Anchoring and Biosynthesis of Small-Intestinal Sucrase–Isomaltase

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The present chapter summarizes some recent and less recent work on the positioning, anchoring and biosynthesis of the small-intestinal sucrase–isomaltase (SI) complex, which is the most abundant integral protein of the brush border membrane; it then discusses the implications of the results as to the possible mechanisms underlying human sucrose–isomaltose malabsorption.

I became interested in this enzyme some years ago because (a) it plays a central role in the digestion of starch (accounting for approximately 80% of the small-intestinal maltase activity); (b) it is the major integral protein of the brush border (accounting for approximately 10% of the proteins in the membrane); (c) there is a simultaneous absence or lack of activity of isomaltase (I) and sucrase (S) in sucrose malabsorption; and (d) it has some interesting properties, which will be discussed below. This chapter will first consider the following: (a) the similarities in properties of the two subunits of the SI complex; (b) their common or related biological control mechanism; and (c) the mode of anchoring of the SI complex in the brush border membrane.

PROPERTIES OF THE SUBUNITS OF THE SI COMPLEX

Most work has been carried out on the SI complex of the rabbit small intestine. Whenever available, the information obtained on the SI from other species indicates that the SIs from other mammals are very similar to the rabbit enzyme. The SI complexes from the various species cross-react immuno-logically.

As obtained from Triton-solubilized SI complex, the I subunit has a molecular weight of approximately 140,000 and the S subunit one of approximately 120,000 (11). Both are glycoproteins composed of a single polypeptide chain (15), and both have one active site each (48). Their large size has discouraged sequencing. There are strong indications, however, that they have at least some degree of homology: (a) the (limited) sequence of part of the known active sites (49) is identical in S and I; (b) fingerprints of the tryptic digests of the SI complex yield only a few more peptides as there are Lys +
Arg residues (15); (c) S and I have an extensive overlapping in substrate specificities, maltose, maltitol, (55) and a number of aryl-α-glucopyranosides (16) being split by both subunits. The major differences in substrate specificities are: sucrose, which is not split by I, and the 1,6-α-glucopyranoside bonds in isomaltose and some limit dextrins, which are not split by S; (d) S and I have an extensive overlapping in their fully competitive inhibitors: common to both are D-glucose (30,61), the tris-hydroxy-methyl-amino-methane, lanthanides, D-1:5 gluconolactone (16), nojirimycin and deoxynojirimycin (28). Competitive inhibitors discriminating between S and I are: acarbose, which does not inhibit I, and dextran, which does not inhibit S (38); (e) the same compound, conduritol-B-epoxide, acts as an active-site-directed irreversible inhibitor of both S and I (48); (f) the secondary deuterium effect is approximately as large in S and I (16); (g) the values of the coefficients in the Hensch-Hammett equation are nearly the same in both subunits (16); (h) $pK_a$-values of the groups in the active sites involved in catalysis and/or substrate binding, as deduced from double-logarithmic Dixon plots, are approximately the same in S and I (23,28,71); (i) both enzymes are activated by Na$^+$; (j) both enzymes have the same kinetic mechanism (30,61); (k) both enzymes have the same minimum catalytic mechanism (16).

A COMMON OR RELATED BIOLOGICAL CONTROL MECHANISM(S) OF S AND I

This is supported by the following observations: (a) simultaneous appearance during development, both in man (in whose intestine both activities appear during intrauterine life (20) and in species in which both activities appear after birth, e.g., the rat (51), and the mouse (3); (b) simultaneous response to the stimulation by sugars and/or corticosteroids in vivo (40; see also for review, ref. 37); (c) simultaneous absence or lack of activities in sucrose–isomaltose malabsorption (8,17,22,25,26,47,60,65), which is a monofactorial genetic disease (32,46,50); (d) constant ratios between S and I activities in random samples of human peroral biopsies (9).

These observations, while not allowing the identification of the common step(s) in the control of biosynthesis and/or membrane insertion of S and I, do nevertheless indicate that these processes in the subunits of the SI complex are mutually related.

THE MODE OF ANCHORING OF THE SI COMPLEX IN THE BRUSH BORDER MEMBRANE

The SI complex is an integral membrane protein, as shown by the failure of neutral buffers of high or low ionic strength to bring it into solution. Solubilization can be achieved either by controlled proteolytic treatment, as for instance with papain (6,62), or elastase (1,41), or with detergents, such as
Triton X-100 (62,69). Most of the information on the positioning and anchoring of SI in the brush border membrane is derived from studies on the mechanism of solubilization with papain and Triton, and by comparing the characteristics of the SI forms thereby obtained.

