COMPOSITION OF CELLULAR MEMBRANES IN THE PANCREAS OF THE GUINEA PIG

III. Enzymatic Activities

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ABSTRACT

A comparative study of the enzymic activities of membrane fractions derived from guinea pig pancreatic homogenates has yielded the following results: Rough microsomal membranes (derived from the rough ER) have the reductase activities of the two microsomal electron transport systems but lack enzyme activities of Golgi-type (TPPase) and plasmalemmal-type (5′-nucleotidase, β-leucyl naphthylamidase, Mg-ATPase). Smooth microsomal membranes (derived primarily from the Golgi complex), zymogen granule membranes, and plasmalemmal fractions possess overlapping enzyme activities of plasmalemmal type, in different relative concentrations for each fraction. In addition, the smooth microsomal membranes exhibit TPPase and ADPase activity and share with rough microsomes the reductase activities of the two electron transport chains. Taken together with recent data on the lipid composition of the same fractions (2), these results indicate that the membranes of the pancreatic exocrine cell are chemically and functionally distinct, and hence do not mix with one another during the transport of secretory products.

INTRODUCTION

In the preceding papers of this series we have shown that there are considerable differences in lipid composition among membrane preparations isolated from guinea pig pancreas homogenates (1, 2). Rough microsomal membranes have a low content of sphingomyelin and cholesterol and a high content of lecithin, whereas in smooth microsomal membranes (Golgi membranes), zymogen granules membranes, and plasmalemmal fractions the situation is reversed: the concentration of cholesterol and sphingomyelin varies from one type of membrane to another but it is in general three- to fivefold higher than in rough microsomal membranes.

Since these results indicate that pancreatic cellular membranes belong to at least two distinct classes, the investigation was extended to enzymatic activities to find out if, and to what extent, they reveal further differentiation among the membranes under study.

MATERIALS AND METHODS

General

Details concerning animals used and procedures applied to prepare cell fractions and isolate membrane subfractions are given in reference 1. Lipid extraction, lipid phosphorus determinations, and protein measurements were carried out as in reference 2.

The following enzyme activities were assayed according to published procedures, modified as indicated: cytochrome *c* oxidase, Cooperstein and

Lazarow (3); rotenone-insensitive nicotinamide adenine dinucleotide (NADH)[1]-cytochrome *c* reductase and nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome *c* reductase, Sottocasa et al. (4); adenosine diphosphatase (ADPase) and Mg^{2+} and Na^+–K^+ adenosine triphosphatases (ATPases) (using Tris ATP as substrate), Emmelot et al. (5); acid *p*-nitrophenylphosphatase, Ostrowski and Tsugita (6); alkaline *p*-nitrophenylphosphatase, Emmelot and Bos (7); phosphodiesterase, Brightwell and Tappel (8); 5′-nucleotidase, Widnell and Unkeless (9); β-leucyl naphthyl-amidase, Goldbarg and Rutemberg (10); glucose-6-phosphatase, Swanson (11); NAD-glycohydrolase, Kaplan (12); NAD-pyrophosphatase, Jacobson and Kaplan (13); arylsulfatase *c*, Roy (14); and monoamine oxidase, Weissbach et al. (15). Thiamine pyrophosphase (TPPase) was assayed by a modification of the method of Novikoff and Heuss (16); the assay contained in 0.3 ml: 0.37 mM TPP, 30 mM Tris buffer, pH 7.4, 5 mM $MgCl_2$, and enzyme. The reaction was stopped after 45 min of incubation at 37°C by the addition of 0.1 ml of 40% trichloroacetic acid (TCA).

Inorganic phosphate release by all phosphatases was determined as described by Ames (17).

All enzymes were assayed in duplicate within 2 hr after the isolation of the cell fractions.

