Translational control of an intestinal microvillar enzyme

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The rates of biosynthesis of adult and foetal pig small-intestinal aminopeptidase N (EC 3.4.11.2) were compared to determine at which level the expression of the microvillar enzyme is developmentally controlled. In organ-cultured explants, the rate of biosynthesis of foetal aminopeptidase N is only about 3% of the adult rate. The small amount synthesized occurs in a high-mannose-glycosylated, membrane-bound, form that is processed to the mature, complex-glycosylated, form at a markedly slower rate than that of the adult enzyme. Extracts of total RNA from adult and foetal intestine contained comparable amounts of aminopeptidase N mRNA, encoding gel-electrophoretically identical primary translation products. Together, these data indicate that the expression of aminopeptidase N is controlled at a translational level.

INTRODUCTION

Aminopeptidase N (EC 3.4.11.2) is one of the major integral hydrolases of the microvillar membrane of the small intestine, participating in the final digestion of nutrients. This group of enzymes is commonly referred to as 'stalked' membrane proteins, owing to their characteristic membrane topology (Kenny & Maroux, 1982; Norén *et al.*, 1986). Aminopeptidase N from adult individuals is synthesized by ribosomes attached to the rough endoplasmic reticulum, where membrane translocation and insertion, together with *N*-linked high-mannose glycosylation, takes place. Intracellular transport to the extracellular site of function proceeds in a membrane-bound state, and the route passes through the Golgi complex, where carbohydrate processing occurs to yield the mature form of the enzyme (Danielsen *et al.*, 1984).

It is well known that the activity of microvillar enzymes, including aminopeptidase N, increases during embryonic and/or early postnatal development, and that glucocorticoids and thyroxine play a major role in inducing or stimulating their expression (Moog, 1979; Kedinger *et al.*, 1980; Henning, 1981). This process is a part of the overall cellular differentiation that leads to the formation of the highly polarized enterocyte, and a study of the mechanisms controlling this differentiation is essential for the understanding of how cell polarity is generated. This paper reports a comparison of the rate of biosynthesis of aminopeptidase N in adult and foetal small intestine, a work performed to determine at what level the expression of this microvillar enzyme is controlled.

MATERIALS AND METHODS

Materials

Equipment for performing organ culture, including Trowell's T-8 medium, foetal-calf serum, sterile plastic dishes with grids and [³⁵S]methionine (specific radioactivity > 1000 Ci/mmol), were obtained as previously described (Danielsen *et al.*, 1982a). Dog pancreatic microsomal membranes were purchased from Amersham International, Amersham, Bucks., U.K.

Pig adult and foetal small intestines were kindly given

by the Department of Experimental Pathology, Rigshospitalet, Copenhagen, Denmark, and De Forenede Andelsslagterier, Ringsted, Denmark, respectively.

Methods

Organ culture of intestinal mucosal explants was performed as described by Danielsen *et al.* (1982a) and preparation of intestinal RNA and cell-free translation in a reticulocyte lysate system were as described by Danielsen *et al.* (1982b). Enzymes were purified by line-immunoelectrophoresis (Danielsen & Cowell, 1983). SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970), and fluorography of gels by the method of Bonner & Laskey (1974).

RESULTS

Organ culture of intestinal explants is a well-established technique for maintaining both adult (Browning & Trier, 1969) and foetal (Simon-Assmann *et al.*, 1982) tissue for periods up to 48 h. We have previously adapted organ culture for pig small-intestinal explants and have shown that the morphology, at both the light- and the electron-microscopic level, is well preserved during culture up to 24 h (Danielsen *et al.*, 1982a). The explants also maintained their contents of protein and microvillar enzymes and were able to sustain a continuous protein synthesis during the entire culture period. The explant system has since been used to study the nature and kinetics of microvillar-enzyme biosynthesis in adult intestine (for review, see Danielsen *et al.*, 1984). The second system used, cell-free translation of total RNA from small intestine, has previously been used to gain information about the primary translation product of aminopeptidase N and its co-translational processing by microsomal membranes (Danielsen *et al.*, 1982b, 1983).

