Partial characterization of the carbohydrate units of rat intestinal sucrase–isomaltase

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Sucrase–isomaltase was purified from rat intestinal microvillus membranes after injection of D-[2-³H]mannose and L-[6-³H]fucose, using a column of monoclonal antibody–protein A–Sephacrose. After Pronase digestion and gel filtration of the glycopeptides labelled from both precursors, a major part of the radioactivity was recovered in asparagine-linked complex oligosaccharides, and a smaller amount in partially alkali-labile high-molecular-weight glycopeptides. Only a small amount of [³H]mannose was found in endo- β -N-acetylglucosaminidase H-sensitive high-mannose oligosaccharides.

Sucrase–isomaltase is an integral membrane glycoprotein that appears in intestinal microvillus membranes during differentiation of intestinal epithelial cells. Although extensive work has been performed on its biosynthesis and on the mechanism of its insertion into the membrane (Semenza, 1976; Frank *et al.*, 1978; Brunner *et al.*, 1979; Hauri *et al.*, 1979, 1980; Cezard *et al.*, 1979), relatively little is known about the structure of the carbohydrate units associated with this enzyme complex. Rat and rabbit sucrase–isomaltase contain 15–20% carbohydrate, whereas the human enzyme contains 30–40% carbohydrate and exhibits blood-group activity (Cogoli *et al.*, 1972; Kelly & Alpers, 1973). The sugars present include fucose, glucosamine, galactosamine, mannose and galactose, and there is some suggestion that a carbohydrate group is attached to threonine at position 11 near the amino terminal (Frank *et al.*, 1978). In the present work, we report the partial characterization of the labelled glycopeptides obtained from sucrase–isomaltase purified by immunoaffinity chromatography after intraluminal administration of D-[2-³H]mannose and L-[6-³H]fucose to rats.

Experimental

Materials

The source of chemicals has been described

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previously (Quaroni *et al.*, 1980; Herscovics *et al.*, 1980). *Charania lampas* α -L-fucosidase was purchased from Miles Laboratories, Elkhart, IN, U.S.A. *Escherichia freundii* endo- β -galactosidase was a gift from Dr. Y.-T. Li, Tulane University, New Orleans, LA, U.S.A.

Preparation of labelled sucrase–isomaltase

Female Sprague–Dawley rats (CD strain), weighing 200–230 g, were obtained from Charles River Co., Wilmington, MA, U.S.A., and were starved overnight before use. Pairs of rats were anaesthetized with diethyl ether, and either D-[2-³H]mannose (2.5 mCi/rat, sp. radioactivity 14.5 Ci/mmol) or L-[6-³H]fucose (0.5 mCi/rat, sp. radioactivity 14 Ci/mmol) dissolved in 0.35 ml of 0.154 M-NaCl was injected directly into the proximal part of the intestinal lumen. At 3 h after injection of [³H]fucose and 4 h after injection of [³H]mannose intestinal microvillus membranes were prepared as described by Kessler *et al.* (1978). The microvillus-membrane fractions were suspended in 6 ml of 14 mM-sodium phosphate buffer, pH 8.0, containing 100 mM-NaCl and 1% Triton X-100 at a protein concentration of 3–4 mg/ml. After brief sonication (3 $\times$ 3 s), the mixture was kept in ice for 90 min to allow solubilization of membrane components. The mixture was then centrifuged at 100 000 g for 1 h, and the supernatant containing solubilized microvillus membrane was passed through a protein A–Sephacrose-4B affinity column to which BB 3/34/12 monoclonal antibody toward sucrase–isomaltase

had been bound (Hauri *et al.*, 1980; Quaroni & May, 1980). The column was first eluted with 0.14 M-sodium phosphate buffer, pH 8.0, containing 1% Triton X-100, and then with 0.1 M-citric acid/1% Triton X-100 at pH 5.0. The fraction eluted at pH 5.0 was neutralized, and dialysed against 10 mM-sodium phosphate buffer, pH 7.2, containing 0.1 M-NaCl. Triton X-100 was removed by chromatography on a column of Bio-Beads SM-2, eluting with 10 mM-sodium phosphate, pH 7.2, containing 0.1 M-NaCl; 90–95% of the applied radioactivity was recovered after Bio-Beads SM-2 chromatography.

Polyacrylamide-gel electrophoresis

Membrane proteins obtained from the solubilized microvillus membranes, and those eluted from the antibody–Sephacrose column with pH 8.0 and pH 5.0 buffers were separated by slab-gel electrophoresis in 7.5% acrylamide gels containing 0.1% sodium dodecyl sulphate, and the gels were then processed for fluorography as described previously (Quaroni *et al.*, 1980).

Preparation of glycopeptides

The protein fractions eluted from the antibody–Sephacrose 4B column with pH 5.0 buffer were freeze-dried and digested with pre-incubated Pronase as described previously (Herscovics *et al.*, 1980). Glycopeptides obtained after Pronase digestion were chromatographed on a column (1 cm × 113 cm) of Bio-Gel P-6 (200–400 mesh) in 0.1 M-pyridine acetate buffer, pH 5.0. Fractions (1 ml) were collected and assayed for radioactivity in Hydrofluor (National Diagnostics, Somerville, NJ, U.S.A.) using a Packard Model 3255 liquid-scintillation spectrometer.

