COMPARATIVE STUDY OF THE INTERACTIONS OF THE TRYPSIN AND DETERGENT FORM OF THE INTESTINAL AMINOPEPTIDASE WITH LIPOSOMES.

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SUMMARY : Gel filtration, gradient centrifugation and interfacial spreading of mixed vesicles of intestinal aminopeptidase and brush border lipids or egg lecithin showed little or no incorporation of the trypsin form of the enzyme. By contrast, the detergent form readily incorporated, thus confirming the role of the hydrophobic tail in the fixation of the molecule to the lipid matrix of the membrane.

Two major enzymes of the intestinal brush border, aminopeptidase (EC 3.4. 1.2) and maltase (EC 3.2.1), have recently been shown by Maroux and Louvard (1,2) to be amphipatic molecules which easily aggregate in a non dissociating medium. These molecules are composed of a large, strongly hydrophilic part bearing the catalytic activity and a much smaller hydrophobic part (the tail) probably anchoring the first to the lipid matrix of the membrane. Treatment of the membrane with neutral detergents solubilizes the entire molecules (the detergent form) whereas papain and trypsin only release the hydrophilic part (the trypsin form) by splitting one or several peptide bonds in an intermediate region.

The role of an anchor assigned to the hydrophobic tail has been confirmed in other cases by reconstitution assays (3-7).Likewise, only the detergent form of aminopeptidase has been found in the course of the present work to incorporate into brush border lipids or egg lecithin liposomes. Little or no incorporation was observed with the trypsin form of the enzyme in which the tail is absent.

Materials and Methods

Previously published techniques were employed for the preparation of vesicles from pig jejunal brush border (8) and for the purification of the trypsin and detergent forms of aminopeptidase (9). Aminopeptidase activity was measured

using L-alanine p-nitroanilide as substrate (8). The specific activity of the preparations was about 20 units per mg proteins. The brush border lipids were extracted from the vesicles by chloroform-methanol (2:1 v/v), washed and dried according to Folch (10). Egg lecithins were isolated on silicic acid and checked for homogeneity by thin layer chromatography. Trace amounts of [^{14}C]-phosphatidyl choline (NEN Chemicals) were added to facilitate detection.

Liposomes and reconstituted lipoprotein particles were generally obtained by sonication (20 times in burst of 15 sec each min) at 0-4° C under nitrogen in a 50 mM phosphate buffer pH 7.0 containing 0.1 M NaCl in 1 mM $MgCl_2$. Non dispersed aggregates were removed by a 5 min centrifugation at 3000 g. An alternative procedure involving dissolution of the lipid in sodium deoxycholate and prolonged dialysis (11) was also employed for some of the gradient centrifugation assays.

Results

The ability of the 2 forms of aminopeptidase to interact with lipids was tested by gel filtration, gradient centrifugation and film spreading.

Gel filtration. Sonicated mixtures of brush border lipids and aminopeptidase were filtered through Sepharose 4 B with the results illustrated by fig. 1. The trypsin form of the enzyme is seen in the left diagram to be normally retarded (mol. wt : 280,000 daltons (9)) in this system whereas the liposomes are excluded. Hence, both components migrate independently. With the detergent form, most of the material is excluded (right diagram). This latter result is somewhat ambiguous for the detergent form, due to aggregation, would migrate fast through Sepharose even in the absence of any interactions with the lipid. But, the indication concerning the trypsin form is significant.

Gradient centrifugation. The above mixtures and their constituents were also centrifuged in 5-20 % linear sucrose gradients under non equilibrium conditions. Fig. 2 shows that the trypsin form, when centrifuged alone, yields a single band as expected from its already reported homogeneity (9). Two bands are observed with the detergent form due, respectively, to aggregation of this form in non dissociating medium and partial conversion into the trypsin form (S. Maroux, personal communication). The brush border lipids remain at the top of the gradient so that any interaction with aminopeptidase should easily be detected by this technique. The results obtained under the same conditions with the sonicated mixtures are reproduced in fig. 3. The trypsin form and the lipids

