N-Terminal sequences of pig intestinal sucrase–isomaltase and pro-sucrase–isomaltase

Implications for the biosynthesis and membrane insertion of pro-sucrase–isomaltase

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The hog sucrase–isomaltase complex is anchored to the small-intestinal brush border membrane, as in the rabbit, via a hydrophobic segment located in the N-terminal region of the isomaltase subunit. The immediate precursor of the 'final' sucrase–isomaltase (i.e., pro-sucrase–isomaltase as prepared from adult hogs whose pancreas had been disconnected from the duodenum) is an amphiphilic single polypeptide chain of M_r 260 000–265 000. Its N-terminal sequence is virtually identical with (not merely homologous to) the corresponding region of the isomaltase subunit of 'final' sucrase–isomaltase. This shows that the isomaltase portion of pro-sucrase–isomaltase is the N-terminal 'half' of the precursor polypeptide chain. Thus the succession of domains in pro-sucrase–isomaltase and its mode of anchoring in the membrane could be deduced. On this basis a likely mechanism of biosynthesis and insertion is proposed.

Biosynthesis *Membrane insertion* *Partial sequence* *Pro-sucrase–isomaltase*

1. INTRODUCTION

Sucrase–isomaltase (EC 3.2.1.48–10), a stalked intrinsic membrane protein most extensively studied from the rabbit small intestine (reviews [1,2]) is built up of two polypeptides, one with sucrase–maltase and the other with isomaltase–maltase activity.

By different approaches it has been demonstrated that sucrase–isomaltase is synthetised as a single-chain, two active sites sucrase–isomaltase (pro-sucrase–isomaltase, M_r ~260 000 [3–8]. An early labelled high-M_r polypeptide precipitable with antibodies against 'final' sucrase–isomaltase was demonstrated in Golgi membranes [3]. An enzymatically active, immunologically cross-reacting, single-chain sucrase–isomaltase (pro-sucrase–isomaltase) could be isolated from enterocytes which had not been in contact with pancreatic enzymes. This had been made possible either by disconnection of the pancreatic duct in pigs [4] or by transplantation of rat fetal small intestine [5]. A single-chain sucrase–isomaltase could also be isolated from the calcium-precipitated membrane fraction, which is expected to contain intracellular and basolateral membranes [6]. Finally, the cell-free in vitro translation of single-chain pro-sucrase–isomaltase has been achieved [7,8].

For the evaluation of the biosynthetic mechanism and membrane insertion it is important to gather information on the order in which the sucrase and isomaltase domains are synthetised. This question may be tackled by the determination of N-terminal sequences. In a parallel paper [9] this has been performed on a preparation from transplants of fetal small intestine. However, we found it imperative to investigate this question also on a pro-sucrase–isomaltase prepared by a totally different biological procedure since transplants might, in principle, develop abnormally in the site of transplantation as compared to an in situ tissue. Thus here we have used intestines from either normal hogs, or hogs in which the pancreatic juice had no access to the intestinal lumen [4].

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2. MATERIALS AND METHODS

Sources of chemicals were as reported in [4,9]. Aprotinin (Kunitz trypsin inhibitor) was a kind gift from Novo (Bagsvaerd).

2.1. *Purification of Triton-solubilised sucrase–isomaltase and pro-sucrase–isomaltase*

Rabbit and pig intestinal sucrase–isomaltase were prepared as in [4,10]. Pig small intestines were kindly delivered by the Dept. Experimental Pathology, Rigshospitalet (Copenhagen). During the purification of the pig enzyme, aprotinin (2.8 mg/l) was present throughout.

2.2. *Separation of sucrase and isomaltase polypeptides*

Separation of the sucrase and isomaltase polypeptides for sequencing was achieved by preparative polyacrylamide gel electrophoresis on 3 mm thick slab gels using a discontinuous sulphate–borate system modified [7] from [11]. A 20 μl aliquot of the sample (20 μg) was ^{125}I-labelled by the chloramine-T procedure [12] and mixed with unlabelled pro- or final sucrase–isomaltase prior to electrophoresis. The protein bands were visualized under a UV-lamp by placing the gel onto a thin-layer chromatography plate with fluorescent additive [13]. The gel strips containing the protein were eluted with an Isco sample concentrator, model 1750, with an efficiency of 60–70% for pro-sucrase–isomaltase and 74–80% for sucrase and isomaltase polypeptides as determined by radioactivity measurements. Purity of the eluates was tested by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. The eluates were lyophilised and subjected to amino acid sequencing.

