Ann. Biol. anim. Bioch. Biophys., 1979, **19** (3 B), 787-790.

Hydrolases bound to the intestinal brush border : An example of transmembrane proteins

par S. MAROUX, D. LOUVARD, M. SÉMÉRIVA, P. DESNUELLE

Centre de Biochimie et de Biologie Moléculaire du C.N.R.S.,
31, Chemin Joseph-Aiguier 13274 Marseille Cedex 2 France.

Summary. Two of the hydrolases attached to the intestinal brush border (aminopeptidase and maltase) had 3 domains : (*a*) a bulky, hydrophilic, enzymatically active « head » which emerged almost entirely from the external surface of the membrane ; (*b*) a short hydrophobic domain inserted in the membrane, stabilizing the head at the surface ; (*c*) a short segment penetrating into the cytoplasm. This type of transmembrane proteins could be implicated in the transfer of any kind of information from one side of the cellular plasma membrane to the other. In particular, the amino acids originating from peptides due to the catalytic activity of the « head » might be directly injected into the carrier system. This mechanism has not yet been experimentally confirmed. However, it would economize energy and explain why amino acids originating from peptides in the lumen were better adsorbed than free amino acids.

Introduction.

The absorbing cells of the small intestine (enterocytes) are generated in the crypts of the villi ; then, they move towards the tips where they are desquamated into the lumen. During this migration the young cells differentiate into mature cells with an adsorbing capacity. One of the effects of this morphological differentiation is the appearance of an invaginated region of the plasma membrane called the brush border or microvilli. Enterocytes are of great interest to cell biologists and biochemists because they constitute an excellent model for studying the differentiation and biosynthesis of cell constituents. Moreover, all the molecules generated during the intraluminal phase of digestion must cross the brush border membrane before their entry into the enterocytes and their later discharge into the blood and lymph. Thus, efficacious transport systems may be expected to exist in this membrane.

The purpose of the present report was to study the mode of integration of certain brush border hydrolases (notably aminopeptidase and maltase) in the lipid matrix of the membrane. The chemical structure of these molecules (fig. 1) is consistent with the idea that they play a role, not only as believed earlier in the last steps of intraluminar digestion, but also in the transport of the digested products across the brush border membrane.

The hydrolases will be shown to include 3 distinct domains : (*a*) a bulky and hydrophilic « head » emerging almost entirely from the external surface of the cell ; (*b*) a short hydrophobic domain anchoring the head at the surface and (*c*) an equally short segment penetrating into the cytoplasm.

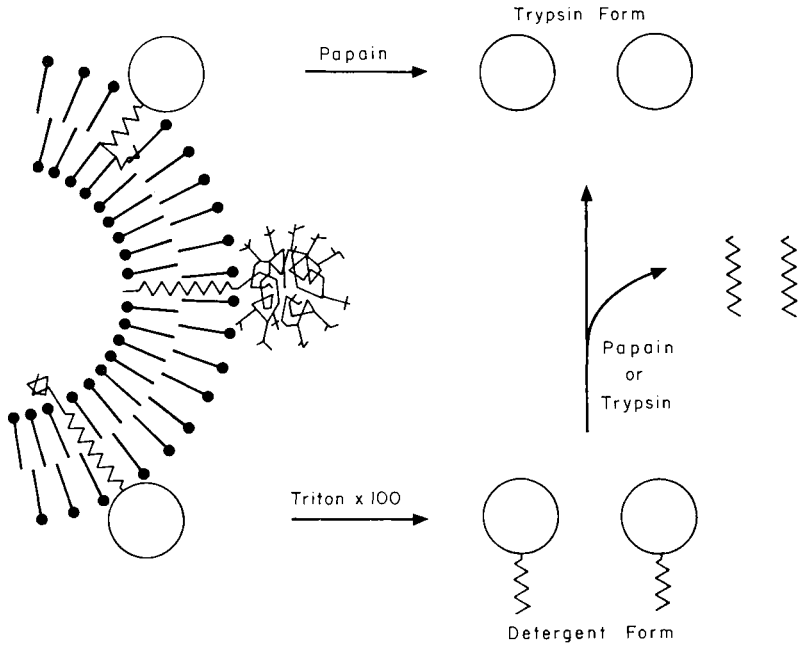

FIG. 1. — *Model of a glycoprotein bound to the surface of a membrane by an hydrophobic anchor.* One of the drawings shows a protein spanning the membrane (transmembrane protein). (From Maroux *et al.*, 1975).

a) The hydrophilic head

The first step of our study was to work out a technique for the purification of the brush border. Electron microscope observations have shown that the preparations thus obtained are entirely composed of closed vesicles having a granular appearance after negative staining (Louvard *et al.*, 1973). These vesicles are all right-side out and therefore constitute suitable material for a detailed investigation of the surface components of the membrane.

One of the techniques employed for « solubilizing » membrane proteins is to incubate the membrane fragments or vesicles with a proteolytic enzyme. Papain treatment of the above-mentioned vesicles induced the loss of their granular appearance and the release of the hydrolase activities into water. It was therefore concluded that the heads (i) were enzymatically active ; (ii) were located on the external side of the membrane and (iii) formed part of the fuzzy coat or glycocalix already shown by cytologists to cover the external surface of the brush border membrane.