When sealed (35) brush border membrane vesicles are subjected to limited papain digestion from the luminal side, SI activity is totally solubilized, although the permeability and transport properties of the vesicles remain unchanged (70). Thus, most of the SI complex can be removed from the luminal side without disrupting the membrane fabric. Also, opening up of the vesicles with detergent does not lead to any apparent increase in S activity (33). By negative staining “lollipops” can be visualized on the luminal surface of the brush border membrane; at least some of them have been identified with the SI complex (44). Ferritin-labeled antibodies have also localized the SI complex at the luminal surface of the brush borders (18). It seems, therefore, that the SI complex is anchored in the membrane in such a way as to protrude from its luminal side, and that only a small part of the protein mass directly interacts with the membrane fabric.

Solubilization by papain is likely to be related to the hydrolysis of one or more peptide bond(s), whereas Triton solubilization is not likely to be. Triton-solubilized SI, but not papain-solubilized SI, easily forms aggregates upon removal of the detergent and can interact with phosphatidyl choline, yielding regularly shaped proteoliposomes (12). Triton-solubilized SI, but not papain-solubilized SI, has (a) hydrophobic segment(s), as detected by charge-shift electrophoresis (72). The irreversible solubilization by papain is accompanied by the loss of one or more hydrophobic segments, which are necessary for the interaction of SI with either natural or artificial membranes. Indeed, papain treatment of isolated Triton-solubilized SI or of Triton-SI-proteoliposomes leads to a protein indistinguishable from papain-solubilized SI plus a rather short highly hydrophobic peptide (11).

This hydrophobic “anchor” is likely to be localized at the N-terminal region of the I subunit (Fig. 1) since papain solubilization does not lead to any change in (a) the C-terminal regions of either subunit, (b) the N-terminal of sucrase, or (c) the apparent molecular weight of S. Papain solubilization, however, produces covalent changes in the N-terminal region of I, since it leads to (a) a decrease (by ~20,000 daltons) of its apparent molecular weight and (b) a change of its N-terminal with appearance of heterogeneity (the substrate specificity of papain is rather broad). The conclusion appears therefore to be that (a) the SI complex is anchored to the brush border membrane via a hydrophobic segment located at the N-terminal region of the I subunit; (b) neither S nor the C-terminal region of I are inserted in the brush border membrane; and (c) since papain solubilizes SI by acting from the luminal side without modifying the C-terminal regions of S or I, or the N-terminal region of S, these polypeptide segments must not span the membrane: these C- and N-termini must therefore be located at the luminal side of the membrane (Fig. 1) (11).
SMALL-INTESTINAL SUCRASE-ISOMALTASE

Sequencing of the N-terminal of I reveals, as expected, a highly hydrophobic sequence—probably the most hydrophobic one ever reported (Fig. 2) (24,64). Some points in this sequence are worth discussing.

Residue 11 is glycosylated (24,64). The neutral sugars galactose, glucose and fucose are associated with this amino acid residue in approximately 1:1:1 molar ratios. Since glycosylation is confined to the luminal compartment of the endoplasmic reticulum and of the Golgi membranes (for reviews see refs. 27,52,53), these observations indicate that residue 11 is located on the extracellular side of the membrane. Since the 1–11 sequence, even if stretched, could barely cross the membrane (and many of its residues are charged) it seems unlikely that Ala-1 is located on the side of the membrane opposite to residue 11.
FIG. 2. Partial N-terminal sequences of ProSI and IS polypeptides. (From Hauri et al., ref. 29, and Sjöström et al., ref. 64, with permission.)
The N-terminal of I is located on the luminal side: direct evidence for this has been provided recently by the use of a new, little permanent acetimidate (3-[dimethyl-2-(acetimidoyethyl)ammonio]-propane-sulfonic acid, DAP) (14). The degree of modification of the I N-terminal (Ala) by this reagent is the same, irrespective of whether the reaction is carried out on right-side out brush border membrane vesicles (35) or on membrane fragments obtained by deoxycholate extraction (36). Ala-1 is as easily accessible to DAP acting on the luminal side only as to DAP acting on both sides. It must, therefore, be located at the luminal side. Similar results have been obtained with Ile-1 (the N-terminal of the sucrase subunit), which we know from other evidence to be located at the luminal side (11). If the isomaltase polypeptide chain has both termini at the luminal side, it must either not cross the membrane fabric or cross it an even number of times. In order to make a statement on these points, the following data on the I anchor have to be collected.

