THE HYDROPHOBIC ANCHOR OF SMALL-INTESTINAL SUCRASE–ISOMALTASE

N-Terminal sequence of the isomaltase subunit

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Received 29 September 1978

1. Introduction

The sucrase–isomaltase complex (SI), a glycoprotein of two subunits of app. mol. wt 140 000–160 000 each, is an intrinsic protein of the small-intestinal brush border membrane [1,2]. The catalytic centres are fully accessible from the lumen, and the bulk of the protein mass probably protudes from the outer, luminal surface of the membrane ([3,4], reviewed [5,6]).

Several ectoproteins have a linear domain structure near the C-terminal which encompasses the cytoplasmic and the intramembraneous regions (e.g. [7]; reviewed [8]). Small intestinal SI, instead, is anchored to the brush border membrane via a segment at the N-terminal region of one of the subunits (i.e., isomaltase) [2]. A significant interaction of the C-terminal regions with the membrane fabric could be ruled out. This different mode of anchoring of an intrinsic protein to a plasma membrane called for additional events to be postulated in the biosynthesis and/or the insertion process(es) of SI.

We report here a partial amino acid sequence of the N-terminal region of the isomaltase subunit. It includes an extremely hydrophobic sequence, which agrees with and supports the conclusion reached previously, that the 'anchor' of SI is located in this area of the molecule.

Abbreviations. SI, sucrase–isomaltase complex; SDS, sodium dodecyl sulfate, PAGE, polyacrylamide gel electrophoresis; PTH, phenyl thio hydantoin

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2. Materials and methods

Triton-solubilized sucrase–isomaltase [9] was isolated from rabbit small intestine [10] and the individual subunits were prepared by SDS–PAGE [2] and eluted [11] as described elsewhere. SDS was removed by ion exchange [12,13].

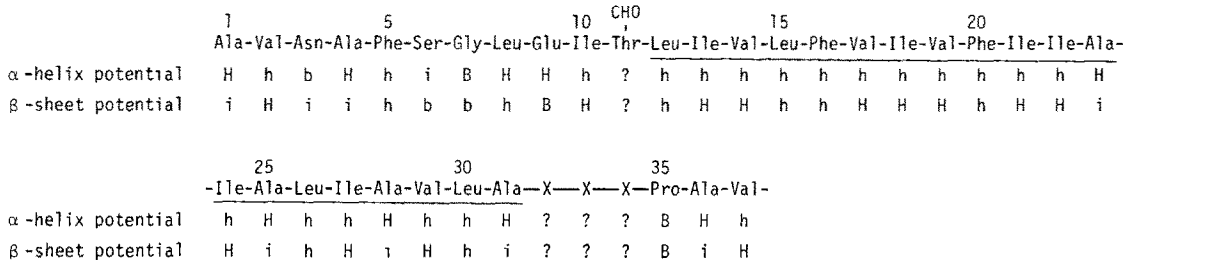
Sequence analysis was carried out automatically on a Sequenator (Beckman Sequencer model 890 C). The program was a slightly modified Beckman standard program. All chemicals were Pierce sequenal grade. Conversion of thiazolinones and identification of PTH-amino acids was carried out as in [14,15]. Where no amino acid residue could be identified, the back hydrolysis method [16] of PTH-amino acids was employed.

All chemicals were reagent grade, unless indicated otherwise.

3. Results and discussion

3.1. *The amino acid sequence (fig.1)*

The sequence in fig.1 contains a highly hydrophobic segment, residues 12–31. Very conspicuously hydrophobic sequences have been reported (e.g. [17,18]) for other intrinsic membrane proteins too. To the best of our knowledge, however, the sequence in fig.1



H, strong α (β) former; h, α (β) former; i, α (β) indifferent; b, α (β) breaker; B, strong α (β) breaker

Fig.1. The N-terminal region of the isomaltase subunit of the sucrase-isomaltase complex from the brush border membrane of rabbit small intestine.

is unique in being the most hydrophobic ever reported: its side chains can contribute a hydrophobic term of about −30 kcal/mol* to the total interaction energy between the polypeptide chain and the hydrophobic layer of the lipid bilayer membrane. This value can be compared, e.g., with the value of −9 kcal/mol calculated for the free energy of transfer of palmitic acid from water to heptane [20,21]. This highly hydrophobic segment is likely to be the reason for the aggregation in the absence of detergent of Triton-solubilised sucrase–isomaltase [9,10], or of the isolated isomaltase [40] or of the peptide which is derived from the N-terminal region of the isomaltase subunit during controlled papain digestion of the protein–lipid vesicles described in [22].