2.3. *Determination of N-terminal amino acid sequences*

N-Terminal sequences were determined by automated Edman degradation in a Beckman sequencer (model 890C) using the Quadrol (*N,N,N′,N′*-tetrakis (2-hydroxypropyl)ethylendiamin) program together with polybrene [14]. The amino acid phenylthiohydantoins were identified by high-performance liquid chromatography [15].

2.4. *Other methods*

Sucrase activity was assayed using sucrose as substrate [16] as in [4].

3. RESULTS

We report here the sequences of the hog enzymes and also those of the rabbit isomaltase (cf. [17]) and sucrase. For comparison, we also report in fig.1 the sequences of rat pro-sucrase–isomaltase (prepared via a totally different biological procedure; i.e., from transplants of fetal small intestine) and of the 'final' polypeptides of the rat enzyme [9], and the partial sequences of 3 glycophorins [22–24].

It is apparent that the N-terminal sequences of (amphiphilic) pro-sucrase–isomaltase are identical* with those of the respective isomaltase polypeptides; that these sequences are homologous in the 3 species considered, and show no detectable similarity with the N-terminal sequences of the sucrase polypeptides.

All the residues at position 11 of the pro-sucrase–isomaltases and the isomaltases are glycosylated, as observed in [17] for Thr-11 in rabbit isomaltase. For the pig peptides, use was made of the phenol/H_2SO_4 procedure [18]: the phenylhydantoin derivative was found to be associated with 2 or 3 monosaccharide residues. The spectrum of the colour obtained was very close to that of a standard mixture (1:1:1) of galactose, glucose and fucose (not shown). These sugars have been identified by thin-layer chromatography as occurring at position 11 of rabbit isomaltase (H.W., unpublished), and they are among those found in the sucrase–isomaltase complex of the rabbit [19].

4. DISCUSSION

In [9] it was shown that the N-terminal part of rat pro-sucrase–isomaltase is identical with the isomaltase domain. This pro-sucrase–isomaltase had been obtained from small intestinal fetal transplants: in principle, it is quite possible that the development of transplants may follow somewhat different pattern(s) (e.g., not produce normal post-translational modifications of otherwise normally synthetised proteins), or produce artefacts as compared to other biological systems. It was thus

* Due to the very small amounts available, the identification of some highly hydrophilic PTH-derivatives was not quite secured. This held true particularly for positions 4 of hog ProSI and 6 of rat ProSI