Fig. 1 shows the polypeptide pattern of membrane-bound and soluble fractions of organ-cultured adult and foetal explants labelled for 5 h. The fuzzy *M_r*-166000 polypeptide of mature aminopeptidase N, which is a predominant component in the adult membrane-bound fraction, was not apparent in foetal tissue. As shown in Table 1, the enzyme only comprises 0.026% of the total protein synthesized in foetal explants, compared with 0.80% in adult tissue, indicating that the rate of synthesis

Fig. 1. Biosynthesis in organ-cultured adult and foetal explants

The explants were labelled for 5 h with 100 μ Ci of [35 S]-methionine/ml and homogenized in 1 ml of 5 mM-Tris/HCl (pH 7.5)/150 mM-NaCl with a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 100 000 g for 1 h in a Beckman Airfuge to obtain a pellet (membrane-bound fraction) and a supernatant (soluble fraction). Samples of these fractions were subjected to SDS/polyacrylamide gel electrophoresis. Apparent M_r values are shown. Tracks: 1, adult membrane-bound fraction; 2, foetal membrane-bound fraction; 3, immunopurified transient (M_r 140 000) and mature (M_r 166 000) forms of adult aminopeptidase N; 4, immunopurified adult sucrase-isomaltase; 5, adult soluble fraction; 6, foetal soluble fraction; 7, immunopurified adult proline dipeptidase; 8, immunopurified adult glycyl-leucine dipeptidase.

of aminopeptidase N during development from the foetal to the adult state is increased about 30-fold. This result was also obtained with labelling periods as short as 10 min and in the presence of the proteinase inhibitor leupeptin, arguing against the possibility that the low amount of aminopeptidase N synthesized in foetal

explants can be ascribed to a rapidly occurring post-translational degradation of newly synthesized enzyme, as has been observed in adult explants organ-cultured in the presence of tunicamycin (Danielsen & Cowell, 1984). The molecular forms of foetal aminopeptidase N were membrane-bound and of similar M_r to the adult transient high-mannose-glycosylated (M_r 140 000) and mature complex-glycosylated (M_r 166 000) forms of the enzyme (Fig. 2). However, the rate of conversion of the foetal transient form of aminopeptidase N into the mature form was very slow; the latter could not be detected after 1.5 h of labelling, whereas it appears after about 30 min in adult explants (Danielsen, 1982) (Fig. 2). Furthermore, the amount of mature form relative to the amount of transient form after 3 h of labelling was about one order of magnitude less than previously observed for the adult tissue (Danielsen & Cowell, 1985) (as judged by densitometric scanning of the fluorograph), indicating that the rate of conversion of aminopeptidase N in foetal tissue is 4–5 times slower. However, after longer periods of labelling (up to 14 h), the relative intensity of the mature form greatly increased, indicating that, although it is generated at a slower rate, it is the quantitatively predominant molecular form present in foetal, as well as in adult, intestine.

Two cytosolic dipeptidases, glycyl-leucine dipeptidase (EC 3.4.13.11) (Norén *et al.*, 1973) and proline dipeptidase (EC 3.4.13.9) (Sjöström *et al.*, 1973), were also found to have a much lower rate of biosynthesis in foetal than in adult tissue (0.006% versus 0.092% and 0.026% versus 0.190%, respectively).

mRNA from guanidinium chloride extracts of total RNA of adult and foetal small intestine was observed to encode primary translation products of aminopeptidase N that are gel-electrophoretically identical (M_r 115 000) (Fig. 3). The similar relative amounts of aminopeptidase N synthesized by the cell-free translation system (Table 1) indicate that adult and foetal small intestine contain comparable relative amounts of mRNA encoding the enzyme. Glycyl-leucine dipeptidase (Fig. 3) and proline dipeptidase (results not shown) were also synthesized in

Table 1. Synthesis of adult and foetal aminopeptidase N in organ-cultured intestinal explants and by intestinal mRNA in cell-free translation