Materials

Enzymes and reagent grade chemicals were obtained from the following sources: Alcohol dehydrogenase: Worthington Biochemical Corp., Freehold, N. J.; Tris ATP, adenosine monophosphate (AMP), *p*-nitrophenylphosphate, bis-*p*-nitrophenylphosphate, NAD^+, NADH, NADPH, rotenone, horse heart cytochrome c (grade III): Sigma Chemical Co., St. Louis, Mo.; glucose-6-phosphate, ADP, TPP, *N*(1-naphthyl)-ethylenediamine HCl, diisopropylfluorophosphate (DFP): Mann Research Laboratories, Inc., New York; diethyl-*p*-nitrophenylphosphate (E 600): K & K Laboratories Inc., Plainview, N. Y.; L-leucyl-β-naphthylamide: Schwarz Bio Research Inc., Orangeburg, N. Y.

[1] *The following abbreviations were used in this article:* ADP, AMP and ATP, adenosine di-, mono-, and triphosphate; DFP, diisopropylfluorophosphate; E 600, diethyl-*p*-nitrophenylphosphate; ER, endoplasmic reticulum; NAD^+(H) and $NADP^+$(H), nicotinamide adenine dinucleotide and dinucleotide phosphate, oxidized and reduced (H) forms; PMS, postmicrosomal supernate; SA_{pl}, specific activity normalized to phospholipid; SA_{pr}, specific activity normalized to protein; TCA, trichloroacetic acid; TPP, thiamine pyrophosphate; Tris, Tris (hydroxymethyl) aminomethane.

RESULTS

Effects of Aging on Enzyme Activity and Distribution

Since pancreatic homogenates contain active digestive enzymes (e.g., amylase and lipase) and activatable phospholipolytic and proteolytic zymogens, and since some pancreatic enzymes are active at 0°C (e.g., lipase and trypsin) we checked, to begin with, whether membrane enzyme activities were lost during the relatively long period needed to isolate cell fractions. To this intent, the effect of a 24 hr aging at 4°C was tested on two different preparations, namely, whole homogenates and mitochondrial supernates. As shown in Fig. 1, this treatment causes severe inactivation of NADH-cytochrome *c* reductase and partial inactivation of Mg^{2+}-dependent ATPase in both preparations. All the other activities tested remained practically unchanged, or increased by ∾40% in the case of 5′-nucleotidase. It appears, therefore, that aging at 4°C in the presence of pancreatic enzymes and zymogens has little effect on membrane associated enzymatic activities, except for NADH-cytochrome *c* reductase. The latter is known to be sensitive to storage and dilution in liver cell fractions (18); hence, its inactivation in our experiments may be due to unspecified factors involved in aging rather than to degradation by pancreatic digestive enzymes.

We next checked the effects of aging on enzyme distribution between microsomes and postmicrosomal supernate (PMS) to find out whether solubilization of microsomal membrane enzymes occurs during cell fractionation. To this intent, mitochondrial supernates were fractionated into microsomes and PMS after 0 hr,[2] 7 hr, and 24 hr of aging at 4°C.

Fig. 2 shows that at 0 hr ∾74% of the 5′-nucleotidase and ∾64% of the NADPH-cytochrome *c* reductase activity remain in the final supernate[3] presumably as soluble proteins or protein complexes. This distribution is quite different

[2] 0 hr is the time when the fractionation of a postmitochondrial supernate into microsomes and PMS is first obtained. It comes ∾1.5–2.0 hr after tissue homogenization.

[3] 5′-AMP hydrolysis cannot be ascribed to nonspecific alkaline phosphatases present in PMS since, under identical assay conditions, PMS hydrolyses β-glycerophosphate seven to 10 times slower than 5′-AMP.

