Biochimica et Biophysica Acta, 684 (1982) 133–136
Elsevier Biomedical Press

BBA 70000

BBA Report

THE AMINO ACID SEQUENCE OF THE HYDROPHOBIC ANCHOR OF RABBIT INTESTINAL BRUSH BORDER AMINOPEPTIDASE N

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(Received May 18th, 1981)

Key words: Aminopeptidase N; N-terminal sequence; Enzyme anchor

The N-terminal sequence (14 residues) of the detergent form of rabbit intestinal aminopeptidase N was shown to be different from that of the protease form of the same enzyme and to be mostly hydrophobic. This finding is fully consistent with a previous assumption according to which this class of enzymes may be anchored to the brush border membrane by their N-terminus. This special mode of assembly may be facilitated by a positively charged lysine residue near the beginning of the sequence (Lys 4) just before an uninterrupted stretch of hydrophobic amino acids.

Brush border aminopeptidases are amphiphilic molecules composed of a large hydrophilic domain protruding from the external side of the membrane, and of a much smaller hydrophobic anchor spanning the membrane [1]. The anchors of pig and rabbit intestinal aminopeptidases N and A [2–5], and that of pig kidney dipeptidylpeptidase IV [6] have been isolated as short, predominantly hydrophobic peptides after limited proteolysis of detergent-extracted enzyme molecules (the 'detergent forms'). The anchor-free hydrophilic domains are called the 'protease forms' of the enzymes.

Pig intestinal aminopeptidase N, which is a dimer with two identical subunits [7], is usually obtained in the state of a trimer due to artefactual cleavages during purification [2,5,7]. One of the N-terminal residues of the detergent form of the trimer was shown to be the same as that of the corresponding hydrophobic peptide, while all N-terminal residues of the protease form were different [2]. This finding suggested for the first time that (unlike several other membrane proteins such as glycophorin [8], the glycoprotein of the vesicular stomatitis virus [9], the histocompatibility antigens [10,11] and the influenza virus haemaglutinin [12]) the enzyme was anchored to the membrane by the N-terminus. This special mode of insertion was extended later to a variety of other intestinal and renal brush border hydrolases listed in Ref. 13 as well as, more recently, to pig intestinal aminopeptidase A [3] and rabbit intestinal aminopeptidase N [4]. Investigations concerning this latter enzyme are facilitated by the fact that it is monomeric in aqueous solution [4].

In the course of the present work, the insertion of rabbit aminopeptidase N by its N-terminus was further confirmed by showing that the N-terminal sequences of the detergent and protease forms of the enzyme are different and that the former is mostly composed of hydrophobic amino acids. Structural similarities were also found between the aminopeptidase anchor and corresponding sequences in a variety of other membrane proteins and secretory preproteins.

The two forms of the rabbit enzyme were purified as in Ref. 4. Sequences are determined on 60 nmol of protein using a Beckman liquid phase Sequencer Model 890 C with the 0.1 M Quadrol

TABLE I

N-TERMINAL SEQUENCES OF THE PROTEASE AND DETERGENT FORMS OF RABBIT INTESTINAL AMINOPEPTIDASE N

The chains of aminopeptidase (detergent form) and of the two proteolipids are aligned with respect to the lysine residue (Lys_4 in aminopeptidase). Homologous residues are underlined. The point of cleavage of the protease form of aminopeptidase is indicated by arrow. The sequence of both aminopeptidase forms were determined twice, except for the 4-residues extension of the longer protease form which was determined only once. X, unidentified residue, ( ), tentative identification.

	1				↓	5							10							
Aminopeptidase (protease form)	Asn-	Thr-	X-	Gln-		Ser-	Pro-	X-		Met-	Ala-	X-		X-		Asn-	X-		X	
	1					5							10							
Aminopeptidase (detergent form)	Tyr-	Ile-	Ser-	Lys	┌	Ala-	Leu-	Gly-	Ile-	Leu-	Gly-	Phe-	X-		(Leu)-	(Gly)				
	11					15							20							
Proteolipid (*N. crassa*) [16]	Glu-	Val-	Ser-	Lys	├	Asn-	Leu-	Gly-	Met-	Gly-	Ser-	Ala-	Ala-	Ile-		Gly				
	6					10							15							
Proteolipid (*S. cerevisiae*) [16]	Leu-	Ala-	Ala-	Lys	└	Tyr-	Ile-	-Gly-	Ala-	Gly-	Ile-	Ser-	Thr-	Ile-		Gly				

program. Conversion of thiazolinones and identification of thiohydantoins were carried out as in Ref. 14.

The N-terminal sequences of both forms are indicated in Table I. When prepared as reported earlier [4], the protease form of rabbit aminopeptidase possesses a N-terminal serine [4]. Omission of the dialysis step between incubation of the detergent form with trypsin and Sepharose filtration of the resulting protease form leads to a new molecular species with four additional residues before the serine. These residues are probably split off during dialysis by autolysis or attack by endogeneous proteases.