During papain-solubilisation of SI from intact brush borders the sole or major event occurring is the cleavage of a 17 000 dalton segment from the N-terminal region of the isomaltase subunit [2]. This segment (of which a part is represented by the sequence in fig.1) must therefore play an important role in anchoring SI to the brush border membrane. The calculated −30 kcal/mol would indeed be sufficient to 'anchor' SI in the membrane, if all the side chains between residue 12 and 31 are in contact with the hydrophobic leaflet of the lipid bilayer in the brush border membrane. Information is needed, therefore, on the secondary and tertiary structure of this segment. Although not experimentally available at present, some predictions based on Chou and Fasman's analysis [23–25] are presented below.

3.2. *Possible secondary structure(s) on the hydrophobic segment 12–31*

Assuming that Chou and Fasman's statistical calculations can be applied to a peptide interacting with the lipid bilayer, the following tentative deductions can be presented. Between residue 12 and 31 any sequence of 6 amino acids has a helical potential $\langle P_\alpha \rangle \geqslant 1.03$ and β-sheet potential $\langle P_\beta \rangle \geqslant 1.05$, with no helical or β-sheet breaking residues. Both conditions A1–A4 and B1–B4 (see [23,24]) are fulfilled. The conclusion is, therefore, that both α-helix and β-pleated sheet structures are possible, although the latter is more probable, since for the whole segment 12–31 $\langle P_\alpha \rangle = 1.18$ and $\langle P_\beta \rangle = 1.38$**.

Chou and Fasman's predictions are based on the statistical analysis of the distribution of residues among regions of different conformations in globular proteins [23–25]. Indeed predictions made on their basis have turned out to be satisfactorily accurate for water soluble globular proteins. However, the hydrophobic environment produced by lipids [26,27] as well as by organic solvents [28,29] are likely to favour α-helix formation at the expense of random coil and β-sheet conformations. It is quite possible, therefore,

* This value was computed using the data of [19] (p. 121), assuming that the contribution of Ile is the same as that of Leu

** In the first half of the hydrophobic segment the calculated $\langle P_\beta \rangle$ is much larger than $\langle P_\alpha \rangle$, in the second half the calculated $\langle P_\alpha \rangle$ is somewhat larger than $\langle P_\beta \rangle$

that Chou and Fasman's calculation tends to overestimate the β-sheet potential of intramembraneous membrane protein regions [29]. A final decision on the secondary structure of the hydrophobic segment 12–31 will be only possible on the basis of experimental evidence. At any rate, it is interesting to note that if the whole sequence 12–31 were in the α-helix or β-sheet conformation, it would be longer than 30 Å, sufficient to span the hydrophobic layer of the membrane.

As expected, the hydrophobic residues of sequence 12–31 have extremely low β-turn probabilities, indicating that this peptide region is linear rather than folded. Whether Pro-35 may be involved in a β-turn or not, is only a reasonable speculation at the moment. (Proline residues have among the highest β-turn potential [23–25].)

3.3. *Possible secondary structure(s) and possible positioning of the 1–11 segment*

The region 1–8 is predicted as random coil.

Residue 11 is, in all likelihood, a glycosylated threonine:

(i) Its PTH derivative cannot be detected in the ethyl-acetate extract;

(ii) Back hydrolysis of the anilino-thioazolinone in HCl yields α-amino-butyrate but no histidine or arginine;

(iii) During back hydrolysis the solution becomes slightly brownish.

