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IDENTIFICATION OF THE PROTEINS EXPOSED ON THE CYTOPLASMIC SURFACE OF THE PANCREATIC ZYMOGEN GRANULE *

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Lactoperoxidase-catalyzed ^{125}I-iodination was used to label pancreatic zymogen granules. Membrane proteins facing the cytoplasmic surface were specifically labeled. Two low molecular weight proteins of 17000 and 15000 were intensely labeled at 0°C. Another small 13 kDa protein was strongly iodinated at 25°C along with some others, including the 29 kDa subunit of the ATP diphosphohydrolase. The major glycoprotein of the granule membrane was not iodinated but the presence of an iodinated 80 kDa protein suggests that proteolytic fragments of the 92 kDa glycoprotein were accessible to iodination on the intact granule. These proteins localized on the cytoplasmic surface of the granule are believed to play a major role in the exocytotic phenomenon of the exocrine pancreas.

Upon hormonal stimulation, the pancreatic zymogen granule fuses specifically and solely with the apical membrane of the acinar cell to release its content into the extracellular space of the pancreatic ductular system. This phenomenon is referred to as exocytosis. First it implies the recognition of the two membranes, and secondly it involves their fusion to release the content of the granule [1]. Proteins on both the zymogen granule and the apical membrane are the most likely candidates for the recognition step of exocytosis [2]. In this report we identified the membrane proteins exposed on the cytoplasmic surface of the zymogen granule. Their availability to enzymatic iodination on intact granules was the criteria by which their orientation was assessed. This is the first time that iodination of intact pancreatic zymogen granules is reported.

Zymogen granules were prepared from pig pancreas according to already published methods [3,4]. They were radioiodinated using the solid phase lactoperoxidase-glucose oxidase system (Enzymobeads, Bio-Rad). Freshly prepared granules (1.94 mg protein in 50 μl) were added to a labeling buffer made of 55 mM Pipes, pH 6.0, 0.6 M sucrose, 50 μl bead suspension, 0.15% β-D-glucose and 40 μCi Na^{125}I. The reaction was performed for 20 min at the indicated temperature and stopped by the addition of 10 μl 200 mM NaN_3. The granules were lysed in order to get their membrane by addition of 5 ml of Hepes 25 mM, pH 8.0, KCl 0.2 M, 0.1 mM PMSF, containing 0.2% NaI. Tubes were kept on ice for 5 min before low speed centrifugation to remove the beads, followed by high speed centrifugation (130000 × *g*) to pellet the membranes. The pellets were solubilized in SDS and run on polyacrylamide gel electrophoresis according to Laemmli [5]. Super-

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Pipes, 1,4-piperazinediethanesulphonic acid; PMSF, phenylmethylsulphonyl fluoride.

natant fractions representing the content of the granule, were also run on gels. Gels were stained with Coomassie blue and autoradiographed using an intensifying screen.

Membranes were characterized by the presence of a 92 kDa protein well-known to be the major protein of this membrane [4,6]. Fig. 1 shows the iodinated proteins. The content of the granule (lane C) was not labeled, indicating that no lysed granules were produced or present during the radioiodination procedure. Only cytoplasmic oriented membrane proteins were therefore responsible for the iodination pattern observed in Fig. 1. A triplet of low molecular weight proteins were preferentially iodinated (Fig. 1, Table I). A band of 15 kDa showed the strongest labeling at 0°C. This radioiodinated band was localized between two Coomassie blue bands of 17 and 14 kDa. It did not correspond to any definite stained band on the gel, thus implying that this protein had a very high labeling. The second and third most intensely labeled bands corresponded to a 17 and a 13 kDa protein, respectively. In contrast these latter bands could be observed on the stained gel. No labeling was observed at the 92 kDa level over the zymogen granule membrane major glycoprotein. However, a similar broad looking band was labeled, corresponding to 80 kDa. It accounted for 6% of the total incorporated ^{125}I. Like the labeled band of 15 kDa, this 80 kDa band was not visible on the stained gel. Such a band of 80 kDa has bever been detected in pig zymogen granule membranes [4]. In order to explain the apparition of this band, one could raise the possibility of a proteolytic degradation of the 92 kDa glycoprotein. Therefore complementary fragments of about 12 kDa should be formed. This was actually the size of the most heavility labeled polypeptides observed. This hypothesis, however, implies that the intact 92 kDa protein which is still present in the membrane preparation, did not have the same accessibility to iodination as did the proteolytic fragments of the protein. Proteolysis would then render accessible to iodination on intact granules, the fragments of the 92 kDa protein. In support of this hypothesis, antibodies against the 92 kDa protein, raised using the excised band from gels, showed reactivity against a lower molecular weight band of approx. 75 000 (LeBel, D., unpublished data). Purified anti-75 000 and purified anti-92 000 antibodies both reacted with the two bands on immunoblots.

TABLE I

RADIOIODINATION OF MEMBRANE PROTEINS ON INTACT ZYMOGEN GRANULES

The radioiodinated bands were cut off the dried gel shown in Fig. 1 and counted for ^{125}I radioactivity. Data represent the % of the total radioactivity in the lane. Incubation conditions are described in the text.

