DOES SUCRASE–ISOMALTASE ALWAYS EXIST AS A COMPLEX IN HUMAN INTESTINE?

Hanne SKOVBJERG, Hans SJÖSTRÖM and Ove NORÉN

Department of Biochemistry C, The Panum Institute, University of Copenhagen and *Medical-Gastroenterological Department C, Herlev Hospital, Denmark*

Received 5 October 1979

1. Introduction

The sucrase–isomaltase of the small intestinal brush border membrane has been isolated as a complex from a number of species [1–3]. In addition studies on human small intestine suggested the presence of free sucrase [4–6] and isomaltase [5,6]. However, papain treatment or autolysis used in these studies to release the enzymes may have induced changes in the peptide chains resulting in dissociation of the sucrase–isomaltase complex. The question whether sucrase and isomaltase exist separately in vivo has therefore not been answered conclusively.

It has been suggested [7] that sucrase–isomaltase is synthesized as a one-chain peptide. The demonstration of free sucrase and/or isomaltase does not fit into that hypothesis and such a finding is therefore, provided it is not a part of the catabolism of the enzyme, of importance for a better understanding of the biosynthetic mechanism of the sucrase–isomaltase complex.

This paper presents evidence for the in vivo existence of free, active isomaltase in addition to the sucrase–isomaltase complex in proximal jejunum but not in ileum.

2. Materials and methods

Agarose type HSA was purchased from Litex, Copenhagen. Aprotinin was a gift from Novo, Copenhagen. Diisopropylfluorophosphate was delivered by Sigma, Saint Louis, MO. 1,10-phenanthroline was obtained from Merck, Darmstadt. Duodenal fluid was collected from the duodenal loop by a peroral tube inserted after an overnight fast. All other chemicals as well as the small intestinal samples were obtained as in [8,9].

2.1. *Triton X-100 solubilized proteins from isolated brush border membranes*

Brush border membranes from proximal jejunum or distal ileum were isolated and the proteins solubilized with Triton X-100 as in [8]. In certain experiments aprotinin (7 μg/ml), 1,10-phenanthroline (1 mM) or diisopropylfluorophosphate (0.15 mM) was added to all buffers during the preparation. In some experiments the preparation was performed at room temperature or at 37°C.

2.2. *Treatment of ileal brush border membranes with duodenal juice*

The isolated brush border membranes (1–2 mg protein/ml) were incubated for 30 minutes at 37°C in 50 mM potassium phosphate buffer (pH 7.4) with an equal volume of undiluted duodenal fluid before solubilization with Triton X-100.

2.3. *Triton X-100 and papain solubilized proteins from unfractionated biopsies*

The results presented here on unfractionated biopsies are based on further evaluations of the biopsy material in [9].

2.4. *Crossed immunoelectrophoresis*

Antiserum against human brush border membrane proteins was prepared as in [8].

The electrophoresis was run in 1% agarose in either 0.037 M sodium barbital, 0.37 M glycine and 0.19 M Tris (pH 8.8) containing 0.1% Triton X-100 and 0.05% sodium deoxycholate (antigen: the Triton X-100

solubilized proteins from isolated brush border membranes [8]) or in 0.020 M sodium barbital (pH 8.6) (antigen: the Triton X-100 and papain solubilized proteins from unfractionated biopsies [9]). The precipitates were stained either for protein with Coomassie Brilliant Blue or for enzymatic activities using sucrose, maltose or palatinose as substrates [8].

3. Results and discussion

Figure 1 shows the typical immunoelectrophoretic pattern of Triton X-100 solubilized proteins from the isolated jejunal brush border membranes. The identification of the sucrase–isomaltase (EC 3.2.1.48–EC 3.2.1.10), lactase–phlorizin hydrolase (EC 3.2.1.23–EC 3.2.1.62), maltase (EC 3.2.1.20), microvillus aminopeptidase (microsomal, EC 3.4.11.2) and dipeptidyl peptidase IV (EC 3.4.14.X) precipitates has been reported [8].