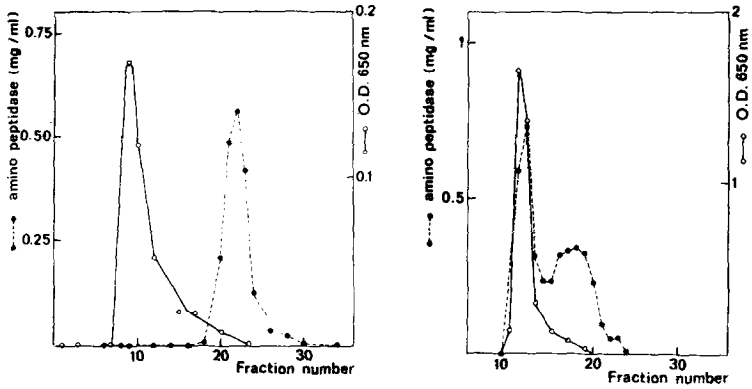

Figure 1. Filtration assays through Sepharose 4 B. Left, trypsin form of aminopeptidase (16.5 mg) sonicated with an equal weight of brush border lipids in 2 ml of the phosphate buffer. Right, detergent form (4.6 mg) and 5.0 mg of lipids. The column (1.5 cm x 120 cm) was equilibrated and eluted with the buffer. Fraction volumes, 2 ml. Flow rate, 6 ml/h. Aminopeptidase recovery, 75-80 %.

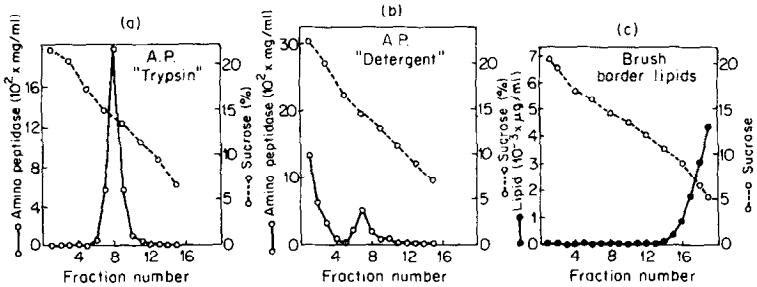

Figure 2. Non equilibrium gradient centrifugation of both forms of aminopeptidase (AP) and of brush border lipid liposomes. The linear 5-20 % sucrose gradient was made with an Isco gradient forming apparatus Model 570 in 4 ml plastic centrifuge tubes. Samples (50 µl) of sonicated lipids (9 mg) or protein (2 mg) in 2 ml of the phosphate buffer containing 0.03 % Emulphogen BC 720 were placed at the top and centrifuged for 5 h at 58,000 RPM in the SW 60 Ti rotor of a Spinco-Beckman centrifuge. Fraction volume, 0.250 ml. The sucrose concentration in the fractions was checked to $\pm$ 0.5 % with an Abbe refractometer.

are again observed to migrate independently (left), each retaining its original behaviour. By contrast, a substantial part of the lipid is carried to the bottom of the gradient by the detergent form (right). Similar results were obtained with egg lecithin liposomes.

In another series of assays, the samples were centrifuged to equilibrium for 16 h and the sucrose gradient was adjusted between 20 and 40 % for optimal separation. The reassociation was done by dialysis rather than by sonica-

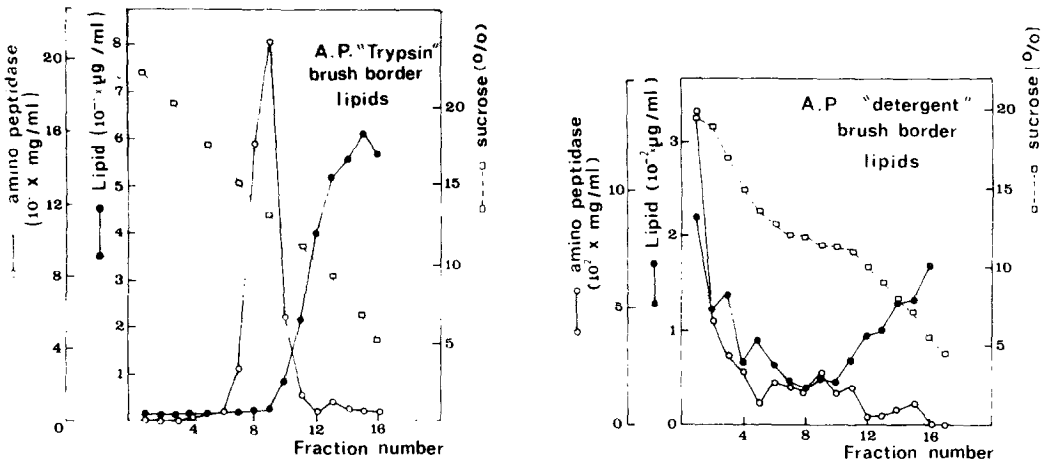

Figure 3. Gradient centrifugation under non equilibrium conditions of sonicated mixtures of brush border lipids and aminopeptidase. Same conditions as in fig. 2 except for the fact that lipids and enzyme are sonicated together in order to evaluate the incorporation extent.

tion. It is noteworthy that the results obtained under these modified conditions (fig. 4) fully confirm the possibility for the detergent form to interact with the lipid (right) and also the absence of any detectable interaction with the trypsin form (left). Peak 1 in the right diagram is probably related to the already mentioned contamination by the trypsin form, whereas the faster migrating peaks marked 2 are likely to correspond to various degrees of aggregation of the detergent form. Little lipid is associated with these latter peaks.

