COMPARATIVE ANALYSIS OF ZYMOGEN GRANULE MEMBRANE POLYPEPTIDES

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SUMMARY: Polypeptides of zymogen granule membranes from rat, beef, dog, pig, and rabbit pancreas were analyzed by polyacrylamide gel electrophoresis in 1% sodium dodecylsulfate. Granule membranes were characterized by a single major polypeptide species detected by the periodic acid-Schiff procedure. The relative mobilities of this component were similar for these mammals. The component was distinct from major polypeptides of mitochondrial or microsomal membranes. The zymogen granule membrane may, therefore, represent a highly specialized intracellular membrane.

Membrane-bound granules containing secretory products are particularly prominent in tissues such as the exocrine pancreas (1). Zymogen granules have been isolated from dog (2), beef (3), and guinea pig (4) and their general chemical composition has been defined. However, the polypeptides of the granule membrane have not been studied. Besides sequestering secretory proteins, the granule membrane may function in the release of these products during secretion (5). Presumably the zymogen granule membrane serves the same specific functions in the pancreas of different mammalian species. From these considerations, it may be proposed that the membrane contains components associated with specialized functions, and that such components may be a general feature of this membrane class.

To study the polypeptide compositions of zymogen granule membranes, we isolated granules from the pancreas of mammalian species representing three different orders: dog; rabbit and rat; and beef, sheep, and pig. The polypeptides of washed membranes from these zymogen granule preparations were fractionated by polyacrylamide gel electrophoresis in SDS.[1] Comparison of the membrane profiles of gels stained for protein and carbohydrate suggests that

[1]Abbreviations used are: SDS, sodium dodecylsulfate; PAS, periodic acid-Schiff.

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zymogen granule membranes from these sources are characterized by a single glycosylated polypeptide species. The granule membrane generally contains fewer polypeptides than either microsomal or mitochondrial membranes and thus may be a less complex membrane.

MATERIALS AND METHODS

Male rabbits (15 lb), were obtained from Center of Laboratory Animal Research, Michigan State University, and male Sprague Dawley rats (250 - 300 g); from Spartan Research Animals, Haslett, Michigan. Fresh dog pancreas was obtained through the courtesy of Dr. M. D. Baillee. Fresh beef, pig, and sheep pancreas were obtained from local slaughter houses. Tissue fractionation was performed at -4° to 0°. The tissues were homogenized in 0.3 M sucrose containing 0.25 mg/ml soybean trypsin inhibitor (Sigma Chemical Co.) by either of two procedures. Up to 20 g wet weight of tissue were homogenized by a procedure similar to that of Jamieson and Palade (4). Larger batches of tissue were homogenized twice for 2 min with a polytron homogenizer (Model PT10, Brinkman Instruments, Westburg, N.Y.) driven at 3,800 rpm. Zymogen granules were prepared from beef pancreas homogenates with minor modifications of the method of Greene et al. (3). Filtered homogenates of other tissues were centrifuged at 500 xg for 10 min to sediment debris. Zymogen granules were next sedimented by centrifugation at 1,730 xg for 30 minutes. Contaminating mitochondria were removed from the top of the white zymogen granule pellet (4). Mitochondria were obtained by centrifuging the 1,730 xg supernatant fraction for 15 min at 8,500 xg. Microsomes were sedimented by centrifuging the 8,500 xg supernatant fraction for 1 hr at 93,700 xg. These fractions were further purified by repeated suspension in 0.3 M sucrose and centrifugation.

Zymogen granules were lysed in 0.2 M $NaHCO_3$, pH 8.2, containing 0.25 mg/ml soybean trypsin inhibitor, and the granule ghosts were separated from mitochondria using a discontinuous gradient similar to the procedure of Meldolesi et al. (6). To remove secretory proteins, the granule membranes were next extracte with 0.25 M NaBr and collected by centrifugation for 45 min at 195,000 xg. Washed