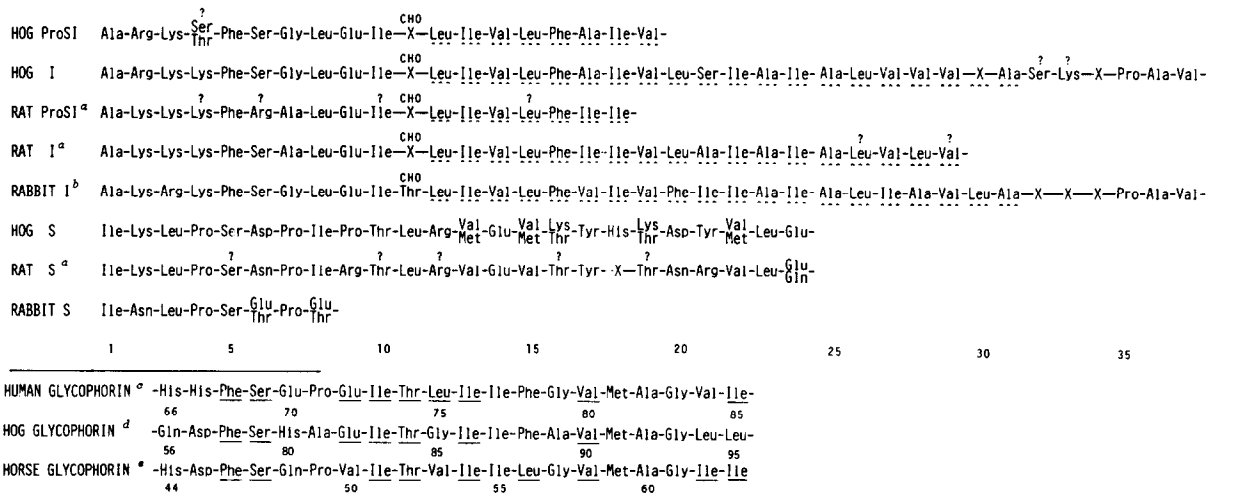

Fig.1. Partial N-terminal sequences of pig pro-sucrase–isomaltase (pro-SI) and of the isomaltase (I) and sucrase (S) polypeptides from 'final' SI. (For comparison, the corresponding N-terminal sequences of rat and rabbit enzymes and some partial sequences of glycophorins are reported.) (~) Hydrophobic segment beginning at residue 12 in the isomaltase and in the pro-SI; (—) residues in the glycophorin which are identical with residues in rabbit isomaltase; (a) from [9]; (b) from [17] and this paper; (c) from [22]; (d) from [23]; (e) from [24]; (?) Due to the very small amount of material available, these residues could not be identified with certainty.

essential to complement the observations on rat transplants on an entirely different and more 'normal' biological system; i.e., on adult pigs whose pancreas had been disconnected from the duodenum some days prior to sacrifice [4]. It is clear from fig.1 that the N-terminal sequence in pig pro-sucrase–isomaltase is identical (not merely homologous) to that of the isomaltase 'final' polypeptide of the same species (for comparison fig.1 also reports the corresponding sequences for the rat polypeptides, [9]).

The identity of the N-terminal sequences observed strongly suggests that pro-sucrase–isomaltase begins with the isomaltase domain. One can exclude the reverse order: a hydrophobic segment (which would be lost during the transformation of pro-sucrase–isomaltase to 'final' sucrase–isomaltase) followed by the sucrase domain, another hydrophobic segment and then the isomaltase domain. In fact, this order of domains would have led to homology, rather than to identity between the N-terminal sequences of pro-sucrase–isomaltase and the isomaltase subunit (fig.1), since no stringent selective pressure can be expected to act on the exact sequence of an anchoring segment, if it only remains hydrophobic. Furthermore, if pro-sucrase–isomaltase, arisen by (partial) duplication of an ancestoral gene, were instead to begin with the sucrase domain (rather than with the isomaltase), then the hydrophobic segment which is eventually found at the N-terminal region of 'final' isomaltase should, in pro-sucrase–isomaltase, occur between the sucrase and isomaltase domains. Preliminary experiments with various approaches do rule out the occurrence of such (an) hydrophobic segment(s) (H.W. et al., in preparation).

The extensive homology among the N-terminal regions of the isomaltase polypeptide from pig and rat with those from the rabbit (it is not clear whether the different amino acids reported at positions 2–4 [17] were due to a difference in breed or to other reasons) shows that they have the same function; i.e., membrane anchoring of sucrase–isomaltase [20]. Some homologies are especially noteworthy with respect to the relation of isomaltase to the membrane. The presence of carbohydrates at positions 11 strongly suggests that

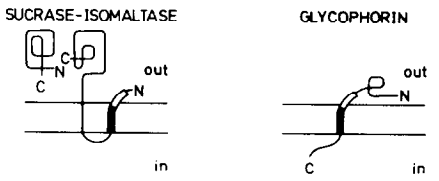

Fig.2. Models of sucrase–isomaltase and glycophorin depicting the analogous segment ▪□ in relation to the position of the molecule in the membrane. The unfilled part indicates the highly homologous segment: N, N-terminus; C, C-terminus.

these residues are located at the luminal side. Furthermore, all the hydrophobic sequences begin at residue 12. Finally, whenever a substitution has taken place, the alternative amino acid has similar properties. Thus the sequence information indicates that the positioning of sucrase–isomaltase in the small intestine of pig (and rat) is the same as has been established for the rabbit [12].

