Biosynthesis of intestinal microvillar proteins

The intracellular transport of aminopeptidase N and sucrase-isomaltase occurs at different rates pre-Golgi but at the same rate post-Golgi

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The kinetics of processing and microvillar expression of aminopeptidase N (EC 3.4.11.2) and sucrose α-D-glucohydrolase–oligo-1,6-glucosidase (sucrase-isomaltase, EC 3.2.1.48 and EC 3.2.1.10) were compared by labelling of pig small intestinal mucosal explants with [^{35}S]methionine. The conversion from transient (high mannose glycosylated) to mature (complex glycosylated) form was 1.7-times slower for sucrase-isomaltase than for aminopeptidase N, indicating a slower rate of migration from the rough endoplasmic reticulum to the Golgi complex. Likewise, sucrase-isomaltase appeared in the microvillar fraction at a slower rate than aminopeptidase N. The relative pool sizes of mature and transient forms of both enzymes in intracellular membranes (Mg^{2+}-precipitated fraction) were determined to obtain information on the relative time, spent pre- and post-Golgi, respectively, prior to microvillar expression. This ratio was 0.24 ± 0.06 (mean ± SD) for sucrase-isomaltase as compared to 0.40 ± 0.04 (mean ± SD) for aminopeptidase N. Considering the slower rate of pre-Golgi transport for sucrase-isomaltase, this indicates that the two microvillar enzymes have rather similar if not identical rates of post-Golgi transport.

Microvillar protein *Biosynthesis* *Intracellular transport* *Aminopeptidase N* *Sucrase-isomaltase*

1. INTRODUCTION

It is generally accepted that newly synthesized proteins, destined either for secretion or for insertion in the plasma membrane, follow a common route of membrane-confined transport leading from the rough endoplasmic reticulum, via the Golgi complex, to the surface of the cell [1]. Likewise, it has become evident that they may do so at different rates within the same cell; thus Strous and Lodish [2] reported the existence of different rates of externalization amongst secretory and (viral) plasma membrane proteins in infected rat hepatoma cells and stated that these differences arose during transport from the rough endoplasmic reticulum into or through the Golgi complex. Scheele and Tartakoff [3], studying the transport of a variety of secretory proteins in guinea pig exocrine pancreas, observed asynchrony at 4 levels; in the exit from the rough endoplasmic reticulum, transit through the Golgi complex, entry into granules and discharge from the cell. Thus, there seems to be no general rule concerning the ways in which a cell organizes the regulation of the consecutive steps in its pathway for protein externalization.

The small intestinal enterocyte expresses a number of peptidases and glycosidases in its apical microvillar membrane [4]. The common nature of their co- and post-translational processing makes it reasonable to assume that newly synthesized microvillar enzymes share the same route of transport from the site of synthesis to the site of function [5]. However, in [6], we observed that sucrose α-D-glucohydrolase–oligo-1,6-glucosidase (sucrase-isomaltase, EC 3.2.1.48 and EC 3.2.1.10)

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is processed slower than aminopeptidase N (EC 3.4.11.2), an observation that has also been reported by other investigators [7]. In this work, we have extended our earlier study by comparing in greater detail the kinetics of processing and microvillar expression of these two enzymes to determine their relative migration rates in the pre- and post-Golgi parts of the pathway leading to the microvillar membrane.

2. EXPERIMENTAL

2.1. *Materials*

Equipment for performing organ culture, including Trowell's T-8 medium, foetal calf serum, sterile plastic dishes with grids and [^{35}S]methionine (spec. radioact. >1000 Ci/mmol), were obtained as described [8].

Pig small intestines were kindly given by the department of Experimental Pathology, Rigshospitalet, Copenhagen, Denmark.

2.2. *Organ culture*

Organ culture of pig intestinal mucosal explants was performed as described [8]. The explants were radioactively labelled for periods of 30–180 min by the addition of [^{35}S]methionine (100 μCi/ml) to the culture medium. Immediately after labelling the explants were frozen and kept at −80°C until further processing.

2.3. *Other methods*

Labelled explants were either solubilized in 1 ml of 5 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% Triton X-100, to obtain a total extract of explant protein or fractioned into Mg^{2+}-precipitated and microvillar membranes as described [6]. Aminopeptidase N and sucrase-isomaltase were purified from total explant extracts and Triton X-100 solubilized Mg^{2+}-precipitated and microvillar membranes by line immunoelectrophoresis [9]. The purified enzymes were subjected to SDS-polyacrylamide gel electrophoresis [10], and the gels prepared for fluorography [11]. Developed films were scanned in a Kipp & Zonen DD2 gel scanner (Delft, The Netherlands).