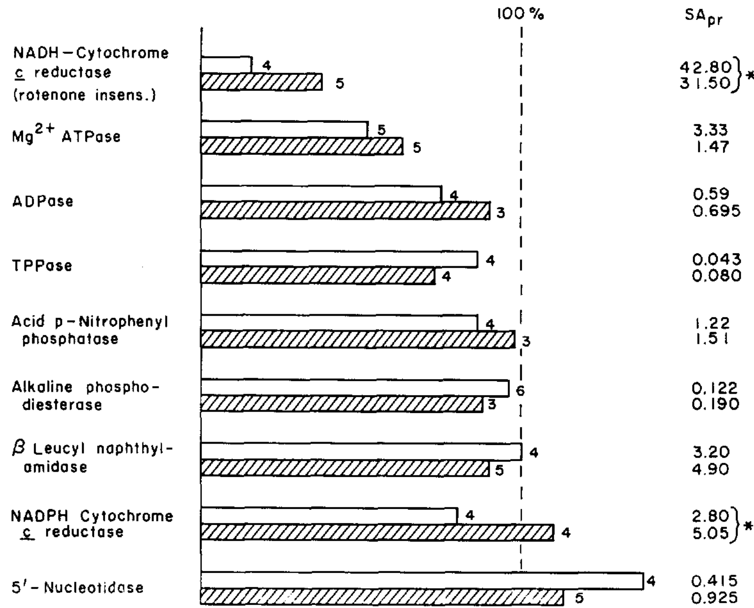

FIGURE 1 Effects of aging on enzyme activities in pancreatic homogenates and postmitochondrial supernates. Enzyme activities were assayed in tissue homogenates and in postmitochondrial supernates derived therefrom immediately upon the isolation of the latter (time 0) and after 24 hr of aging at 0°C. The bars give residual activities as per cent of activities at time 0. The figures on the right are SA_{pr}'s at time 0. Solid bars, total homogenate; striped bars, postmitochondrial supernate. *, mμmoles of cytochrome *c* reduced/min at 25°C per mg protein. For all phosphatases the figures are: μmoles of substrate hydrolyzed/60 min at 37°C per mg protein.

from that found in liver cell fractions in which NADPH-cytochrome *c* reductase is strictly a microsomal membrane enzyme (4, 19, 20), whereas 5'-nucleotidase is associated with membranes in both the plasmalemmal and microsomal fractions (5, 9, 20–23).

Aging over 7 hr increases both the total activity of the two enzymes and, in the case of NADPH-cytochrome *c* reductase, the relative concentration in PMS, suggesting partial solubilization, but aging of pancreatic microsomes over the same period in 0.3 M sucrose or liver PMS has no effect on either activity or distribution of the enzymes considered. Some of the soluble or solubilized enzymes, e.g. 5'-nucleotidase, seem to be less stable than membrane-bound forms. Another enzyme recovered in large proportion in the final supernate is TPPase but the effect of aging on its activity and distribution was not studied.

β-leucyl naphthylamidase activity probably is the result of several different peptidases (24). In the liver, one of them is associated with the plasmalemma (22, 25), whereas others are found in the lysosomes (26) and final supernate (24). In the pancreas, ~80% of the β-leucyl naphthylamidase of the mitochondrial supernate is recovered in the final supernate at 0 hr and the distribution is not affected by aging over 7 hr. NADH-cytochrome *c* reductase and Mg^{2+}-ATPase are recovered mostly in the microsomal fraction; aging causes progressive inactivation of both enzymes, and only limited solubilization of the reductase.

So far, our results indicate that there is no extensive inactivation of membrane enzymes—contrariwise, some of them are activated by 1.5- to 2.0-fold—and no striking redistribution of enzyme activities over the interval needed for cell fractionation, if the situation found at 0 hr is taken as a reference base. But the validity of this base can be questioned for the following reason: 0 hr comes ~2 hr after tissue homogenization, and during this interval lipase produces sizable amounts of lysophosphatides and free fatty acids (2) which may solubilize certain membrane components; hence, the discrepancy noted in 5'-nucleotidase and NADPH-cytochrome *c* reductase distribution between hepatic and pancreatic cell fractions may be the result of extensive enzyme solubilization. Attempts to check this assumption on pancreatic fractions prepared in the presence of lipase in-