Film spreading. The spreading of membrane or reconstituted lipoprotein vesicles at an air-water interface can be used for quantitatively investigating the interactions of a protein with a lipid matrix. The vesicles are spread at zero surface pressure with the aid of an original technique recently worked out in the Laboratory (R. Verger and F. Pattus, submitted for publication) and the components remaining at the surface are determined after lateral aspiration of the film (J. Rietsch, R. Verger and P. Desnuelle, submitted for publication). When sonicated vesicles of egg lecithin and aminopeptidase were used in the assays, the percentage of recovery was 65 % for the lipid due to the spreading of only a part of the vesicles during their passage through the interface. It was 36 % for the detergent form of the enzyme and 12 % for the trypsin form. This latter value is of the same order as that observed with the

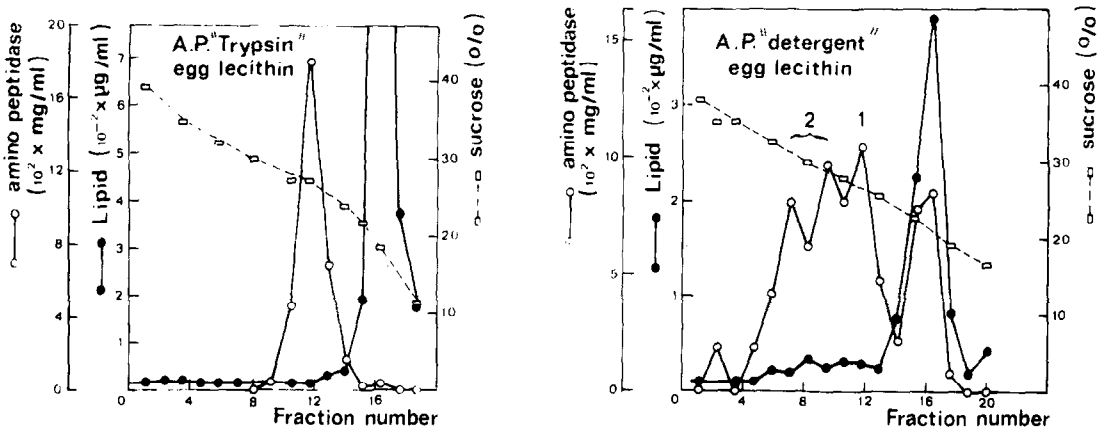

Figure 4. Equilibrium gradient centrifugation. Egg lecithin (40 mg) was dissolved in 2 ml of 5 % sodium deoxycholate. An aliquot (0.5 ml) was added to 16 ml of the phosphate buffer containing 2 mg of aminopeptidase and the mixtures were dialyzed for 4 days against 4 changes of the buffer. The material collected by a 2 h centrifugation at 250,000 g was suspended in 1 ml of the buffer from which 50 µl served for each assay.

protein alone. Therefore, the spreading technique confirms the lipid binding capacity of the detergent form as compared with the trypsin form.

Discussion

Two main conclusions emerge form the above data : (a) The detergent form of aminopeptidase containing the hydrophobic tail can incorporate into liposomes and form a stable lipid-protein film at the air-water interface. This incorporation is substantial but never complete because of the presence of sizeable amounts of the trypsin form in the preparations and also of the possibility clearly suggested by fig. 4 that aggregation may lower the affinity of the detergent form for lipids, perhaps by masking the tail in the interior of the aggregates. In other words, a high yield of incorporation would require that the tail dips into the lipid before inducing aggregation. (b) The interactions of the trypsin form of the enzyme with lipids appear to be weaker or inexistent. This finding confirms the role of the tail in the fixation of the enzyme to the lipid (1,2) but it is at variance with the statement made by Storelli et al. (12) that another brush border enzyme, the sucrase-isomaltase complex, can incorporate into a lipid bilayer after previous solubilization by papain. The origin of this discrepancy is not known. It will be of interest to see whether the reconstituted associations reported herein with the detergent form of the enzyme can serve as models for checking the active transport properties of the brush border membrane.

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