3. RESULTS AND DISCUSSION

It has previously been shown that the processing of newly synthesized microvillar enzymes takes place in the Golgi complex [12]. Here, intermediate molecular forms between transient (high mannose glycosylated) and mature (complex glycosylated) forms could be visualized only when the intracellular transport was arrested in the Golgi complex by use of the ionophore monensin, indicating that the Golgi-located processing reactions of trimming and complex glycosylation of *N*-linked oligosaccharides under normal conditions occur extremely rapidly after another. The transient and mature forms of newly synthesized microvillar enzymes can thus be taken to represent the pre- and post-Golgi stages of the intracellular transport, respectively, and the rate of conversion from transient to mature form to be a measure of the rate of migration from the rough endoplasmic reticulum into the Golgi complex.

For both aminopeptidase N and sucrase-isomaltase, the relatively large size difference between transient and mature forms makes an efficient separation of these forms feasible and thus permits their quantification by densitometric scanning of fluorographs (fig.1). It was therefore possible to

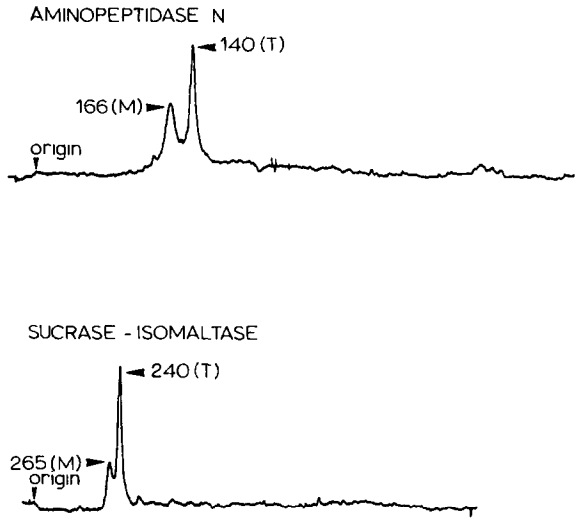

Fig.1. Densitometric scanning of purified microvillar enzymes. Aminopeptidase N and sucrase-isomaltase were immunopurified from the Mg^{2+}-precipitated fraction of explants, labelled for 2 h, and subjected to SDS-PAGE. After electrophoresis, the gels were prepared for fluorography and developed film analyzed by densitometric scanning. Apparent M_r values ($\times 10^{-3}$) are shown. T, transient forms; M, mature forms.

compare more accurately the kinetics of carbohydrate processing and microvillar expression of the two enzymes to obtain information on the pre- and post-Golgi parts of their intracellular transport.

Fig.2 shows the time-course for the conversion from transient to mature form, expressed as intensity of mature form/intensity of transient form. With labelling periods of 70–180 min, this conversion for both enzymes progressed linearly with time, indicating that the labelling of the transient forms had reached a maximum. (This was not the case with shorter labelling periods, presumably because cells of organ cultured tissue are not in full synchrony.) The conversion progressed 1.7-times slower for sucrase-isomaltase than for aminopeptidase N, indicating that the two newly synthesized microvillar enzymes migrate from the rough endoplasmic reticulum to the Golgi complex at different rates.

Precipitation with divalent cations [13,14] is a rapid procedure for separating intracellular (and basolateral) membranes from microvillar membranes and soluble protein. The method works well with organ cultured tissue; thus, in contrast to the mature form of aminopeptidase N from the microvillar fraction, both transient and mature forms of the enzyme from the Mg^{2+}-precipitated fraction were fully protected from proteolytic cleavage, indicating a minimal cross-contamination between the two fractions [15]. Using the Mg^{2+}-precipitation method, a difference in the kinetics of microvillar expression of aminopeptidase N and sucrase-isomaltase could be observed; as shown in fig.3, the relative amount of mature sucrase-isomaltase compared to that of mature aminopeptidase N in the microvillar fraction increased during labelling periods up to 3 h.

