Volume 228, number 1, 102–104 FEB 05547 February 1988

Biosynthesis of intestinal microvillar proteins

Effect of castanospermine on cell-free synthesis of aminopeptidase N

E. Michael Danielsen and Jørgen Olsen

Department of Biochemistry C, Panum Institute, University of Copenhagen, Copenhagen, Denmark

Received 14 December 1987

Pig small intestinal mRNA was translated in a rabbit reticulocyte lysate system supplemented with microsomal membranes. Castanospermine, an inhibitor of glucosidase I, induced a high mannose-glycosylated form of microvillar aminopeptidase N (EC 3.4.11.2) of increased molecular mass, indicating the blocked removal of glucose residues. In contrast to its reduced expression in a mucosal explant system [(1986) Biochem. J. 240, 777–782], this molecular form of aminopeptidase N was at least as abundant in cell-free translation as its normal high mannose-glycosylated counterpart, ruling out degradation taking place in the rough endoplasmic reticulum. Degradation of newly produced , malprocessed enzyme must therefore occur at a later stage during intracellular transport, presumably in the sarcoplasmic reticulum or in transitional elements between this organelle and the Golgi complex.

Castanospermine; Aminopeptidase N; mRNA; Cell-free translation

1. INTRODUCTION

In cell-free translation, mRNA extracted from pig small intestine encodes a 115 kDa polypeptide which represents the microvillar enzyme aminopeptidase N (EC 3.4.11.2) [1]. In the presence of microsomal membranes, the primary translation product translocates the endoplasmic membrane without cleavage of the signal and becomes high mannose-glycosylated, resulting in an increase in molecular mass to 140 kDa which is the size of the transient, intracellular form of aminopeptidase N [2]. Other microvillar peptidases as well as glycosidases appear to follow a similar biosynthetic mechanism [3,4]. We have observed previously that inhibitors such as tunicamycin and castanospermine severely reduce the expression of aminopeptidase N in a mucosal explant system, indicating the rapid degradation of malprocessed molecules [5,6].

Here, we studied the effect of castanospermine on cell-free synthesis of aminopeptidase N to determine the subcellular site of degradation of newly produced, malprocessed enzyme, a mechanism whose function may be to act as a 'quality control' in the biosynthesis of this type of protein.

Correspondence address: E.M. Danielsen, Dept of Biochemistry C, Panum Institute, 3 Blegdamsvej, DK-2200 Copenhagen N, Denmark

Abbreviations: RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

2. EXPERIMENTAL

2.1. *Materials*

Rabbit reticulocyte lysate, dog pancreatic microsomal membranes and [^{35}S]methionine (spec. act. > 1000 Ci/mmol) were purchased from Amersham (England) and castanospermine from Boehringer Mannheim (FRG).

Pig small intestine was kindly delivered by the Department of Experimental Pathology (Rigshospitalet, Copenhagen, Denmark).

2.2. *Methods*

RNA was extracted and isolated from nitrogen-frozen pig small intestine and translated in the rabbit reticulocyte lysate system as in [1,7]. In translations with castanospermine, the in-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

hibitor was present at 0.1 mg/ml. Immunopurification of aminopeptidase N and SDS-PAGE were performed as in the above-cited works. Gel tracks were scanned in an LKB Ultroscan XL densitometer (LKB, Bromma, Sweden).

3. RESULTS AND DISCUSSION

Fig.1 shows cell-free translation of intestinal mRNA. Castanospermine did not affect the overall incorporation of radioactivity into protein and did not alter the electrophoretic mobility of the major polypeptides, encoded by the intestinal mRNA. Cell-free synthesis of aminopeptidase N is depicted in fig.2. Here, the non-glycosylated, primary translation product of 115 kDa was also visible in the presence of microsomal membranes, showing that membrane translocation and attachment of *N*-linked high mannose oligosaccharides are not fully efficient in this system. In the presence of castanospermine, the glycosylated form migrated markedly slower than the control, indicating a higher molecular mass. This agrees well with the reported inhibition by castanospermine of glucosidase I, resulting in high mannose oligosaccharides containing the three initial glucose residues [8]. An identical molecular form of the enzyme has been observed in the mucosal explant system [6]. It is noteworthy, however, that the glycosylated form, seen in cell-free translation in the presence of castanospermine, is at least as abundant as the corresponding form from control experiments (as judged by densitometric scanning of similarly loaded gel tracks), whereas in the explant system, its expression was reduced to 35%. Prolonged incubation at 37°C of the translation mixture (up to 3 h) caused equally extensive degradation of the normal and Glc-containing high mannose-glycosylated forms of the enzyme (not shown). These findings indicate that the latter form of aminopeptidase N within the RER lumen is equally stable to its normally trimmed counterpart.

