoprotein showed the presence of both A and H activity. No blood group B activity was found.

Discussion

Rat duodenal-gland mucus glycoprotein was obtained from homogenates by extraction with water; no reduction, high salt extraction or any other harsh method was necessary. Thus it was the intact glycoprotein that was studied. The relatively low density (1.37 g/ml) of this glycoprotein in a CsCl density gradient suggests a relatively low molar ratio of hexose to protein, as compared with, for instance, rat stomach (1.47 g/ml) (Spee-Brand *et al.*, 1980) or rat small-intestinal mucus glycoprotein (1.49 g/ml) (Fig. 1b). This is in agreement with the chemical composition, which shows a relatively high protein content (35%) and consequently a low molar ratio of sugar to protein (1.2:1.0). Many other purified mucus glycoproteins possess much lower protein contents. This value varies between 5% for the major glycoprotein of rat stomach (Spee-Brand *et al.*, 1980) and about 15% for, for instance, rat small-intestinal glycoprotein (Wold *et al.*, 1974; Jabbal *et al.*, 1976). Even the latter value might be an overestimation, since it is well known that non-covalently bound protein is not easily removed by the gel-filtration techniques used for the purification of glycoprotein in that work.

As compared with other mucus glycoproteins, the sedimentation coefficients observed for the non-reduced (6.13 S) and reduced (1.35 S) glycoprotein are rather low. Much higher sedimentation coefficients are found for, for instance, rat (Spee-Brand *et al.*, 1980), pig (Snary *et al.*, 1970; Scawen &

Allen, 1975) and human (Pearson *et al.*, 1980) gastric mucus glycoprotein and for rat (Jabbal *et al.*, 1976), pig (Mantle *et al.*, 1981) and human (Jabbal *et al.*, 1976) small-intestinal mucus glycoprotein. These values range from about 8S for reduced glycoprotein to about 20S for non-reduced glycoprotein. As the degree of glycosylation of the duodenal-gland mucus glycoprotein is relatively low, this might well explain the rather low sedimentation coefficients observed.

The marked change in sedimentation coefficient on reduction by 0.5M-2-mercaptoethanol (from 6.13S to 1.35S) and the difference in elution pattern on Sepharose 2B before and after reduction (Figs. 3a and 3b) clearly demonstrate the multi-glycopeptide structure of the glycoprotein and the involvement of interchain disulphide bridges in the linkage of the subunits. A similar multi-glycopeptide structure has been described previously for, for instance, pig (Snary *et al.*, 1970; Allen & Snary, 1972; Allen, 1977) and human gastric (Pearson *et al.*, 1980) mucus glycoprotein and for pig small-intestinal mucus glycoprotein (Mantle *et al.*, 1981). For rat gastric mucus glycoproteins (Spee-Brand *et al.*, 1980) evidence for such a multi-glycopeptide structure has been presented also. However, a multi-glycopeptide structure was not observed for rat small-intestinal mucus glycoprotein (Forstner *et al.*, 1973), but, as has been pointed out (Mantle *et al.*, 1981), this might be due to the relatively mild reduction conditions used in that study.

The complete loss of cysteine and the conservation of serine and threonine as well as of carbohydrate on digestion of the reduced glycoprotein with trypsin indicate that the protein components of the glycoprotein consist of two regions: a glycosylated region rich in serine and threonine, and a region devoid of carbohydrate with a high cysteine content. Whereas the non-glycosylated region is easily digested, the glycosylated region is not. Such a structure of the glycopeptide is in conformity with the structure of the glycopeptide of many other mucus glycoproteins (Donald, 1973; Scawen & Allen, 1977; Allen, 1978; Gibbons, 1978; Mantle *et al.*, 1981).

Marked differences exist between carbohydrate chains of mucus glycoproteins of different origin along the digestive tract of the rat. Relatively simple oligosaccharides are present in sublingual mucins (Slomiany & Slomiany, 1978a,b). More-complex oligosaccharides with 2 to 13 sugar residues are present in colonic mucus glycoprotein (Slomiany *et al.*, 1980). A mean number of about 8 sugar residues was found for small-intestinal mucus glycoprotein (Forstner *et al.*, 1973; Bella & Kim, 1972), but their exact structure is unknown. The oligosaccharides of gastric mucus glycoproteins are

probably even more complex. They possess a mean number of 19 sugar residues (Spee-Brand *et al.*, 1980).