Explants of adult and foetal small intestine were labelled continuously for 5 h with 100 μ Ci of [35 S]methionine/ml. The radioactivity incorporated into both adult and foetal explants was about 10^6 c.p.m./mg of explant protein. After culture, the explants were solubilized in 1 ml of 5 mM-Tris/HCl (pH 7.5)/150 mM-NaCl/5% (v/v) Triton X-100. After 15–30 min on ice with frequent vortex-mixing, the homogenate was centrifuged for 1 min in a Beckman Microfuge to obtain a supernatant of extracted protein. For cell-free translation, the translation mixtures (280 μ l) contained 100 μ l of nuclease-treated rabbit reticulocyte lysate, 100 μ Ci of [35 S]methionine and guanidinium chloride extracted adult or foetal intestinal RNA in amounts yielding maximal incorporation of radioactivity into protein (3000–6000 c.p.m./ μ l of translation mixture). In both explant and cell-free translation experiments, total protein synthesis was determined as trichloroacetic acid-precipitable radioactivity (measured in 10 μ l samples), and aminopeptidase N synthesis was determined by measuring the radioactivity incorporated into the carefully washed immunoprecipitate. The synthesis of aminopeptidase N is expressed as $100 \times$ radioactivity of aminopeptidase N/radioactivity of trichloroacetic acid precipitate. Foetuses of 14–20 cm in length were used, and each value listed is the mean of three to five experiments.

Type of experiment	Aminopeptidase N synthesis (% of total protein synthesis)	
	Adult	Foetal
Organ culture of explants	0.80	0.026
Cell-free translation of mRNA	0.18	0.19

Foetal explants were labelled with 200 μ Ci of [35 S]methionine/ml for 1.5 h (1), 3 h (2), 7 h (3) and 14 h (4), and after culture, aminopeptidase N was purified from Triton X-100 extracts as described in the legend to Table 1. The purified enzyme was subjected to SDS/polyacrylamide-gel electrophoresis (apparent M_r values are shown). Track 5 shows the transient form of adult aminopeptidase N for comparison.

relative comparable amounts by mRNA from adult and foetal intestine.

The properties of adult and foetal mRNA encoding aminopeptidase N were further compared by supplementing the cell-free translation system with microsomal membranes. For adult mRNA, microsomal membranes have previously been shown to process the primary translation product of aminopeptidase N into an

Fig. 3. Cell-free translation of adult and foetal intestinal mRNA

SDS/polyacrylamide-gel electrophoresis of immunopurified adult and foetal aminopeptidase N and glycyL-leucine dipeptidase from cell-free translation of intestinal mRNA (apparent M_r values are shown). Tracks: 1, adult aminopeptidase N; 2, foetal aminopeptidase N; 3, adult glycyL-leucine dipeptidase; 4, foetal glycyL-leucine dipeptidase; 5, adult total translation products; 6, foetal total translation products.

Fig. 4. Processing of primary translation products by microsomal membranes

SDS/polyacrylamide-gel electrophoresis of immunopurified adult and foetal aminopeptidase N from cell-free translation of intestinal mRNA in the presence of microsomal membranes (apparent M_r values are shown). Tracks: 1, adult aminopeptidase N; 2, foetal aminopeptidase N; 3, adult total translation products; 4, foetal total translation products.

endoglycosidase H-sensitive polypeptide of M_r 140 000, identical with the transient form of the enzyme from organ-cultured explants (Danielsen *et al.*, 1983). As shown in Fig. 4, microsomal membranes likewise glycosylated the primary translation product of foetal mRNA. However, in parallel incubations, the microsomal membranes processed the foetal primary translation product with only about half the efficiency of that with which the adult M_r -115 000 polypeptide was glycosylated and did not stimulate its translation, as observed for the adult enzyme (judged by densitometric scanning of fluorographs).