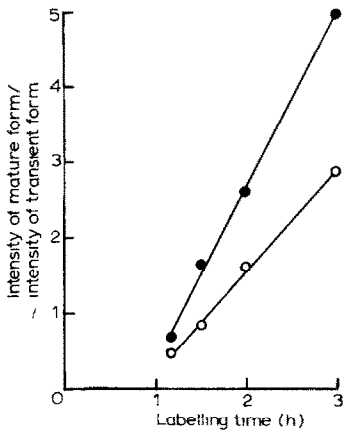

Fig.2. Time-course for carbohydrate processing from transient to mature forms. Aminopeptidase N and sucrase-isomaltase were immunopurified from total Triton X-100 extracts of explants, labelled for 30–180 min, and subjected to SDS-PAGE. Fluorographs of the gels were analyzed by densitometric scanning and the intensity of the mature forms relative to that of the transient forms, was determined. The curves were constructed by linear regression analysis of the data from two series of experiments, omitting the 30 min and 50 min time points, where maximal labelling of the transient forms had not yet been achieved. (●) Aminopeptidase N; (○) sucrase-isomaltase.

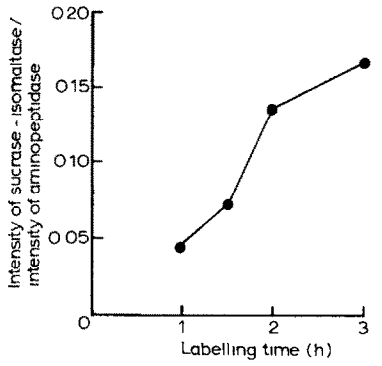

Fig.3. Time-course of appearance of newly synthesized enzymes in the microvillar membrane. Aminopeptidase N and sucrase-isomaltase were immunopurified from the microvillar fraction of explants, labelled for 60–180 min, and subjected to SDS-PAGE. Fluorographs of the gels were analyzed by densitometric scanning and the intensity of the mature form of sucrase-isomaltase relative to that of aminopeptidase N, was determined. The data shown are the mean of two series of experiments.

In explants labelled for 2 h, a period exceeding the time required for microvillar expression of newly synthesized enzymes by 0.5–1 h, the intracellular route of transport becomes maximally labelled [6]. To determine whether aminopeptidase N and sucrase-isomaltase have different rates of post-Golgi transport, the relative amount of mature vs transient form in the Mg^{2+}-precipitated fraction from such explants was measured. This ratio indicates the relative pool size of the pre- and post-Golgi transport compartments for a given

component, and assuming steady-state conditions, also the relative periods of time spent by this component pre- and post-Golgi, respectively. In a series of 3 experiments, this ratio was found to be 0.40 ± 0.04 (mean ± SD) for aminopeptidase N. For sucrase-isomaltase, purified from the same subcellular fractions, this ratio was considerably lower, namely 0.24 ± 0.06 (mean ± SD). Considering a pre-Golgi transport for sucrase-isomaltase that is 1.7-times slower than for aminopeptidase N, this indicates that the two enzymes have very similar migration rates for the post-Golgi transport.

The kinetic data of the present work demonstrate that newly synthesized aminopeptidase N and sucrase-isomaltase are expressed in the microvillar membrane at different rates. Furthermore, whereas the results clearly show a difference in the rate of pre-Golgi transport, as measured by the rate of conversion from transient to mature form, they also indicate that the kinetics of the post-Golgi transport for the two enzymes are rather similar if not identical. We have recently presented evidence suggesting that the sorting of newly synthesized microvillar enzymes (i.e., their accumulation in areas of the membrane from where other proteins with other destinations are excluded) takes place concomitantly with – or shortly after – the conversion from transient to mature form, that is, in the Golgi complex or soon after exit from this organelle [16]. The accomplishment of this sorting process is likely to be the formation of transport vesicles, destined for the microvillar membrane; in fact, vesicles containing aminopeptidase N have been localized by immunoelectron microscopy in the apical portion of the cytoplasm of enterocytes (Dr Gert Hansen, personal communication). Varying rates of transport for different microvillar enzymes prior to sorting (between the rough endoplasmic reticulum and the Golgi complex) can be explained by differing affinities for either the transport system, operating between these two organelles, or for the enzymes, responsible for the carbohydrate processing. However, after sorting and formation of transport vesicles, different microcillar enzymes should be expected to have a similar rate of transport (assuming the existence of a common type of transport vesicles for all microvillar-destined enzymes). The kinetic data presented here are consistent with this general view of post-Golgi transport and strengthen the notion that the sorting process occurs simultaneously with or shortly after the conversion from transient to mature form.

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