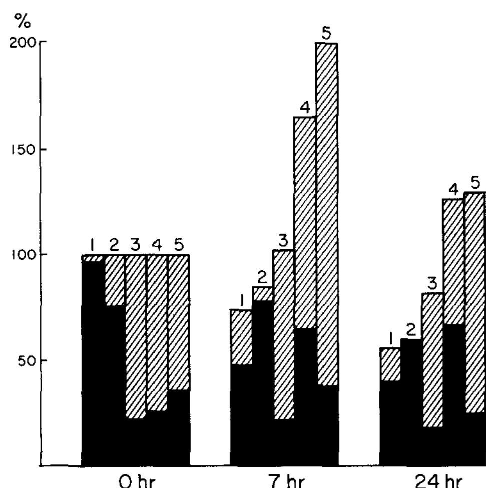

Figure 2 Effects of aging upon enzyme activity and distribution in cell fractions derived from pancreatic postmitochondrial supernates. Postmitochondrial supernates, prepared from homogenates of guinea pig pancreas, were fractionated into microsomes and postmicrosomal supernates after 0 hr, 7 hr, or 24 hr of aging at 0°C. The ensuing fractions were assayed for enzyme activities as given under Methods. The results, which are the averages of two highly consistent experiments, are expressed as percentages of total activities in postmitochondrial supernates at 0 hr. Solid bars, microsomes; striped bars, PMS. Numbers denote the following enzyme activities: 1, NADH-cytochrome *c* reductase; 2, Mg^{2+}-ATPase; 3, β-leucyl naphthylamidase; 4, 5′-nucleotidase; 5, NADPH-cytochrome *c* reductase.

hibitor E 600 (27) were unsuccessful, since the compound proved to be an equally strong inhibitor of 5′-nucleotidase, NADPH-cytochrome *c* reductase, and β-leucyl naphthylamidase. Since NADPH-cytochrome *c* reductase is known to be solubilized from liver microsomes by trypsin digestion (19), enzyme distribution was also studied in cell fractions derived from pancreatic homogenates prepared in the presence of 0.8 mM DFP, but this trypsin inhibitor did not change the distribution pattern for NADPH-cytochrome *c* reductase and 5′-nucleotidase.

The assumption was also checked in two mixing experiments in which isolated guinea pig liver microsomes were resuspended in either hepatic or pancreatic PMS (see reference 2 for experimental details). After 2 hr aging at 4°C, microsomes and PMS's were separated and assayed for NADH- and NADPH- cytochrome *c* reductase. The results showed that incubation in pancreatic PMS caused neither inactivation nor solubilization of the reductases. 5′-nucleotidase was not tested since its activity is very low in guinea pig hepatic microsomes.

Normalization of the Activity of Membrane Enzymes

Since some of our fractions were contaminated with nonmembrane proteins, which come from content and attached ribosomes in the case of the microsomes and from cytoplasmic filaments and basement membrane fragments in the case of the plasmalemmal fraction (1), we decided to normalize specific enzymatic activities not only to protein (SA_{pr}) as usually done in the literature, but also to phospholipid (SA_{pl}), the other major membrane component, which should be less sensitive to the contaminants mentioned. Although the latter procedure gives a better basis for comparison it is not fully satisfactory, since phospholipid concentration remains unknown in the plasma membrane (1) and appears to be considerably lower in rough microsome membranes than in smooth microsome- and zymogen granule membranes (1). The SA_{pl} of the smooth microsome and zymogen granule fractions could be roughly normalized to a phospholipid (PLP) concentration equivalent to that in rough microsome membranes by multiplying the corresponding figures by 1.6. When activities are normalized to phospholipid, data obtained in assaying microsomal fractions appear to apply equally well to microsomal membranes, since we have shown that after NaCl-$NaHCO_3$ extraction (which releases microsomal contents [1]) microsomal phospholipids are quantitatively recovered in these membranes and since, in preliminary experiments, we have found that the same applies to NADH- and NADPH-cytochrome *c* reductase, Mg^{2+}-ATPase, TPPase, 5′-nucleotidase, alkaline phosphodiesterase, and β-leucyl naphthylamidase activities. Only acid *p*-nitrophenylphosphatase and ADPase activities are incompletely (50–60%) recovered in membranes after the alkaline extraction of microsomes. In the rest of the text we will primarily rely on SA_{pl} to describe the enzymatic activities of pancreatic cell fractions.