The fact that the head emerged almost completely from this external surface was proved by comparing the number of accessible antigenic sites in the free (solubilized)

and the bound form of the enzymes. A monospecific antibody raised against intestinal aminopeptidase was purified by passage through an immuno-absorbent column and labeled by radioactive iodine. A simple titration then showed that 8 antigenic sites out of a total of 12 were fully accessible in the bound form of the enzyme, 2 were fully masked and 2 had a limited accessibility, probably due to the proximity of the plane of the membrane surface (Louvard, Maroux and Desnuelle, 1975). Antibodies are excellent probes for evaluating the accessible surface of an antigen in a multimolecular complex.

b) **The hydrophobic domain**

Besides treatment of the membrane by proteolytic enzymes, producing the so-called papain or trypsin forms, membrane proteins are also known to be released by mild detergents (detergent form). The two forms were found to be different in the case of intestinal and renal aminopeptidase and maltase. In particular, the papain form of both enzymes was freely soluble in water, whereas the detergent form was observed to aggregate in non-dissociating medium, suggesting the presence in the latter form of an hydrophobic peptide split off by the protease. Actually, digestion of the detergent form of aminopeptidase and maltase by trypsin led to the purification of a peptide of about 90 mainly hydrophobic residues. The corresponding domain may be assumed to penetrate into the hydrophobic matrix of the membrane and to anchor the hydrophilic head at the surface (Maroux and Louvard, 1976).

c) **The cytoplasmic segment**

In order to prove that one of the extremities of the preceding domain had emerged from the internal surface of the membrane and reached the cytoplasm, vesicles were formed in the dark in a solution containing an unspecific, radioactively labeled, photosensitive reagent. The vesicles were thoroughly washed again in the dark in order to remove the reagent adsorbed at the surface ; they were then illuminated for a suitable period of time in order to start the reaction between the reagent and any part of the molecules accessible from the inside. In this way, the above peptide was shown to be significantly labeled and therefore to protrude into the cytoplasm (Louvard, Séméria and Maroux, 1976).

Conclusions.

The model represented in figure 1 shows how a protein may be anchored to a membrane by a short hydrophobic domain of its peptide chain. In the case of intestinal (and also renal) aminopeptidase and maltase, this domain is very close to one extremity of the chain so that the protein is quite asymmetrically distributed with respect to the plane of the membrane. The largest part of the molecule is on the external side of the cell and it bears the enzymatically active site. Therefore, it is ideally situated for acting on incompletely hydrolyzed peptides before their entry into the cell.

The model is also interesting for two other reasons. First, the molecule is amphipathic, with a head made strongly hydrophilic by a number of attached sugar chains and a strongly hydrophobic part penetrating into the membrane. This type of structure

is very rigidly oriented. Free rotation and lateral diffusion are possible, but tumbling, and alternately presenting a region of the molecule on each surface of the membrane, is unlikely for thermodynamic reasons. The second factor is that the molecule is a transmembrane protein theoretically capable of transferring any kind of information across the membrane.

Experiments are now in progress in our laboratory to demonstrate that aminopeptidase is involved in amino acid transport. It is indeed conceivable that incompletely hydrolyzed peptides in the lumen are split into amino acids by the enzymatically active head and then, instead of being released in water, are injected into the carrier. This mechanism would probably save energy and explain why amino acids originating from peptides in the lumen are better adsorbed than free amino acids.

Commission CNERNA Digestion-Absorption/Association des Physiologistes, Paris 5-6 octobre 1978.

Résumé. Deux des hydrolases attachées à la bordure en brosse intestinale (aminopeptidase et maltase) sont composées de 3 domaines : (*a*) une « tête » volumineuse, hydrophile et enzymatiquement active qui émerge presqu'entièrement de la face externe de la membrane ; (*b*) un court domaine hydrophobe qui plonge dans la membrane et de ce fait stabilise la tête à la surface ; (*c*) un court segment émergeant dans le cytoplasme. Ce type de protéines transmembranaires peut en principe être impliqué dans n'importe quel transfert d'information d'un côté à l'autre de la membrane plasmique des cellules. On peut en particulier imaginer que les amino acides produits à partir de peptides grâce à l'activité catalytique de la « tête » soient directement injectés dans le système transporteur. Ce mécanisme n'a pas encore été expérimentalement confirmé. Il aurait néanmoins l'avantage d'économiser de l'énergie et d'expliquer pourquoi les amino acides fournis à l'état de peptides sont mieux absorbés que les amino acides initialement libres dans la lumière.

References

LOUVARD D., MAROUX S., BARATTI J., DESNUELLE P., MUTAFSCHIEV S., 1973. On the preparation and some properties of closed vesicles from hog duodenal and jejunal brush border. *Biochim. biophys. Acta*, **291**, 747-763.

LOUVARD D., MAROUX S., DESNUELLE P., 1975. Topological studies on the hydrolases bound to the intestinal brush border membrane. *Biochim. biophys. Acta*, **389**, 389-400.

LOUVARD D., SÉMÉRIVA M., MAROUX S., 1976. The brush border intestinal aminopeptidase : a transmembrane protein as probed by macromolecular photolabelling. *J. mol. Biol.*, **106**, 1023-1038.

MAROUX S., LOUVARD D., 1976. On the hydrophobic part of aminopeptidase and maltases which binds the enzyme to the intestinal brush border membrane. *Biochim. biophys. Acta*, **419**, 189-195.

MAROUX S., LOUVARD D., DESNUELLE P., 1975. The intestinal brush border aminopeptidase (β-naphthyl amidase) as a model of an enzyme bound to the surface of a membrane, 55-69. In DESNUELLE P., MICHELSON A. M., *Proceed. 10th FEBS Meet., Vol.* **40**, North Holland.