DISCUSSION

The combined use of an explant system and cell-free translation in a comparative study on the biosynthesis of adult and foetal aminopeptidase N should be able to

give information about the level at which the expression of the enzyme is controlled during embryonic development. Before drawing any conclusions, however, one must take into account the overall difference in structure and function between adult and foetal intestine (for review, see Smith, 1985) and consider if potential inaccuracies or shortcomings of the approach could lead to an erroneous interpretation of the data. Thus a lower absorptive function of the immature foetal epithelium might conceivably cause a decreased uptake of radioactive precursor, leading to poor incorporation of radioactivity into protein. As it turned out, however, foetal explants synthesized about the same amount of protein as adult tissue, as judged by the trichloroacetic acid-precipitable radioactivity (Table 1). Furthermore, by expressing the biosynthesis of aminopeptidase N relative to the overall protein synthesis, any difference in uptake of radioactive precursor will be cancelled as a source of error. The multi-cellular composition of intestinal mucosa could also be thought to influence the results obtained, both with the explant system as well as with cell-free translation of total intestinal RNA, for instance if the contribution by non-epithelial cells varies significantly between adult and foetal tissue. However, in the explant system the polypeptide pattern of total adult and foetal protein synthesis showed a high degree of overlap (Fig. 1); this was even more pronounced in cell-free translation (Figs. 3 and 4), arguing against this possibility being of major importance.

The very low rate of biosynthesis of aminopeptidase N in foetal, as compared with adult, tissue agrees well with the general notion that only small amounts of enzyme activity are present at this stage of development. Determinations of antibody-suppressible aminopeptidase activity in Triton X-100 extracts of foetal intestine likewise indicate a low amount of enzyme compared with adult values (results not shown). The fact that the foetal enzyme is membrane-bound and occurs in molecular forms characteristic of a 'normal' co- and post-translational processing is evidence that the expression of aminopeptidase N is controlled at a stage before polypeptide synthesis. Surprisingly, adult and foetal intestine contain comparable amounts of mRNA for aminopeptidase N, arguing against the possibility of a transcriptional control. Taken together, the data from organ culture and cell-free translation experiments indicate that the mechanism that controls the expression of aminopeptidase N operates at a translational level. A similar finding has not previously been reported for a microvillar enzyme; in fact, it has been suggested that sucrase-isomaltase (EC 3.2.1.48–3.2.1.10) and maltase-glucoamylase (EC 3.2.1.20) are regulated at a transcriptional level (Simon-Assmann *et al.*, 1984). However, in that study, which reported the effect of actinomycin D on microvillar enzyme activities in organ-cultured rat small-intestinal explants, the culture period was of a length comparable with the life-span of the cells (48 h), obscuring a clear-cut interpretation of the data.

Translational control of protein expression is a phenomenon known from other organisms, most notably the sea urchin in its early embryonic development (Gross *et al.*, 1964), where the overall rate of protein synthesis increases after fertilization (Brandhorst, 1976). Examples of a more selective type of translational control have been reported to occur in higher organisms (Shull & Theil, 1982; Maundrell *et al.*, 1983; McGuire *et al.*, 1984). For

proinsulin, it has been suggested that its biosynthesis induced by glucose in pancreatic islets of the rat is mainly achieved by an enhancement of the translational efficiency of proinsulin mRNA pre-existent on membrane-bound polyribosomes (Itoh & Okamoto, 1980): The observed lower affinity of the foetal primary translation product of aminopeptidase N for microsomal membranes in cell-free translation can hardly in itself account for the 30-fold difference in the rate of synthesis in the explant system, but could be suggestive of a structural difference between adult and foetal mRNA species. Likewise, the markedly slower rate of conversion of the foetal enzyme from the transient into the mature form is indicative of a slow rate of migration from the rough endoplasmic reticulum into (and through) the Golgi complex, and could be suggestive of a minor structural difference between adult and foetal primary translation products, altering the 'signals' for processing and intracellular transport (Sabatini *et al.*, 1982). Alternatively, this phenomenon may reflect a poorly developed pathway for processing and transport of newly synthesized plasma-membrane proteins in foetal tissue. The results with the two cytosolic dipeptidases indicate that translational control might be a general phenomenon in the expression of proteins that have a similar developmental pattern, be they membrane-bound or soluble. The mechanism whereby this translational control is achieved is as yet unknown, but is an attractive subject for further study.

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