Enzyme Activities in Pancreatic Cell Fractions

We could not detect in any of our pancreatic fractions glucose-6-phosphatase, NAD-glycohydro-

TABLE I

Specific Activities for Cytochrome c Oxidase, Rotenone-Insensitive NADH-Cytochrome c Reductase, and NADPH-Cytochrome c Reductase in Subcellular Fractions Isolated from Guinea Pig Pancreas

Enzymes	Rough microsomes		Smooth microsomes		Zymogen granule membranes		Plasma membranes	
	SA_{pr}	SA_{pl}	SA_{pr}	SA_{pl}	SA_{pr}	SA_{pl}	SA_{pr}	SA_{pl}
Cytochrome *c* oxidase*	*0–1* (5)	*0–9.8* (5)	*0–1.1* (5)	*0–4.4* (5)	42.6 (10) *26.5–57.3*	97.0 (10) *53.0–136.0*	19.6 (8) *9.0–37.4*	91.0 (8) *44.5–170.0*
NADH-cytochrome *c* reductase (rotenone insensitive)*	17.9 (8) *10.6–29.4*	181.0 (8) *100.0–327.0*	32.0 (8) *18.8–40.1*	130.0 (8) *82.0–200.0*	15.0 (4) *10.6–20.0*	32.8 (4) *21.1–51.4*	9.3 (6) *4.9–13.9*	47.2 (6) *22.0–65.0*
NADPH-cytochrome *c* reductase*	2.7 (7) *1.6–3.4*	27.7 (7) *16.0–38.0*	4.7 (7) *4.1–6.1*	17.8 (7) *15.5–34.0*	0 (4)	0 (4)	0.7 (4) *0.6–0.8*	3.5 (4) *2.2–4.2*

* mμmoles of cytochrome *c* oxidized or reduced at 25°C/min per mg protein (SA_{pr}) or per mg phospholipid (SA_{pl}).
Values given are averages. Number of experiments is shown in parentheses. Ranges are given in italics.

TABLE II

Specific Activities for Plasma Membrane-Type Enzymes in Subcellular Fractions Isolated from Guinea Pig Pancreas

Enzymes	Rough microsomes		Smooth microsomes		Zymogen granule membranes		Plasma membranes	
	SA_{pr}	SA_{pl}	SA_{pr}	SA_{pl}	SA_{pr}	SA_{pl}	SA_{pr}	SA_{pl}
5'-nucleotidase*	0 (9)	0 (9)	3.3 (9) *1.7–3.9*	13.2 (9) *6.0–18.9*	1.9 (7) *1.2–3.1*	4.2 (7) *2.2–8.1*	3.9 (8) *2.5–4.9*	18.3 (8) *12.0–27.3*
β-leucyl naphthylamidase‡	*0–0.2* (5).	*0–1.8* (5)	10.2 (5) *7.6–14.2*	41.2 (5) *28.0–50.5*	3.1 (3) *3.0–3.3*	6.7 (3) *6.5–6.8*	8.6 (4) *7.9–9.1*	44.3 (4) *35.6–49.2*
Mg^{++}-ATPase*	0 (7)	0 (7)	2.5 (6) *1.9–4.0*	11.0 (6) *6.6–18.0*	17.6 (6) *11.2–22.3*	38.1 (6) *20.0–57.0*	9.2 (6) *8.3–11.1*	46.0 (6) *39.0–58.3*