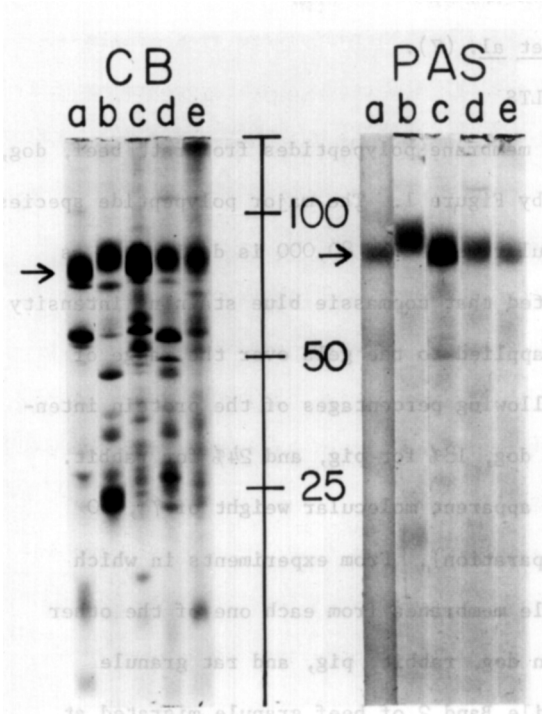

Fig. 1.

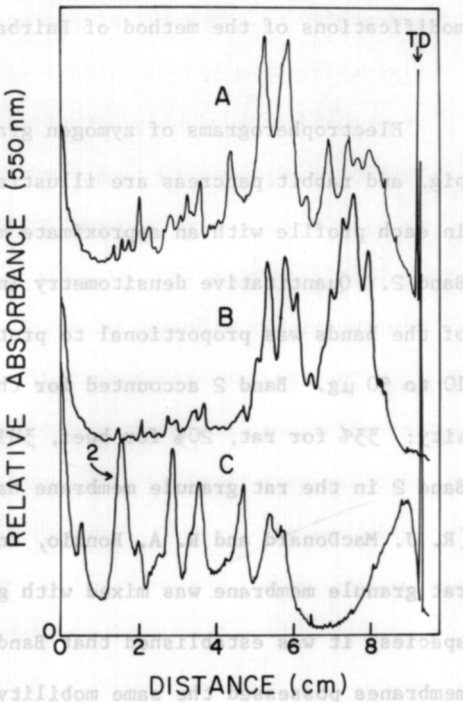

Fig. 2.

Figure 1. Electrophoretic comparison of zymogen granule membrane polypeptides from different mammalian species. Granule membranes, prepared as described in Materials and Methods were dissolved in 1% SDS, 10 mM Tris-Cl, pH 8.0, 5 mM EDTA, and 2% 2-mercaptoethanol, by heating at 60° for 30 min. Between 20 and 40 µg of membrane protein from (a) rat, (b) beef, (c) dog, (d) pig, and (e) rabbit zymogen granules were applied to 9% acrylamide gels containing 1% SDS (7). After 4 hr at 10 volts/cm, electropherograms were developed with either coomassie blue (CB) by the procedure of Johnson et al. (8), or by the PAS procedure (PAS) using a modification of the procedure of Fairbanks et al. (7). Gels were photographed with a yellow filter (K2) using Kodak Extapan. Band 2 is indicated by arrows. The ordinant indicates approximate molecular weights ($X10^{-3}$) based upon mobilities of ribonuclease (13,400); bovine serum albumin (68,000), and subunits of P. putida RNA polymerase [β,β', 165,000; σ, 98,000; α, 44,000 (8)], relative to the tracking dye, pyronin B.

Figure 2. Electrophoretic comparison of mitochondrial, microsomal, and zymogen granule membrane polypeptides from pig pancreas. Membrane fractions were prepared as described in Materials and Methods. Electrophoresis was performed and the gels were stained with coomassie blue as described in Figure 1. The gels were scanned at 550 nm with a Gilford Spectrophotometer using a linear transport. Tracking dye (TD) indicates the distance of migration, and 2 designates Band 2. A--mitochondrial membrane, B--microsomal membrane, C--zymogen granule membrane.

membranes were prepared from mitochondria and microsomes by sequential extractions with $NaHCO_3$ and NaBr. Polyacrylamide gel electrophoresis was performed with minor

modifications of the method of Fairbanks et al. (7).