The hydrophobic anchor is some 60 amino acid residues long. A highly hydrophobic photolabel has been developed by Dr. J. Brunner in the author's laboratory (trifluoromethyl-3-(m-[125]I)iodophenyl)-diazirine, TID) (13). With the use of this reagent it can be shown that the anchoring segment of I is indeed confined to its N-terminal region (66). The isolated anchor is approximately 60 amino acid residues long and has a helical configuration, interrupted around residue 35 (a Pro) by a β turn. The most likely conformation of the anchor and its interaction with the membrane core is, therefore, that indicated in Fig. 1.

BIOSYNTHESIS AND MEMBRANE INSERTION OF THE SI COMPLEX

In order to explain the positioning of the two subunits (Fig. 1), their homologies, and their common or related biosynthetic control mechanism, the author suggested in 1979 (56,57) that SI is synthesized and inserted in the membrane as a single very long (260,000 daltons at least) polypeptide chain ("pro-sucrase-isomaltase" (ProSI)) which is processed into the "final" two-subunit complex by (extracellular) proteolysis. Since then, direct evidence has been obtained for the existence of an immunologically cross-reacting precursor and of a fully active ProSI in brush borders not previously exposed to pancreatic protease(s) (21,29,43,63,64). Subsequently, other brush border two-subunit enzymes have been shown to be likewise synthesized as a single-chain proform (renal γ-glutamyl-transpeptidase), intestinal glucoamylase, and the β-glycosidase complex (Sjöström et al., personal communication).

In vitro cell-free translation of ProSI has been achieved recently (73). This protein is the largest identified membrane polypeptide chain successfully translated thus far in a cell-free system from total RNA. In addition to providing further evidence that SI is synthesized as a one-chain precursor, the cell-free
*in vitro* translation will hopefully help to solve problems related to the detailed mechanism of biosynthesis and membrane insertion. Prior to this, however, the following questions on the positioning and structure of ProSI have to be answered.

1. Does ProSI begin with the I or the S portion? Figure 2 shows the N-terminal sequences of rat (29), and hog (64) ProSIs. Clearly, they are identical (not merely homologous!) to those of the corresponding I subunits. This identity strongly indicates that the I portion corresponds to the N-terminal part of ProSI (Fig. 1).

2. How many hydrophobic segments (and thus potential anchors) does ProSI carry? In the original suggestion (Fig. 1) (56,57) no mention was made as to the possible existence of a hydrophobic segment at the C-terminal region and/or in the “loop”. The latter possibility can now be ruled out, at least for the ProSI found in the brush border membrane prior to its processing by pancreatic proteases. As a matter of fact, in addition to the amphipathic (Tri-ton-solubilized) ProSI, a water-soluble, nonamphipathic form can be prepared. This form, which shows no charge shift in the electrophoresis in detergents, has an apparent molecular weight only slightly smaller than the amphipathic form: thus, the loop (if it exists at all) between the I and the S portions does not contain any detectable hydrophobic sequence (H. Sjöström et al., personal communication).

**PHYLOGENETIC CONSIDERATIONS**

From a survey of the literature on the occurrence of disaccharidase activities in vertebrates (55), it is clear that I activity is found in all species investigated; this is not the case for S, which in mammals is not found in Pinnipedia [Otarioidea or Phocoidea (31,39,68)], nor in a few species of Marsupiala and Monotremata, and, in birds, in some penguins. In general, S activity is found in terrestrial species feeding on fruits and vegetables. The scattered data do not allow a phylogenetic pedigree to be put forward. No species, however, has been reported as having S but no I activity (which, incidentally, makes physiological sense). This is the reason why the author suggested that the product of the original partial gene duplication would be a “double I” rather than a “double S”. The author has therefore investigated whether the small-intestinal I of a species not endowed with S activity, the sea lion (*Zalophus califomianus*), has two active sites per polypeptide chain and thus mimics the phylogenetic precursor of ProSI (or is a phylogenetic “dead end” thereof) (Table 1).