* μmoles of P_i released at 37°C/60 min per mg protein (SA_{pr}) or per mg phospholipid (SA_{pl}).
‡ μmoles of naphtylamine released at 37°C/60 min per mg protein (SA_{pr}) or per mg phospholipid (SA_{pl}).
Values given are averages. Number of experiments is shown in parentheses. Ranges are given in italics.

lase, NAD-pyrophosphatase, Na^+-K^+-ATPase or arylsulfatase *c*, i.e., activities which in liver and other tissues are considered characteristic enzymes of the microsomal or plasmalemmal fractions (5, 20, 22, 28, 29).

As shown in Table I, both rough and smooth microsomes have NADH- and NADPH-cytochrome *c* reductase activities; their SA_{pl} is higher in rough microsomes, although with the correction mentioned the activities of the two fractions become nearly equal. Since in the pancreas the smooth microsomal fraction represents primarily smooth vesicular elements of the Golgi complex, the situation appears to be quite different from that found in the liver: a Golgi fraction isolated from beef liver (30) has both reductase activities, but a highly purified fraction obtained from rat liver (31) has no NADPH-cytochrome *c* reductase. Our zymogen granule membrane fraction has no NADPH-cytochrome *c* reductase activity and its low rotenone-insensitive NADH-cytochrome *c* reductase[4] could be accounted for entirely by contaminating mitochondria (4), since its concentration relative to cytochrome *c* oxidase is the same as in mitochondrial fractions. Hence, notwithstanding their apparent derivation from the Golgi complex, zymogen granule membranes seem to lack the flavoproteins of the two microsomal electron transport systems which in the pancreas are found associated with both rough (rough-surfaced ER) and smooth (primarily Golgi complex) microsomes.

Enzyme activities associated with the cell membranes in other cell types, i.e. 5′-nucleotidase (5, 9, 20–23, 25, 33–35), β-leucyl naphthylamidase (22, 25, 33, 36, 37), and Mg^{2+}-ATPase (5, 20, 22, 34–38) (Table II), are also highly concentrated in the pancreatic plasmalemmal fraction. In addition, they are present in different concentrations in smooth microsomes and zymogen granule membranes. For instance, lower concentrations than in the plasmalemmal fractions are found in smooth microsomes for Mg^{2+}-ATPase, and in zymogen granule membranes for 5′-nucleotidase and β-leucyl naphthylamidase. The rough microsomes were markedly different: they show no enzymatic activity of plasmalemmal type.

Further differences in enzyme distribution among pancreatic cell fractions are seen when other enzyme activities, usually associated with membranes, are considered (Table III). TPPase, a Golgi complex-ER enzyme in the liver (31, 39), is found only in smooth microsomes (Golgi complex) in the pancreas. The latter fraction also shows the highest SA_{pl} for ADPase, acid and alkaline *p*-nitrophenylphosphatase, and alkaline phosphodiesterase. All these activities are absent or present in considerably lower concentrations in rough microsomes, zymogen granule membranes, and plasmalemmal fractions.

[4] The other possible marker for outer mitochondrial membranes, i.e. monoamine oxidase (32), could not be used since it is present in extremely low concentrations in pancreatic mitochondrial fractions.