Treatment with endo- β -N-acetylglucosaminidase H

Glycopeptide fractions obtained from Bio-Gel P-6 were freeze-dried and treated for 16–18 h at 37°C with 2 m-units of endo- β -N-acetylglucosaminidase H in 100 μ l of 0.15 M-citrate buffer, pH 5.5, containing 25 μ g each of cycloheximide and chloramphenicol, as described previously (Herscovics *et al.*, 1980). The products were then chromatographed on Bio-Gel P-6 as described above.

Treatment with α -L-fucosidase

Glycopeptide fractions were freeze-dried and incubated for 22 h at 37°C with 18 m-units of α -L-fucosidase per 100 μ l of 0.1 M-citrate/phosphate buffer, pH 4.0, containing 25 μ l each of cycloheximide and chloramphenicol. In some cases this treatment was done twice. The products were then chromatographed on Bio-Gel P-6 as described above.

Results and discussion

To obtain maximum labelling of sucrase–isomaltase, the labelled precursors were administered by direct injection into the intestinal lumen. Under these conditions about 5% and 25% of the radioactivity in the intestinal cell homogenates was recovered in the microvillus-membrane fraction from labelled mannose and fucose respectively. The labelled sucrase–isomaltase was purified by adsorption to a column of monoclonal antibody attached to protein A–Sephacrose; it was specifically eluted with buffer at pH 5.0, which dissociates the complex between protein A and immunoglobulin G monoclonal antibody (Ey *et al.*, 1978). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis showed that this pH 5 eluate contained two labelled bands that corresponded in size to the isomaltase and sucrase subunits (Figs. 1b and 1e). The sucrase subunit was more heavily labelled from both precursors than the isomaltase subunit, in agreement with previous studies showing that sucrase contains more carbohydrate than isomaltase (Kelly & Alpers, 1973; Cogoli *et al.*, 1973). Although not apparent in the photograph, a very faint labelled band, which corresponded to the previously identified sucrase–isomaltase precursor (Hauri *et al.*, 1979), was also seen on the gels. These results demonstrate that the sucrase–isomaltase prepared by this technique was radiochemically pure. In contrast, the original microvillus-membrane fraction (Figs. 1a and 1d) and the pH 8 eluate (Figs. 1c and 1f) contained many labelled glycoproteins.

After D-[2-³H]mannose injection, gel filtration of the glycopeptides obtained by Pronase digestion of the labelled sucrase–isomaltase yielded two glycopeptide fractions (Fig. 2a). About 10% of the radioactivity was found in fraction I, which was excluded from Bio-Gel P-6, but most of the radioactivity was recovered in the included glycopeptides. After treatment of the excluded glycopeptides with endo- β -N-acetylglucosaminidase H, two labelled fractions were obtained (Fig. 2b). Fraction II, which was resistant to the endoglycosidase, contained about 80% of the radioactivity, whereas fraction III, which was formed as a result of endo- β -N-acetylglucosaminidase H treatment, contained about 10% of the radioactivity in the glycopeptides. A single treatment of glycopeptides in fraction II with α -L-fucosidase released about 10% of the radioactivity, indicating that some conversion of the labelled mannose into fucose had occurred, as shown previously (Herscovics *et al.*, 1980). A similar treatment of glycopeptides in fraction I could not be done as insufficient material was available.

Two labelled glycopeptide fractions were obtained after injection of L-[6-³H]fucose (Fig. 2c).

Fig. 1. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of labelled rat intestinal microvillus membranes after injection of D-[2- 3 H]mannose and L-[6- 3 H]fucose

Lanes (a) and (d), unfractionated solubilized microvillus membranes; lanes (b) and (e), labelled sucrase-isomaltase that had been specifically bound to the monoclonal antibody-protein A-Sepharose column and subsequently eluted with buffer at pH 5.0; lanes (c) and (f), labelled glycoproteins that had not been adsorbed to the antibody-Sepharose column and were eluted at pH 8.0. Since the amount of label incorporated from [3 H]fucose was about 20 times greater than that incorporated from [3 H]mannose, much larger amounts of the [3 H]mannose- than of the [3 H]fucose-labelled proteins had to be applied to the gels. This difference in loading accounts for the differences in mobility of the enzyme subunits. The [3 H]mannose-labelled isomaltase band that was present on the film did not reproduce well in the photograph. P, sucrase-isomaltase precursor; I, isomaltase; S, sucrase.

Fig. 2. Gel-filtration of labelled glycopeptides obtained from sucrase-isomaltase after injection of D-[2- 3 H]-mannose and L-[6- 3 H]fucose

The labelled sucrase-isomaltase obtained by immunoaffinity chromatography was digested with Pronase and the glycopeptides were fractionated on Bio-Gel P-6. (a), Glycopeptides obtained after injection of D-[2- 3 H]mannose; (b), products obtained after treatment of the included glycopeptides from (a) with endo- β -N-acetylglucosaminidase H; (c), glycopeptides obtained after injection of L-[6- 3 H]fucose. Fraction I: tubes 25–30; Fraction II: tubes 35–45; Fraction III: tubes 46–55.