The Lys and Arg residues in positions 2 through 4 of the isomaltase polypeptide are obviously not cleaved off by the trypsin present in the lumen under in vivo conditions. This may be due to some steric hindrance.

The extensive homology among the sucrase polypeptides is of relevance to the proteolytic transformation of pro-sucrase–isomaltase into final sucrase–isomaltase: it strongly suggests that the mechanism of this transformation is identical in the 3 species considered.

A computer-assisted search for homologies of the sequences in fig.1 with other published sequences was carried out. The only significant homology found was between segment 68–80 of human glycophorin and segment 5–17 of the isomaltases which had >61% identity. Furthermore, if one restricts the comparison to segment 68–76 of glycophorin and segment 5–13 of rabbit isomaltase, the identity becomes as high as 78%. The analogy between the isomaltase and glycophorin sequences extends much further, encompassing His 66 and His 67 in glycophorin at positions corresponding to Arg 3 and Lys 4 in rabbit isomaltase and the long hydrophobic sequences starting at positions 75 in glycophorin and positions 12 in isomaltase. Note that the homology and analogy refer to parts of glycophorin and of isomaltase which

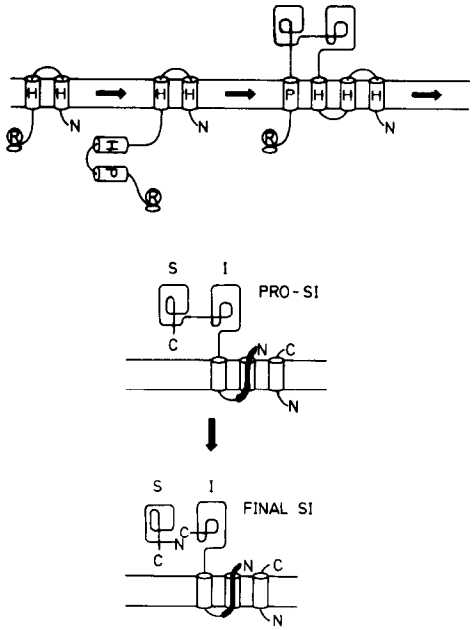

Fig.3. Suggested minimum mechanism of folding, membrane insertion and processing (by signalase and pancreatic protease(s)) of an as yet hypothetical pre-pro-sucrase–isomaltase. Note: the proposed scheme puts emphasis on the folding and insertion only, without indicating any possible interaction with recognition systems. R, ribosome; H, hydrophobic; P, polar; N, N-terminus; C, C-terminus; S, sucrase; I, isomaltase. Segments indicated by a bold line are those the sequence of which is known (fig.1).

are highly conserved in the species considered.

The significance of such long analogous segments containing highly homologous subsegments is not immediately obvious. The localization of the segment in the polypeptide chains in relation to the membrane is depicted in fig.2. It can be noted that these segments are located at the transition between the extracellular medium and the hydrophobic membrane layer of $N_{out} \rightarrow C_{in}$ polypeptide chains. The positioning of the rest of the protein mass is different: In the case of glycophorin, the hydrophobic segment crosses the membrane only once and most of the extracellular protein mass is located at the N-terminal end (i.e., 'before' this analogous segment); in the case of isomaltase, the hydrophobic anchor crosses the membrane twice [20] and most of the extracellular mass is located at the C-terminal end (i.e., 'after' this analogous seg-

ment). The significance of this segment is not known. It has been suggested [21] that this sequence interacts with a collecting and/or transporting protein of the endoplasmic reticulum, and thus is a part of the address for transport to the plasma membrane.

From the information now available on the succession of domains in pro-sucrase–isomaltase, from the most likely absence of a hydrophobic segment between the isomaltase and sucrase regions in pro-sucrase–isomaltase, and from its presumable mode of anchoring in the membrane it seems that the most probable mechanism of biosynthesis and insertion follows the sequence of events depicted in fig.3. In the scheme offered, pro-sucrase–isomaltase is synthetised as pre-pro-sucrase–isomaltase and is inserted by forming two loops [25–27] or 'hair pins' [28] in the membrane.

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