DISCUSSION

The validity of our data depends on the extent of inactivation and redistribution of enzymatic activities during the unequal and, in some cases, relatively long intervals required for the separation of each cell fraction from pancreatic homogenates. Our experiments indicate that there is no inactivation of enzymatic activities in homogenates and mitochondrial supernates over the relevant intervals, but the data concerning enzyme redistribution are more equivocal. At the time of the isolation of the microsomes (∾2 hr after homogenization), two enzymic activities associated with membrane fractions in the liver, i.e. NADPH-cytochrome *c* reductase and 5′-nucleotidase (4, 5, 9, 19–23), are recovered in large proportion in the pancreatic postmicrosomal supernate. The result is particularly striking for 5′-nucleotidase, which in liver membranes is presumably present as an enzyme-sphingomyelin complex (9) and so far has not been solubilized, except by detergents (9, 21). The reductase is more easily detached from hepatic microsomal membranes either by extraction with low detergent concentrations (18) or by digestion with trypsin (19, 40) or steapsin (41). After 2 hr, the extent of redistribution among pancreatic fractions is rather limited, but there is considerable activation of the two enzymes mentioned. So far, our results could be explained by differences in enzyme localization from one cell type to another, or, alternatively, by rapid and extensive redistribution of membrane enzymes within the first 2 hr of the cell fractionation procedure. The negative results obtained by incubating liver microsomes in pancreas PMS support the first alternative, but out data should be considered with caution since for the moment there is no direct evidence that rules out redistribution. As they stand, they suggest considerable diversity among pancreatic cellular membranes.

TABLE III

Specific Activities for Thiamine Pyrophosphatase, ADPase, Acid and Alkaline p-Nitrophenylphosphatase, and Alkaline Phosphodiesterase in Subcellular Fractions Isolated from Guinea Pig Pancreas

Enzymes	Rough microsomes		Smooth microsomes		Zymogen granule membranes		Plasma membranes	
	SA_{pr}	SA_{pl}	SA_{pr}	SA_{pl}	SA_{pr}	SA_{pl}	SA_{pr}	SA_{pl}
Thiamine pyrophosphatase*	0 (3)	0 (3)	0.37 (3) *0.35–0.42*	1.49 (3) *1.34–1.78*	0 (3)	0 (3)	0 (3)	0 (3)
ADPase*	*0–0.2* (7)	*0–2.4* (7)	2.4 (7) *1.0–3.1*	8.7 (7) *4.2–13.2*	0 (4)	0 (4)	0 (5)	0 (5)
Acid *p*-nitrophenylphosphatase‡	*0–0.2* (6)	*0–1.5* (6)	2.8 (7) *2.5–3.0*	11.6 (7) *9.1–13.7*	2.4 (5) *1.9–2.9*	5.3 (5) *3.9–6.9*	1.3 (3) *1.1–1.6*	6.7 (3) *5.1–8.1*
Alkaline *p*-nitrophenylphosphatase‡	*0–0.03* (3)	*0–0.30* (3)	0.24 (3) *0.19–0.27*	0.96 (3) *0.67–1.12*	0 (3)	0 (3)	0 (3)	0 (3)
Alkaline phosphodiesterase‡	0.03 (5) *0.02–0.05*	0.34 (5) *0.24–0.51*	0.80 (5) *0.69–0.87*	3.30 (5) *2.90–3.90*	0.69 (3) *0.66–0.81*	1.50 (3) *1.40–1.60*	0.22 (3) *0.12–0.40*	0.63 (3) *0.45–0.81*

* μmoles of P_i released at 37°C/60 min per mg protein (SA_{pr}) or per mg phospholipid (SA_{pl}).
‡ μmoles of nitrophenol released at 37°C/60 min per mg protein (SA_{pr}) or per mg phospholipid (SA_{pl}).
Values given are averages. Number of experiments is shown in parentheses. Ranges are given in italics.

The rough microsomes are well characterized by the presence of the reductases of the two microsomal electron transport chains, and by the apparently complete absence or extremely low concentrations of plasmalemmal enzymes (5'-nucleotidase, Mg^{2+}-ATPase, β-leucyl naphthylamidase), a situation which differs from that repeatedly found in the liver (5, 9, 18, 20–23, 25, 29). To these features, we should add the absence of low concentration of TPPase, ADPase, and acid and alkaline phenylphosphatases, as well as a characteristic lipid composition which is low in sphingomyelin and cholesterol (2). The findings indicate not only the specificity of the rough membranes but also the purity or near purity of the fraction as isolated: its contamination by other cellular membranes is below detectability by the assays we used.