Since the PTH-derivative of unglycosylated threonine is extractable in ethyl-acetate, these 3 observations are highly suggestive of residue 11 being a CHO-Thr, as indicated in fig.1.

To the best of our knowledge, no example of glycosylation of the cytoplasmic portion of intrinsic plasma membrane proteins has been reported thus far. This, and the current ideas on biosynthesis and glycosylation of membrane proteins (as well as of export proteins) [30,31] suggest that residue 11 resides on the luminal side of the membrane, as most of the glycoprotein mass of sucrase–isomaltase does [4]. This would imply that the polypeptide chain between residue 11 and the bulk of the protein mass crosses that hydrophobic layer of the membrane zero or an even number of times.

3.4. *Biosynthetic considerations*

Biosynthesis of SI takes place in the small intestine (e.g. [32–34]).

The biosynthetic implications of SI being anchored to the plasma membrane via a N-terminal rather that a C-terminal region, and via one of the two subunits only, have been briefly mentioned in [2,35]. The absence of a significant role of the C-terminal regions of either subunit in the anchoring of SI to the membrane may possibly be explained as follows: freshly synthetised (and inserted) sucrase and/or isomaltase may possess C-terminal segment(s) spanning the membrane. Endopeptidases (e.g., of pancreatic origin) would cleave off the(se) segment(s) by acting from the lumen. SI would not be released into the lumen due to its hydrophobic anchor at the N-terminal of the isomaltase subunit. Alternatively, it is also quite possible that SI is synthetised as an 'export' protein (i.e., without hydrophobic segment at the C-terminal region) but remains associated with the brush border membrane via the N-terminal region of the isomaltase subunit.

In the biosynthesis of all or most of export proteins, hydrophobic segments (sometimes preceded by a short hydrophilic segment) are synthetised prior to the polypeptide chain which will be exported eventually. This 'pre-piece' (the 'signal') is split off shortly after its biosynthesis (for the 'signal hypothesis' see [30,36,37]). One can speculate that the sequence of fig.1 is a kind of modified (actually, extended) 'signal': in SI, contrary to export proteins, the signal would not be split off, but rather 'stick' in the hydrophobic leaflet of the rough endoplastic reticulum during the biosynthesis of the later part of the same polypeptide chain. There may be a number of plausible reasons for this segment not being susceptible to the action of 'signal peptidase', a different amino acid composition and/or sequence, a different secondary structure, a special substrate specificity of small intestinal 'signal peptidase'. But perhaps the most appealing of the hypothetical mechanisms is that the isomaltase 'signal' is not available to the action of 'signal peptidase' just because it is embedded in the hydrophobic leaflet of the membrane. Indeed, calculation of the hydrophobic term in the energy of interaction of the hydrophobic segments in the 'pre-pieces' (listed, e.g., in [38,39] and the references quoted therein) lead to figures less

negative*, and in some cases much less negative, than the −30 kcal/mol calculated for sequence 12–31 in fig.1.

But, is the sequence of fig.1 related to those of the 'signals'? The sequences reported for signals, in spite of being all considerably hydrophobic, show little homology. Preliminary calculations indicate, however, that their secondary structures frequently encompass an α-helix region followed by a β-turn (personal communication from Dr G. D. Fasman, 1978). As pointed out above, this secondary structure is indeed possible for the sequence 12–35 in fig.1.

* The pre-piece of trypsinogen (see table 1 from [38]) is the only one having a hydrophobic term of energy interaction approaching that of sequence 12–31 in fig.1

Table 1

Comparison between the N-terminal amino acid sequence of the isomaltase subunit from rabbit intestinal sucrase–isomaltase (fig.1) and those of pre-trypsinogen from dog pancreas (from [38]) and of a part of glycophorin, including its intramembraneous segment (from [7])