RESULTS

Electropherograms of zymogen granule membrane polypeptides from rat, beef, dog, pig, and rabbit pancreas are illustrated by Figure 1. The major polypeptide species in each profile with an approximate molecular weight of 80,000 is designated as Band 2. Quantitative densitometry indicated that coomassie blue staining intensity of the bands was proportional to protein applied to the gels over the range of 10 to 50 μg. Band 2 accounted for the following percentages of the protein intensity: 33% for rat, 20% for beef, 38% for dog, 18% for pig, and 24% for rabbit. Band 2 in the rat granule membrane has an apparent molecular weight of 74,000 (R. J. MacDonald and R. A. Ronzio, in preparation). From experiments in which rat granule membrane was mixed with granule membranes from each one of the other species, it was established that Band 2 in dog, rabbit, pig, and rat granule membranes possessed the same mobility, while Band 2 of beef granule migrated at a slower rate, corresponding to a molecular weight of 85,000. The yield of sheep zymogen granules was uniformly low, approximately 10% of that obtained for beef (3). However, preliminary results suggest that the sheep zymogen granule membrane resembles beef zymogen granules, rather than rat.

In gels stained for carbohydrate, a major band was detected which corresponded to Band 2 of the coomassie blue profiles (Figure 1). A second PAS-positive band was sometimes observed near the tracking dye. This region was also stained by coomassie blue (see also Figure 2,C). There was considerable variation in the degree of staining by both procedures; this material is probably glycolipid or lipid (9).

Comparison of scans of electropherograms of washed membranes of mitochondria, microsomes, and zymogen granules from pig pancreas (Figure 2) suggests that major mitochondrial and microsomal membrane polypeptides were absent from the granule membrane. Similar results have been obtained from the granule preparations from other animals. Figure 2 illustrates other important features of the zymogen granule membrane. For all species examined, the number of dif-

ferent polypeptide classes in profiles of the zymogen granule membranes was usually less than one-half the number in mitochondrial or microsomal membranes. Polypeptide bands in profiles of the latter membranes with mobilities similar to Band 2 accounted for less than 5% of the coomassie blue stain intensity of either microsomal or mitochondrial membranes.

Several observations suggested that membrane degradation was minimized. The protein and phospholipid content of subcellular fractions from adult rat pancreas were not altered by storage with soybean trypsin inhibitor up to 18 hr at 0°, (R. A. Ronzio, in preparation), nor were there any detectable alterations in electropherograms of membrane polypeptides under these conditions. Less than 0.1% of the amylase activity in rat granules remained in purified granule membranes. (R. J. MacDonald and R. A. Ronzio, in preparation). Thus, secretory proteins were effectively removed from granule membranes.

DISCUSSION

This study revealed a striking similarity among zymogen granule membranes from five mammalian species. Each granule membrane contained a major, PAS-positive polypeptide species with a molecular weight of approximately 80,000. These experiments do not distinguish whether this species is an identical component in each membrane. Since the degree of staining by coomassie blue may be reduced by the presence of oligosaccharide moieties (7), and the electrophoretic mobility of glycoproteins in SDS gels may not be proportional to their molecular weights (10), the abundance and mobility of this component are tentative.

Comparison of the granule membrane profiles with those of mitochondria and microsomal membranes, representing the most likely contaminants (6), suggests that the granule membranes were relatively pure. Furthermore, Band 2 was not present in more than trace amounts in microsomes and mitochondria. Hence it is likely that Band 2 represents a glycosylated polypeptide species which is a distinctive feature of the zymogen granule membrane. This species may be involved in the mechanism of secretion; for example, it may specify the interaction with the plasma membrane, resulting in fusion and the release of granule contents (5).

The properties and possible functions of this component are currently being studied in our laboratory. It is of interest that the membrane of zymogen granules is comparatively simple, in terms of the number of polypeptide classes present. Chromaffin granule from the adrenal medulla (11) and possibly salivary gland granules (12) also contain a small number of polypeptide species. Thus storage granules may represent a highly specialized class of membranes.

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