Protein M_r	Percent incorporated ^{125}I	
	0°C	25°C
80 000	6.3	6.4
17 000	12.6	8.5
15 000	12.5	7.0
13 000	11.0	12.4
Others	57.6	65.7

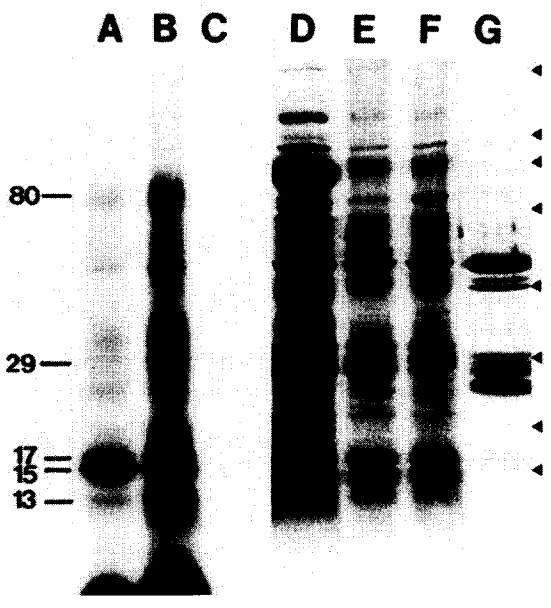

Fig. 1. Lactoperoxidase-catalyzed ^{125}I-iodination of membrane proteins on intact zymogen granules at 0°C (A, E) and 25°C (B, C, F, G). Proteins were run on a 6–15% polyacrylamide gradient gel according to Laemmli. Iodinated granules were lysed and the membranes pelleted at 130 000 × g (A, B, E, F). Lanes C and G show the supernatant. Lanes A, B, C are the autoradiographs of the Coomassie-blue stained gel (E, F, G). Lane D, 150 μg of purified zymogen granule membrane proteins according to Ref. 4. Arrowheads correspond to molecular weight standards of 94 000, 67 000, 43 000, 30 000, 20 100 and 14 400.

This argues in favor of a proteolytic degradation of the 92 kDa glycoprotein in the purified zymogen granule membrane. It would be appropriate here to point out that the proteolytic degradation of membrane proteins has been implied in membrane-fusion reaction, including secretion by exocytosis [7]. In the fusion of rat erythrocyte for example, loss of band 3 (M_r 95 000) was accompanied by the production of a diffuse band moving slightly faster (M_r approx. 80 000) [8]. At 25°C some other bands in the region of 30 kDa were also labeled. A broad band was iodinated at 29 kDa. This molecular mass corresponded to one of the major proteins observed in the purified fraction of the ATP diphosphohydrolase [9]. The two other subunits were shown to be intrinsic glycoproteins having molecular weights of 58 000 and 46 000 [4,9]. None of the former proteins were significantly labeled in our conditions. Since no glycoprotein has so far been shown to have its oligosaccharide chain located on the cytoplasmic side of a membrane [10], these results suggest that these two subunits of the ATP diphosphohydrolase are intrinsic membrane proteins with their oligosaccharides located on the intragranular surface of the membrane. This location is consistent with the intragranular localization of the P_i produced by the catalyzed reaction [11] supporting that the catalytic site of the enzyme would be on the internal surface of the granule. Should the ATP diphosphohydrolase be made up of subunits, there is no direct evidence yet that one of these would be transmembrane. The present study shows however that the 29 kDa major subunit of the ATP diphosphohydrolase is located on the cytoplasmic surface of the granule. This subunit could then act as the cytoplasmic port of the ATP diphosphohydrolase if this 29 kDa protein was shown to be a functional subunit of the holoenzyme. Such a configuration of the subunits would argue for a transmembrane role of the ATP diphosphohydrolase in the zymogen granule membrane.

It can be concluded from this study that a doublet of low molecular weight proteins constitute the most prominent proteins on the surface of intact zymogen granules. The major protein of the membrane was surprisingly not labeled, but the results suggest that a portion of the molecule sensitive to proteolysis, could be accessible to iodination on the intact granule after proteolytic degradation. One subunit of the ATP diphosphohydrolase was also iodinated. We believe that these proteins could play an important role in the processes of recognition and fusion between the zymogen granule membrane with the apical membrane of the acinar cell during exocytosis.

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References

1 Jamieson, J.D. (1981) in Methods in Cell Biology, Vol. 23, (Hand, A.R. and Oliver, C., eds.), pp. 547–558, Academic Press, New York
2 Ekerdt, R., Dahl, G. and Gratzl, M. (1981) Biochim. Biophys. Acta 646, 10–22
3 Pâquet, M.R., St-Jean, P., Roberge, M. and Beaudoin, A.R. (1982) Eur. J. Cell Biol. 28, 20–26
4 LeBel, D. and Beattie, M. (1984) Biochim. Biophys. Acta 769, 611–621
5 Laemmli, U.K. (1970) Nature (Lond.) 227, 680–685
6 MacDonald, R.J. and Ronzio, R.A. (1972) Biochem. Biophys. Res. Commun. 49, 377–382
7 Ahkong, Q.F., Blow, A.M.J., Botham, G.M., Launder, J.M., Quirk, S.J. and Lucy, J.A. (1978) FEBS Lett. 95, 147–152
8 Ahkong, Q.F., Botham, G.M., Woodward, A.W. and Lucy, J.A. (1980) Biochem. J. 192, 829–836
9 Laliberté, J.F., St-Jean, P. and Beaudoin, A.R., (1982) J. Biol. Chem. 257, 3869–3874
10 Sabatini, D.D., Kreibich, G., Morimoto, T. and Adesnik, M. (1972) J. Cell Biol. 92, 1–22
11 Beadudoin, A.R., Laliberté, J.F., LeBel, D., Lord, A., Grondin, G., Phaneuf, S. and St-Jean, P. (1980) Biology of Normal and Cancerous Exocrine Pancreatic Cells, pp. 273–280, Elsevier/North-Holland, Amsterdam