Isomaltase	Dog pancreas pre-trypsinogen		Glycophorin from human erythrocytes	
	Minimum number of bases mutated		Minimum number of bases mutated	With one deletion assumed in isomaltase
1 Ala				
Val				
Ala	1 Ala	0	His	His
5 Phe	Lys,Phe	0	Phe 0	Phe
Ser	Leu,Pro	1	Ser 0	Ser
Gly	Phe	2	70 Glu 1	70 Glu
Leu	5 Leu	0	Pro 1	Pro
Glu	Phe	3	Glu 0	Glu
10 Ile	Leu	0	Ile 0	Ile
Thr-(CHO)	Ala	1	Thr 0	Thr
Leu	Leu,Phe	0	75 Leu 0	75 Leu
Ile	10 Leu	0	Ile 0	$(Ile)_2$
Val	Leu	1	Ile 1	Phe
15 Leu	Ala	2	Phe 1	Gly
Phe	Tyr	1	Gly 2	80 Val
Val	Val	0	80 Val 0	Met
Ile	15 Ala	2	Met 1	Ala
Val	Phe	1	Ala 1	Gly
20 Phe	Pro	2	Gly 2	Val
Ile	Leu	0	Val 1	85 Ile
Ile	Leu	0	85 Ile 0	Gly
Ala	Asp_4		Gly 1	Thr
Ile	...		Thr 1	Ile
25 Ala			Ile 2	Leu
Leu			Leu 0	90 Leu
Ile			90 Leu	Ile
...			Ile	Ser
			Ser	Tyr
			Tyr	Gly
			Gly	Ile
			Ile	Arg_2
			Arg_2	
			...	

[a] The system used in [38] did not differentiate between Leu and Ile. In addition, more ambiguities were present, as indicated. For the purpose of this orientative comparison the 'most favourable' residue in pre-trypsinogen was assumed

Amino acid residues identical with those in the N-terminal sequence of the isomaltase subunit are underlined

In addition, we have compared the sequence in fig.1 with the pre-piece of trypsinogen, which is among the longest signal sequences reported, and which is synthetised in the pancreas, an organ embryologically related with the small intestine (table 1). Between the first 19 amino acid residues of Blobel's pre-trypsinogen [38] and sequence 4–22 of fig.1 there is a maximum identity of 9/19 = 47.5%. Of the remaining 10 non-identical amino acid residues, 5 are compatible with a single-base codon mutation, 4 with 2-base mutations, and only one would require all three bases of the codon to be mutated. Encouraging as this comparison may be, it should be clearly pointed out that Blobel's analysis could not differentiate between Leu and Ile and, moreover, as indicated in the table, further ambiguities were also present; in the above calculation the most 'favourable' amino acid residue was assumed in Blobel's sequence. On the other hand, no deletion was assumed. As a whole, therefore, only future studies will be able to decide whether the sequence in fig.1 includes a modified ('pseudo') signal or whether a pre-piece is synthetised prior to it, or whether the sequence does not play the role of a signal and is not preceded by a signal either.

The possibility has been discussed [35] that the two subunits of SI might first be synthetised as a single, high mol. wt polypeptide chain.

3.5. *Partial homology with glycophorin*

The sequence of the N-terminal region of isomaltase was also compared with that of glycophorin [7] (table 1). To our surprise a better secured homology was found in this case: between amino acid residues 5–27 of isomaltase and 68–90 (or 91) of glycophorin 10 out of 22 i.e., 45% (or 11 out of 23, i.e., 48% if a deletion is assumed to have occurred in isomaltase) are identical. The codons of the other amino acid residues differ by one or two bases. The homology observed encompasses the beginning of the extracellular parts of the proteins and the beginning of their adjacent hydrophobic, intramembranous segments. The amino acids involved in this homology are not the same involved in the possible homology with pre-trypsinogen (table 1). A more limited homology was also found with other membranous segments of intrinsic proteins, e.g., with the myelin proteolipid P7 apoprotein [18].

The significance of the homology between a part of the N-terminal region of isomaltase and a part of glycophorin which is located not far from the C-terminal [7] is not obvious. It may indicate that polypeptide segments interacting with the membrane must have yet unidentified structural features in common, or it may have an as yet ill-understood, biosynthetic significance.

Acknowledgements

This work was partially supported by the SNSF, Berne, and by Nestlé Alimentana, S. A., Vevey. To them sincere thanks are addressed.

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