Lodish and Kong [9], studying the effect of 1-deoxynojirimycin on synthesis and secretion of α_1-antitrypsin and α_1-antichymotrypsin, reported that transport out of the RER requires removal of glucose residues and hypothesized that the normally processed oligosaccharide forms part of the recognition site of a transport receptor for exported proteins. This, together with our previous reports on rapid degradation of newly produced,

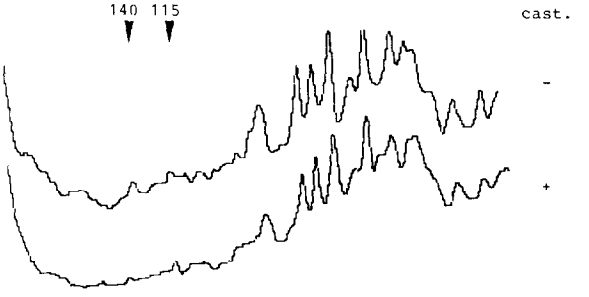

Fig.1. Cell-free translation of intestinal mRNA. Intestinal mRNA was translated in the reticulocyte lysate system in the presence of microsomal membranes for 1 h at 37°C in the absence (−) or presence (+) of castanospermine (cast.). After incubation, 15 μl of the translation mixture was subjected to SDS-PAGE. The positions of the primary translation product of aminopeptidase N (115 kDa) and the high mannose-glycosylated form (140 kDa) are indicated by arrows.

malprocessed microvillar enzymes would implicate the RER in the degradatory process. However, the present result strongly argues that the quality control mechanism operates outside the membrane-translocating and -glycosylating organelle. Theoretically, it is conceivable that a malprocessed aminopeptidase N from the pig cannot be recognized by the degradatory system from the dog owing to species differences. This explanation, however, must be considered unlikely, given the extensive species compatibility of other RER-confined functions such as recognition and cleavage of signal sequences for membrane translocation and attachment and trimming of *N*-

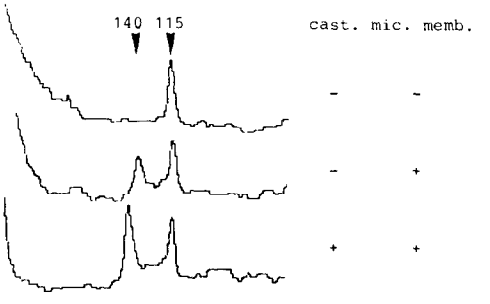

Fig.2. Effect of castanospermine on aminopeptidase N. Intestinal mRNA was translated in the reticulocyte lysate system for 1 h at 37°C in the absence (−) or presence (+) of microsomal membranes and castanospermine. Aminopeptidase N was immunopurified from 200 μl translation mixtures and subjected to SDS-PAGE. Molecular masses (kDa) are indicated by arrows.

linked carbohydrate. Candidates likely to harbour the degradatory system could be the SER or transitional elements between this organelle and the Golgi complex. Interestingly, this part of the intracellular route to the microvillar membrane is the most time-consuming for newly produced enzymes to pass through and also the location where individual enzymes travel at different velocities [10,11]. Taken together, these data therefore suggest that the lag period in biosynthesis of microvillar enzymes which precedes processing in the Golgi complex is actively used by the enterocyte to recognize and sort malprocessed molecules from the mainstream of newly produced proteins.

Acknowledgements: Ms Lotte Wetterberg is thanked for excellent technical assistance. E.M.D. holds a 'Hallas-Møller' stipend from the Novo Foundation. Drs Gillian Cowell, Hans Sjöström and Ove Norén are thanked for valuable discussions concerning the manuscript.

REFERENCES

[1] Danielsen, E.M., Norén, O. and Sjöström, H. (1982) Biochem. J. 204, 323–327.
[2] Danielsen, E.M., Cowell, G.M., Norén, O. and Sjöström, H. (1984) Biochem. J. 221, 1–14.
[3] Semenza, G. (1986) Annu. Rev. Cell Biol. 2, 255–313.
[4] Cowell, G.M. (1987) Biochem. Ed. 15, 166–172.
[5] Danielsen, E.M. and Cowell, G.M. (1984) FEBS Lett. 166, 28–32.
[6] Danielsen, E.M. and Cowell, G.M. (1986) Biochem. J. 240, 777–782.
[7] Danielsen, E.M., Norén, O. and Sjöström, H. (1983) Biochem. J. 212, 161–165.
[8] Saul, P., Chambers, J.P., Molyneux, R.J. and Elbein, A.D. (1983) Arch. Biochem. Biophys. 221, 593–597.
[9] Lodish, H.F. and Kong, N. (1984) J. Cell Biol. 98, 1720–1729.
[10] Danielsen, E.M. and Cowell, G.M. (1985) FEBS Lett. 190, 69–72.
[11] Hauri, H.-P., Sterchi, E., Bienz, D., Fransen, J.A.M. and Marxer, A. (1985) J. Cell Biol. 101, 838–851.