About 25% of the radioactivity was recovered in the high-molecular-weight glycopeptide fraction (fraction I), which was excluded from Bio-Gel P-6. The major part of the radioactivity was found in the included fraction II. Two successive incubations with α -L-fucosidase released about 40% and 53% of the radioactivity as labelled fucose from fractions I and II respectively. Fraction I was only partially degraded during mild alkali treatment (0.05 M-KOH in 1 M-NaBH₄ for 15 h at 45°C) giving rise to a mixture of products of various sizes that could not be clearly fractionated on Bio-Gel P-6. Treatment of the glycopeptides in fraction II under the same

conditions did not alter their elution from the column.

These results show that sucrase-isomaltase yields glycopeptides that contain a large proportion of asparagine-linked oligosaccharides of the complex

type (fraction II) since they are labelled with both [^3H]mannose and [^3H]fucose, and are resistant to endo- β -N-acetylglucosaminidase H and to mild alkali. The sucrase-isomaltase glycopeptides contain only a small amount of high-mannose oligosaccharides (fraction III), which are sensitive to endo- β -N-acetylglucosaminidase H treatment and are labelled with [^3H]mannose, but not with [^3H]fucose. Labelled sucrase-isomaltase also yields a significant proportion of high-molecular-weight glycopeptides (fraction I) that can be labelled from both [^3H]mannose and [^3H]fucose. Attempts to characterize this glycopeptide fraction have not been conclusive. Additional incubation with Pronase and thermolysin did not cause any further degradation of the glycopeptides, neither did incubation with endo- β -galactosidase, an enzyme that has been shown to degrade high-molecular-weight glycopeptides obtained from membranes of other cells (Jarnefelt *et al.*, 1978; Li *et al.*, 1980). Since galactosamine has been found in sucrase-isomaltase (Cogoli *et al.*, 1972; Kelly & Alpers, 1973), it seems likely that fraction I contains at least some O-glycosidically-linked carbohydrate units. The carbohydrate groups may be clustered along the peptide backbone in a way that creates steric hindrance to proteolytic degradation, and that renders β -elimination of O-glycosidically-linked oligosaccharides under alkaline conditions difficult.

In previous work, we obtained a similar pattern of labelled glycopeptides from intestinal microvillus membranes and from crypt-cell luminal membranes (Herscovics *et al.*, 1980). It was, however, not possible to rule out contamination of the luminal membranes with labelled glycopeptides derived from mucins or from other cellular membranes. The present results demonstrate that these glycopeptides, including the high-molecular-weight glycopeptides of fraction I, are characteristic of the luminal membranes of intestinal epithelial cells, since they can be obtained from sucrase-isomaltase, a highly purified intrinsic membrane glycoprotein of intestinal epithelial cells.

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References

- Brunner, J., Hauser, H., Braun, H., Wilson, K. J., Wacker, H., O'Neill, B. & Semenza, G. (1979) *J. Biol. Chem.* **254**, 1821–1828
- Cezard, J.-P., Conklin, K. A., Das, B. C. & Gray, G. M. (1979) *J. Biol. Chem.* **254**, 8969–8975
- Cogoli, A., Mosimann, H., Vock, C., von Balthazar, A.-K. & Semenza, G. (1972) *Eur. J. Biochem.* **30**, 7–14
- Cogoli, A., Eberle, A., Sigrist, H., Joss, C., Robinson, E., Mosimann, H. & Semenza, G. (1973) *Eur. J. Biochem.* **33**, 40–48
- Ey, P. L., Prowse, S. J. & Jenkin, C. R. (1978) *Immunochemistry* **15**, 429–436
- Frank, G., Brunner, J., Hauser, H., Wacker, H., Semenza, G. & Zuber, H. (1978) *FEBS Lett.* **96**, 183–188
- Hauri, H.-P., Quaroni, A. & Isselbacher, K. J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5183–5186
- Hauri, H.-P., Quaroni, A. & Isselbacher, K. J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6629–6633
- Herscovics, A., Bugge, B., Quaroni, A. & Kirsch, K. (1980) *Biochem. J.* **192**, 145–153
- Jarnefelt, J., Rush, J., Li, Y.-T. & Laine, R. A. (1978) *J. Biol. Chem.* **253**, 8006–8009
- Kelly, J. J. & Alpers, D. H. (1973) *J. Biol. Chem.* **248**, 8216–8221
- Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. & Semenza, G. (1978) *Biochim. Biophys. Acta* **506**, 136–154
- Li, E., Gibson, R. & Kornfeld, S. (1980) *Arch. Biochem. Biophys.* **199**, 393–399
- Quaroni, A. & May, R. J. (1980) *Methods Cell Biol.* **21B**, 403–427
- Quaroni, A., Kirsch, K., Herscovics, A. & Isselbacher, K. J. (1980) *Biochem. J.* **192**, 133–144
- Semenza, G. (1976) in *The Enzymes of Biological Membranes* (Martonosi, A., ed.), vol. 3, pp. 349–382, Plenum, New York