It is noteworthy that no sialic acid and little sulphate (2%) are present in the duodenal-gland glycoprotein. The oligosaccharides of mucus glycoproteins of other regions of the rat gastro-intestinal tract are negatively charged, with either sulphate and/or sialic acid to various degrees. Sublingual (Slomiany & Slomiany, 1978a,b) and colonic (Slomiany *et al.*, 1980) mucus glycoproteins both possess considerable amounts of sialic acid. The major mucus glycoprotein of the stomach contains 1–2% sulphate and the minor one 10%, but both contain little if any sialic acid (Spee-Brand *et al.*, 1980). In small-intestinal mucus glycoprotein (Bella & Kim, 1972; Forstner *et al.*, 1973) both sialic acid and sulphate are present. The physiological significance of these differences, however, is unknown. A similar distribution of oligosaccharide chain length, sulphate and sialic acid is found along the digestive tract of the pig (Mantle & Allen, 1981). Pig duodenal-gland mucus glycoprotein, however, has not been investigated so far.

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References

- Allen, A. (1977) in *Mucus in Health and Disease* (Elstein, M. & Parke, D. V., eds.), pp. 275–299, Plenum Press, New York and London
- Allan, A. (1978) *Br. Med. Bull.* **34**, 28–33
- Allen, A. & Snary, D. (1972) *Gut* **13**, 666–672
- Bella, A. & Kim, Y. S. (1972) *Arch. Biochem. Biophys.* **150**, 679–689
- Carlson, D. M. (1968) *J. Biol. Chem.* **243**, 616–626
- Creeth, J. M. & Denborough, M. A. (1970) *Biochem. J.* **117**, 879–891
- Donald, A. S. R. (1973) *Biochim. Biophys. Acta* **317**, 420–436
- Forstner, J. F., Jabbal, J. & Forstner, G. G. (1973) *Can. J. Biochem.* **51**, 1154–1166
- Gibbons, R. A. (1978) *Br. Med. Bull.* **34**, 34–38
- Gottschalk, A. (ed.) (1972) *Glycoproteins*, Elsevier, Amsterdam and New York
- Horowitz, M. I. (1977) in *The Glycoconjugates* (Horowitz, M. I. & Pigman, W., eds.), pp. 189–213, Academic Press, New York

- Jabbal, J., Kells, D. I. C., Forstner, G. G. & Forstner, J. F. (1976) *Can. J. Biochem.* **54**, 707–716
- Mantle, M. & Allen, A. (1981) *Biochem. J.* **195**, 267–275
- Mantle, M., Mantle, D. & Allen, A. (1981) *Biochem. J.* **195**, 277–285
- Pearson, J. D., Allen, A. & Venables, C. W. (1980) *Gastroenterology* **78**, 709–715
- Press, E. M., Piggot, P. J. & Porter, R. R. (1966) *Biochem. J.* **99**, 356–366
- Scawen, M. & Allen, A. (1975) *Biochem. Soc. Trans.* **3**, 1107–1109
- Scawen, M. & Allen, A. (1977) *Biochem. J.* **163**, 363–368
- Slomiany, A. & Slomiany, B. L. (1978a) *J. Biol. Chem.* **253**, 7301–7306
- Slomiany, B. L. & Slomiany, A. (1978b) *Biochem. Biophys. Res. Commun.* **81**, 392–396
- Slomiany, B. L., Murty, V. L. N. & Slomiany, A. (1980) *J. Biol. Chem.* **256**, 9719–9723
- Snary, D., Allen, A. & Pain, R. H. (1970) *Biochem. Biophys. Res. Commun.* **40**, 844–851
- Spee-Brand, R., Strous, G. J. A. M. & Kramer, M. F. (1980) *Biochim. Biophys. Acta* **621**, 104–116
- Starkey, R. J., Snary, D. & Allen, A. (1974) *Biochem. J.* **141**, 633–639
- Wold, J. K., Midtvedt, T. & Jeanloz, R. W. (1974) *Acta Chem. Scand. Ser. B* **28**, 277–284