The smooth microsomes have a mixed spectrum of enzymatic activities: they share with rough microsomes, in probably equal concentration, the two reductases of the electron transport chains; they have in common with the cell membrane fraction, but at different concentrations, all the plasmalemmal enzymes for which we assayed; and finally, they appear to have some activities of their own which are absent (TPPase), or present only in considerably lower concentration (ADPase, alkaline *p*-nitrophenylphosphatase, phosphodiesterase), in all the other fractions. In their case, contamination by plasma membrane fragments is not excluded, but appears to be limited since the ratios of the common enzymatic activities are widely different. Contamination by rough microsomes is also limited as indicated by the low RNA content of the fraction (1). Hence, the presence of electron transport enzymes appears to reflect the situation in situ, which is therefore quite different from that found in rat liver in which Golgi membranes have no detectable NADPH-cytochrome *c* reductase activity (30).

The zymogen granule membranes lack NADPH-cytochrome *c* reductase, TPPase, ADPase, and alkaline *p*-nitrophenylphosphatase activities and thereby differ from both rough and smooth microsomes. They exhibit the usual plasmalemmal enzyme activities but in rather different concentrations from both smooth microsomes and cell membrane fractions. Zymogen granule membranes are known to be contaminated by mitochondrial membranes which could account for all of their rotenone-insensitive NADH-cytochrome *c* reductase activity and a good measure of their Mg^{2+}-ATPase activity. Mitochondrial Mg^{2+}-ATPase activity is known to increase in damaged mitochondria, and does increase in NaCl-NaHCO_3–treated mitochondiral fractions. If the ratio cytochrome *c* oxidase: Mg^{2+}-ATPase activity of such fractions is considered, then 30–40% of the ATPase activity of the zymogen granule membranes could be accounted for by mitochondrial contamination.[5]

Finally, the cell membrane fraction, morphologically the least satisfactory of our preparations, has the usual type of plasmalemmal enzyme activities and little else. In contrast to the situation in other tissues (7, 25, 33, 34, 36, 37) it has no alkaline *p*-nitrophenylphosphatase activity, and its NADH-cytochrome *c* reductase, as well as part of the Mg^{2+}-ATPase activity could be accounted for by mitochondrial contamination as indicated by experiments and calculations similar to those described in the case of zymogen granule membranes.[5]

Smooth microsomes, zymogen granule membranes, and plasmalemmal fractions have a rather similar lipid composition characterized by high sphingomyelin and cholesterol content (2). This common feature and their overlapping enzymatic activities suggest that we are dealing with related yet different types of membranes, a conclusion which is supported by results recently obtained with liver cell fractions. In that case, a Golgi fraction, the equivalent of our pancreatic smooth microsomes, has been recently obtained (30, 31) and a plasmalemmal fraction has been available for some time (5, 9, 21, 22). Their enzymatic patterns appear to be quite different (the equivalent of a zymogen granule membrane fraction is not available in the liver).

Taken as a whole, the results obtained support the hypothesis of nonrandom removal of membranes involved in the intracellular transport of secretory proteins (31), since the participating membranes retain a specific pattern of enzymatic activities and, to a certain extent, of lipid composition. The evidence for nonrandom removal is quite convincing for transport from the rough ER to the Golgi complex, and already substantial although

[5] When the average cytochrome *c* oxidase:Mg^{2+} ATPase ratio of NaCl-NaHCO_3–treated mitochondrial fraction is expressed as *1*, the corresponding values for the zymogen granule membrane and the plasma lemmal fraction turn out to be 0.34 and 0.26, respectively.

in need of further strengthening for the transport from the Golgi complex to the cell surface. Since in pancreatic homogenates chances for artifact production are so numerous, it would be highly desirable to check these data and their interpretations on other less vulnerable cell systems.

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