Sea lion maltase-isomaltase has been isolated by detergent solubilization and immunoabsorption using antibodies directed against final rabbit SI. With the exception of sucrose, which it does not attack, sea lion I has the same substrate specificity as rabbit SI. It has two active sites (shown by labeling with the substrate-site-directed irreversible inhibitor conduritol-B-epoxide) per poly-
TABLE 1. A hypothetical scheme of part of the phylogenesis and of the biosynthesis of small-intestinal I and S in mammals

<table>
<thead>
<tr>
<th>Major characteristics of the enzyme(s)</th>
<th>Possible example</th>
</tr>
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<tbody>
<tr>
<td>Early gene</td>
<td>None found thus far</td>
</tr>
<tr>
<td>By (partial?) gene duplication</td>
<td>(Sea lion I may be a model of this original double I. See text)</td>
</tr>
<tr>
<td>By mutation(s) producing a change in the substrate specificity of one of the sites (i.e., isomaltose → sucrose)</td>
<td>ProSI found in transplants of fetal rat small intestine and in the small intestine of adult hogs, whose pancreas had been disconnected from the duodenum 3–4 days before sacrifice</td>
</tr>
<tr>
<td>By splitting of the single polypeptide chain by pancreatic protease(s)</td>
<td>The SI complex from the small intestine of most mammals; the bands arising from ProSI upon treatment with pancreatic protease(s) in vitro</td>
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</table>

peptide chain of approximately 250,000 molecular weight. The active sites are identical, each of them splitting maltose, isomaltose, and palatinose, with no sign of deviation from Michaelis behavior (72).

It is still premature, however, to take sea lion I as an exact copy of the hypothetical double I. In fact, S activity is found both in species which have branched off from the phylogenetic tree prior to the Otarioidea (the "eared seals", to which the Californian sea lion belongs), and in others which have branched off later on. As a result, additions to the simplistic scheme must be considered. For example, (a) the "one-chain SI" (i.e., fully active ProSI) may have back-mutated to a double I in Otarioidea, in penguins and possibly in other species; (b) a mutation in the one-chain SI may have led to inactivation or loss of S. Subsequent protein fusion (and modification?) would have produced a pseudo-double I in the sea lion. No obvious advantage seems to be connected with events (a) or (b). (c) The appearance of S activity (that is, the mutation presumed to have transformed the hypothetical double I into the single-chain ProSI) may have occurred more than once. SI in phylogenetically remote species would thus be the product of convergent evolution, perhaps owing to the advantage of including sucrose-containing foodstuff in the diet of land animals feeding on vegetables and fruits. Pending further information
on these points, sea lion I should be regarded only as an interesting possible analogy to the hypothetical double I in the phylogenetic and ontogenetic pedigree of the usual SI complex (Table 1).

Polypeptides carrying more than one enzymatically active site are rare, particularly among eukaryotes (for reviews see refs. 10,34,67). In general, enzymes associated in the same polypeptide chain catalyze a sequence of different reactions belonging to the same metabolic pathway, which may be an advantage in eukaryotes (67). It is, however, quite exceptional for two similar or identical active sites (acting "in parallel", rather than "in series") to occur on the same polypeptide chain. The only other example in addition to ProSI and sea lion I is probably the ATPase of myosin filaments. What evolutionary advantage, if any, may be associated with two-enzyme one-chain structure in the case of the intestinal carbohydrases is not apparent at present.

**SUCROSE-ISOMALTOSE MALABSORPTION**

Sucrose-isomaltose malabsorption was identified in 1960–1961 (2,46,74,75). It is less rare than it was originally thought to be: it may occur in as many as 0.2% of North Americans (45) and 10% of Greenland Eskimos (4,42). It is characterized by a complete, or almost complete, lack of S activity, a very strong reduction of I activity and a decrease of maltase activity to about one-third of the normal level (8). In the clinical history the intolerance to sucrose is usually prominent. The tolerance to starch is also reduced (5), more so than to palatinose (isomaltulose). Thus, the clinical picture is that expected if the subunits of the SI complex were both either absent or inactive. In the Sephadex chromatography of the disaccharidase activities of a small-intestinal biopsy from a patient, the peaks of S, I and maltase activities of the SI complex are not found (59). The residual I and maltase activities are likely to be due, in most cases, to the "heat-stable" maltases, i.e., to the maltase-glucoamylases (4) and/or to an I devoid of S activity (25,65).

Sucrose-maltose malabsorption is an hereditary, autosomic (46), monofactorial recessive condition (32,50). The actual enzyme defect underlying this disease, however, is not quite clear. In view of what is known on the positioning and anchoring of the SI complex in the brush border membrane, and of the possible existence of precursors, the following possibilities should be considered: (a) an "operon mutation", or (if S and I arise from two contiguous, fused genes) an extensive deletion of the two-cistron unit; (b) formation of an unstable or ineffective messenger RNA; (c) failure to respond to the hormonal stimulation during development, located at the translational or at a later step; (d) point mutation in the I gene, with loss (or perhaps strong reduction) of I activity and lack of capacity of I to keep S attached; (e) point mutation in the "precursors" with defective activation and/or insertion in the membrane; (f) point mutation (e.g., in I) making the SI complex abnormally susceptible to
intracellular proteases or to those of the intestinal lumen. Other possibilities may also exist.