The sucrase–isomaltase precipitate is very distinct and hydrolyzes sucrose, palatinose and maltose. The anodal leg is split into two precipitates one of which (B) is the continuation of the sucrase–isomaltase precipitate hydrolyzing both sucrose (fig.1b), palatinose (fig.1c) and maltose (fig.1d). The colour reaction of B when palatinose is used as substrate is only faintly seen in the photo (fig.1c), but is clearly visible during incubation of the immunoelectrophoretic plate. The other precipitate (C) hydrolyzes maltose (fig.1d) and palatinose (fig.1c) but not sucrose (fig.1b). The interfering precipitate pattern indicates partial immunological identity between the sucrase–isomaltase complex and precipitate C. On basis of the substrate specificity and the partial immunological identity precipitate C is suggested to represent the free isomaltase enzyme.

Enzymatic staining with sucrose did not reveal any precipitate that corresponds to free sucrase.

The immunoelectrophoretic pattern of ileal brush border membrane proteins did not reveal any free isomaltase precipitate. This might indicate that the free isomaltase detected in jejunum was induced by proteolytic enzymes in the duodenal fluid in vivo. However, incubation of ileal brush borders with duodenal juice did not produce a free isomaltase precipitate.

To test the possibility that the free jejunal isomaltase precipitate was caused by a proteolytic

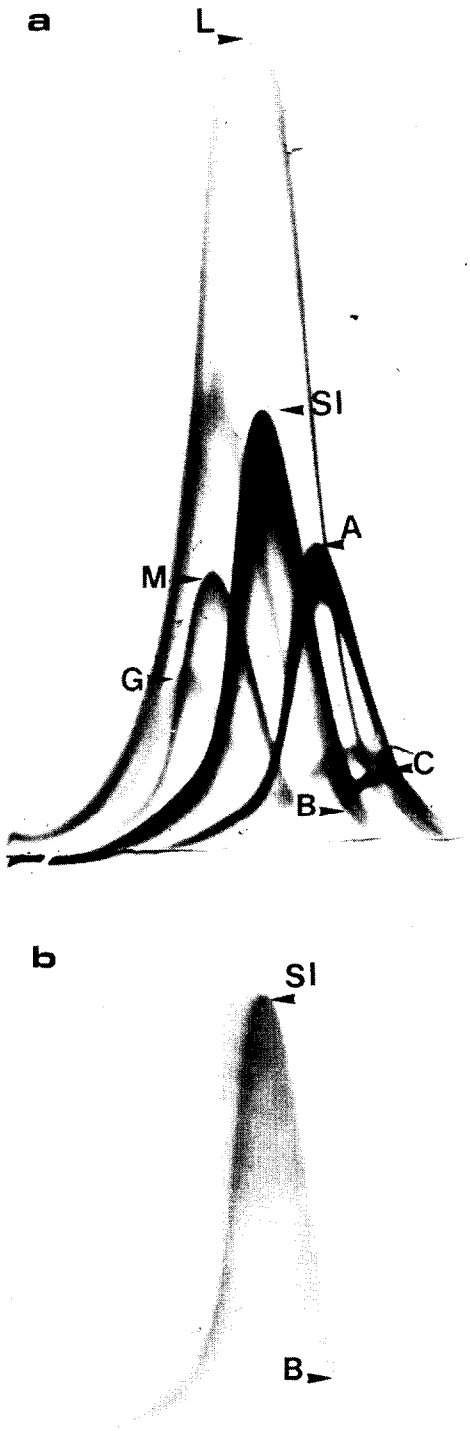

Fig.1a,b.