As shown by Table I, the N-terminal sequence of the detergent form of rabbit aminopeptidase N may be broken down into two distinct parts. One contains two positive charges born by the N-terminal tyrosine and the lysine at position 4. An uncharged hydrophilic residue (Ser 3) is also present in this region. The other part starting just after the lysine is entirely hydrophobic up to the last identified residue (Gly 14). By contrast, the N-terminal sequence of the protease form is rich in hydrophilic residues as already reported for dipeptidyl peptidase [6]. The abnormally high number of non identifiable residues in this form may be due to attached glycan chains which are very abundant in the whole enzyme molecule. These data and the already reported accumulation of hydrophobic residues early in the sequence of isomaltase [15] and dipeptidyl peptidase [6] are fully consistent with the view that several brush border hydrolases are anchored to the membrane by their N-termini. The possibility that changes also occur in the C-terminal region of the enzyme chains during conversion of the detergent into the protease form has not yet been explored, mainly because of the difficulties encountered with the carboxypeptidase technique when applied to large protein molecules exposed to enzymatic degradations during their purification.

The sequence of the detergent form of aminopeptidase, compared to the amino acid composition of the corresponding peptide [4], suggests that this peptide is composed of a hydrophobic core of about 20 residues sandwiched between the above reported, positively charged N-terminal segment and a C-terminal hydrophilic segment probably cut off from the hydrophilic domain of the enzyme during the limited proteolysis leading to the protease form. This uneven distribution of hydrophobic and hydrophilic residues is in good agreement with the strongly amphiphilic properties of these peptides.

A charged amino acid residue just before a hydrophobic sequence has already been characterized in the H^+-ATPase proteolipids from *Neurospora crassa* and *Saccharomyces cerevisiae* [16], the coliphage M 13 precoat protein [17] and a number of leader sequences in eukaryotic [18] and prokaryotic [19] secretory preproteins. A plausible

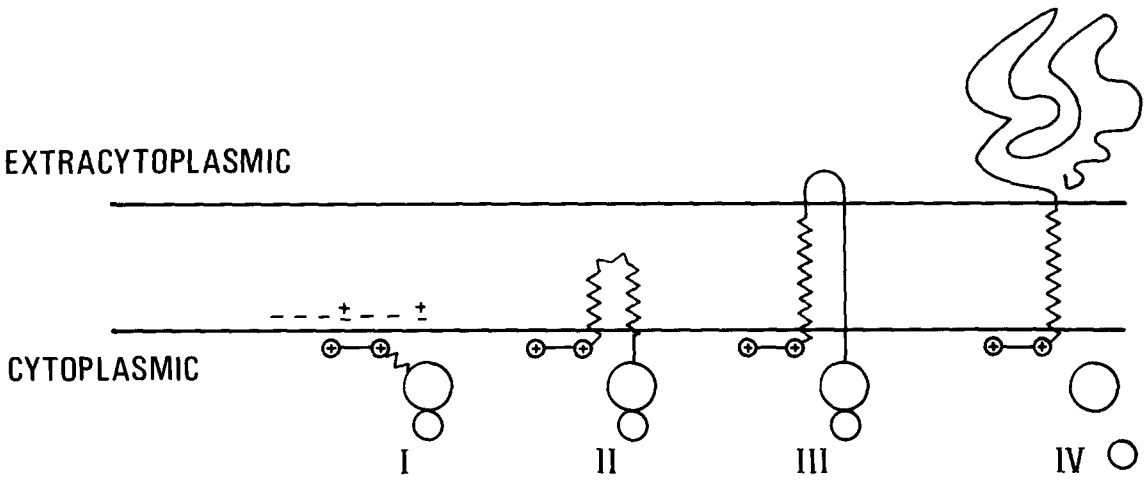

Fig. 1. Model for the insertion of brush border aminopeptidase by the N-terminus. ~, hydrophobic sequence; +, N-terminal α-NH_3^+ or ϵ-NH_3^+ of lysine, − or ±, negative or zwitterions of the phospholipids polar heads. I, binding of the nascent chain to the membrane by charged residues. II and III, chain growth. IV, folded hydrophilic domain held on the extracytoplasmic side of the endoplasmic reticulum or the external side of the brush border by a N-terminal anchor.

hypothesis is that this residue and the N-terminal α-NH_3^+ group play a role in the recognition of nascent protein chains by the phospholipid polar heads rich in negative charges [20] present on the cytoplasmic surface of the endoplasmic reticulum membrane. For proteins which, like the brush border hydrolases, remain bound to the membrane by their N-terminus, these charges may constitute an electrostatic bolt [1,21,22] which holds the first residues of the growing chain on the cytoplasmic membrane face (Fig. 1, I). Then, the hydrophobic segment can penetrate into the bilayer accordingly to the 'direct transfer' theory of Von Heijne and Blomberg [22] and the chain can continue to grow via the so-called 'hair pin loop mechanism' first proposed by Inouye et al. [23] for secretory preproteins (Figs. 1, II and 1, III). Synthesis stops after emergence of the bulk of the molecule from the extractytoplasmic face of the membrane (Fig. 1, IV). An alternative hypothesis is that recently put forwards by Engelman and Steitz [24] which predicts spontaneous insertion of a preformed hairpin. However, even in this case, the initial charged segment of the chains may be assumed to remain at the surface of the membrane and stabilize the insertion by adding electrostatic interactions to the normal hydrophobic forces inside the membrane.

In rabbit brush border isomaltase, the charged lysine residue is replaced by a presumably glycosylated threonine [15] which may also stabilize the insertion. However, the N-terminal sequence of dipeptidylpeptidase has been reported to start immediately with hydrophobic amino acids [6]. Therefore, more work, especially on possible relationships between anchors and leader sequences, is required before the role of the lysine in the assembly of aminopeptidase and other proteins within the membrane can be definitely ascertained.

As shown by Table I, some other structural similarities between the aminopeptidase anchor and two H^+-ATPase proteolipids are noted when the chains are aligned with respect to their common lysine residue.

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