Preiser et al. (47) conclusively demonstrated the absence of SI and of any additional abnormal band in the SDS-polyacrylamide gel electrophoresis patterns of brush border membranes from some patients, and Gray et al. (26) failed to detect by a very sensitive radioimmunoassay any cross-reacting material in the intestinal homogenates or in the brush border membranes of other patients. It thus appears that in these patients mechanisms (d) and (e) alone are unlikely.

The existence in the cytosol of the small intestine of patients with sucrose-isomaltose malabsorption of an enzymatically inactive protein with an apparent molecular weight (by SDS-polyacrylamide gel electrophoresis under nondenaturing conditions) of approximately 220,000 has recently been reported. This observation would be compatible with mechanism (e) above. Characterization of this extra protein occurring in the cytosol could provide decisive evidence on whether or not it qualifies as an intracellular precursor of brush border SI.

Other mechanism(s), i.e., (d) and/or (e) above, are suggested by the observations of other authors. Dubs et al. (22), reported the presence of catalytically inactive, immunologically cross-reactive material in the brush border region of biopsies from sucrose–isomaltose-intolerant patients (by immunofluorescence). Freiburghaus et al. (25) and Skovbjerg and Krasilnikoff (65) found that the residual I activity in some patients has an electrophoretic mobility different from that of the normal SI complex. Changes involving other brush border enzymes were also observed. Furthermore, Cooper et al. (17) have found in three cases, using a highly sensitive fluorogenic assay, that both S and I, although very severely reduced, were nevertheless detectable. In density-gradient centrifugations of the homogenates, S activity of the patients sedimented at the same location as normal S, which indicated that the patient's S was located in the same organelle as the enzyme from normal tissue.

Summing up, it is difficult to draw conclusions which should be valid for all cases of sucrose–isomaltose malabsorption. Rather, it seems most likely that this syndrome may not be genetically homogeneous and that different mechanisms may be operative in the various pedigrees. Examination of the same patients by the various techniques may shed additional light in the future.

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I want to thank most heartily my co-workers—they are the ones who have actually carried out the experimental work and have contributed significantly to our ideas. It is a pleasure to thank those clinicians and physiopathologists with whom we shared our problems. This study was partially funded by the SNSF, Berne and by Nestlé Alimentana, Vevey.
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DISCUSSION

Dr. Ribadeau Dumas: Unless I missed it, you did not mention any data supporting the hypothesis you choose for anchoring in the membrane. Also, did you get confirmation of cell-free translation of signals?

Dr. Semenza: It seems likely that the NH$_2$ terminus of pre-proteins is initially located at the cytosolic rather than at the cisternal surface of the rough endoplasmic reticulum membrane, and that the growing polypeptide chain crosses the bilayer folded as a "loop" or "helical hairpin"; the first hydrophobic helix of the hairpin would pull the polar portions of the polypeptide chain as a second helix into and across the membrane. The first half of the hairpin (i.e., the first hydrophobic helix) would be the pre-piece (the "signal"). Along these lines it has been suggested that ProSI is synthesized as pre-ProSI and inserted into the membrane as follows. The first hairpin establishing the first insertion would be composed of a hydrophobic helix (the pre-piece, not demonstrated yet), followed by a $\beta$-turn which encompasses the short hydrophilic sequence 1 to 11 of ProSI; this hydrophilic sequence is extruded until the hydrophobic sequence which follows terminates the extrusion process. A second hydrophobic-hydrophilic hairpin would follow immediately, and the rest of ProSI could be synthesized and extruded either completely or up to another hydrophobic segment situated close to the COOH terminus. Signal peptidase would split the loop between the two helices of the first hairpin, thus creating a secondary (extracellular) NH$_2$ terminus and producing the final positioning of ProSI. Some of the predictions made by the helical hairpin hypothesis still lack experimental confirmation. (a) Is ProSI originally translated with a pre-piece? In vitro translation of the message should give an answer. (b) Following the hydrophobic sequence which is to span the membrane should be a second hydrophobic piece which enables the anchor to loop back. A proline residue found in position 35 of rabbit I could indicate this "turning point".