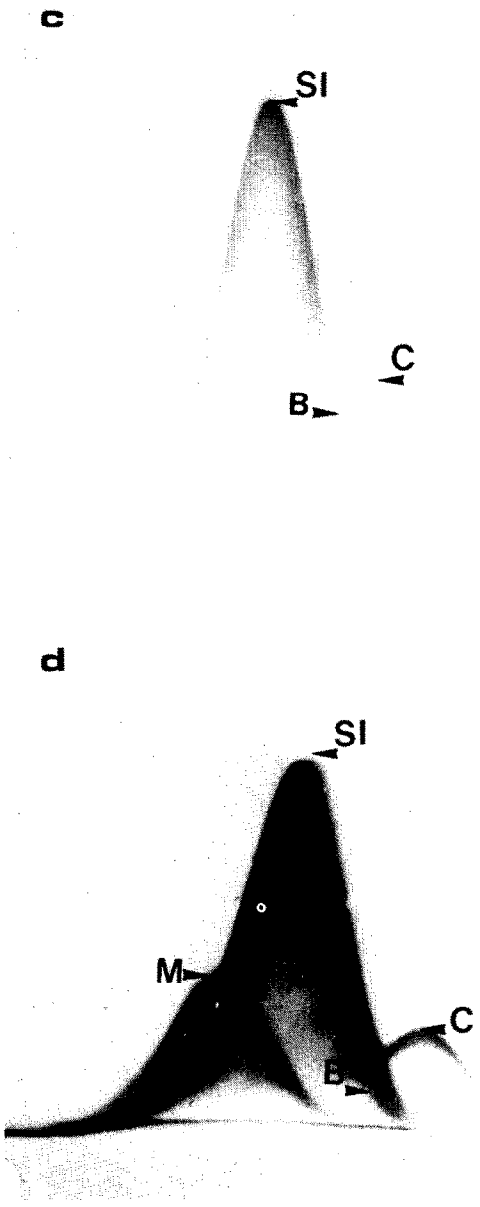

Fig.1. Crossed immunoelectrophoresis of Triton X-100 solubilized proteins from isolated jejunal brush border membranes. (a) Protein staining. (b) Enzymatic staining with sucrose as substrate. (c) Enzymatic staining with palatinose as substrate. (d) Enzymatic staining with maltose as substrate. *Designations:* SI, sucrase–isomaltase; L, lactase–phlorizin hydrolase; M, maltase; A, microvillus aminopeptidase; G, dipeptidylpeptidase IV; (B,C), see text.

artefact generated during the preparation, the brush border membrane proteins were prepared in the presence of different proteolytic inhibitors which, however, did not abolish or reduce the formation of the isomaltase precipitate. No increase in the height of the isomaltase precipitate was seen when the brush border membranes were prepared at room temperature or at 37°C. This further indicates that the free isomaltase is not generated by proteolytic enzymes during the preparative procedure.

Studies on the unfractionated biopsies showed a similar immunoelectrophoretic pattern as above. Also in this system precipitate C is likely to represent the free isomaltase (fig.2). It interferes with the sucrase–isomaltase precipitate and displays the same enzymatic staining properties, except that no hydrolysis of palatinose is observed, probably because isomaltase is easily inactivated by the papain [10,11] used in the solubilization procedure. The isomaltase precipitate was evident in 17 of 21 different jejunal biopsies and absent in all 20 ileal biopsies (fig.3) investigated. The isomaltase precipitate had a different size in different patients. Biopsies taken simultaneously from 9 patients were analyzed more than once at intervals up to 20 months. The isomaltase precipitate showed an

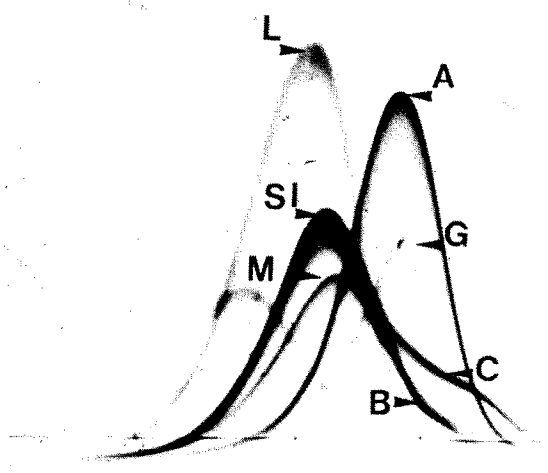

Fig.2. Crossed immunoelectrophoresis of Triton X-100 and papain solubilized biopsies from unfractionated jejunal biopsies. The designation of the precipitates are the same as in fig.1.

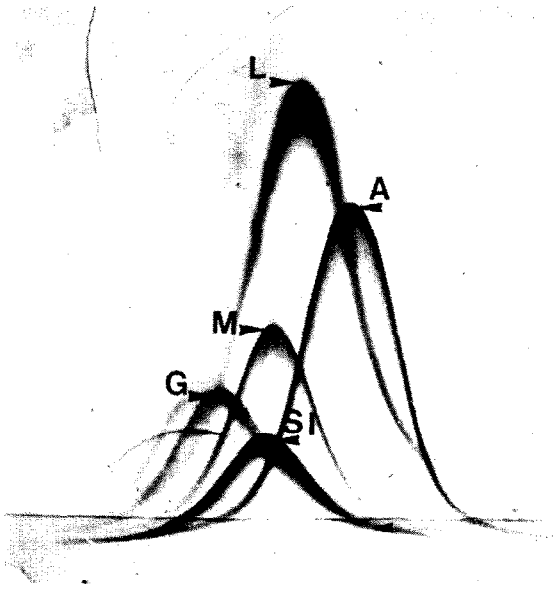

Fig.3. Crossed immunoelectrophoresis of Triton X-100 and papain solubilized proteins from unfractionated ileal biopsies. The designation of the precipitates are the same as in fig.1.

apparently constant size in each patient. This shows that the isomaltase precipitate is subjected to individual rather than preparative variations.

The results presented suggest that in addition to the sucrase–isomaltase complex, free active isomaltase is present in vivo in proximal human jejunum. In contrast, free isomaltase seems not to be present in distal ileum. Theoretically ileal isomaltase might, however, move more slowly than jejunal isomaltase in the first dimension electrophoresis, and thereby co-precipitate with the sucrase–isomaltase complex.

The observation of free isomaltase without equimolar amounts of free sucrase indicates that the two subunits may be synthesized as two distinct peptide chains. However, the possibility that the sucrase–isomaltase complex is synthesized as a single polypeptide chain can not be ruled out. Though we have not been able to demonstrate free sucrase, this enzyme might co-precipitate with the sucrase–isomaltase complex due to a similar migration rate in the first dimension electrophoresis. Another possibility is that sucrase is more rapidly degraded and that the observed pattern reflects part of the physiological catabolism of the sucrase–isomaltase complex.

Since free isomaltase exists in vivo in normal jejunal biopsies this may also be the case in those sucrase–isomaltase deficient patients with residual isomaltase activity. In fact several of the sucrase–isomaltase deficient biopsies described [12,13] as having a cross-reacting protein in immunofluorescent staining using antibodies against the undissociated sucrase–isomaltase complex, have a high residual isomaltase activity. Studies by means of crossed immunoelectrophoresis on peroral biopsies from such patients are in progress in our laboratory.

Acknowledgements

We thank Mrs D. Anthonsen for skillful technical assistance. This work was supported by the Danish Medical Research Council (no. 512–10175), P. Carl Petersens Fond (no. B 1076) and The Novo Foundation.

References

[1] Kolinska, J. and Semenza, G. (1967) Biochim. Biophys. Acta 146, 181–195.
[2] Cummins, D. L., Gitzelmann, R., Lindenmann, J. and Semenza, G. (1968) Biochim. Biophys. Acta 160, 396–403.
[3] Kolinska, J. and Kraml, J. (1972) Biochim. Biophys. Acta 284, 235–247.
[4] Dahlqvist, A. and Telenius, U. (1969) Biochem. J. 111, 139–146.
[5] Asp, N. G. and Dahlqvist, A. (1973) FEBS Lett. 35, 303–305.
[6] Conklin, K. A., Yamashiro, K. M. and Gray, G. M. (1975) J. Biol. Chem. 250, 5735–5741.
[7] Frank, G., Brunner, J., Hauser, H., Wacker, H., Semenza, G. and Zuber, H. (1973) FEBS Lett. 96, 183–188.
[8] Skovbjerg, H., Norén, O. and Sjöström, H. (1973) Scand. J. Clin. Lab. Invest. 38, 723–729.
[9] Skovbjerg, H., Sjöström, H., Norén, O. and Gudmand-Høyer, E. (1979) Clin. Chim. Acta. 92, 315–322.
[10] Auricchio, S., Dahlqvist, A. and Semenza, G. (1963) Biochim. Biophys. Acta 73, 582–587.
[11] Eggermont, E. and Hers, H. G. (1969) Eur. J. Biochem. 9, 488–496.
[12] Dubs, R., Steinmann, B. and Gitzelmann, R. (1973) Helv. Paediat. Acta 28, 187–198.
[13] Freiburghaus, A. U., Dubs, R., Hadorn, B., Gaze, H., Hauri, H. P. and Gitzelmann, R. (1977) Eur. J. Clin. Invest. 7, 455–459.