LIPORIBONUCLEOPROTEIN COMPLEX AS AN INTEGRAL PART OF ANIMAL CELL PLASMA MEMBRANES

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There is growing evidence that plasma membrane (PM) preparations isolated from rat liver contain RNA ranging from 0.5 [1] to 2.1–4% [2] (dry weight). Plasma membranes from hepatoma cells were also reported to contain 1–2% [3] and 2.5% [1] RNA. Wallach et al. [3] emphasized that RNA seemed to be tightly bound to cancer cell plasma membranes.

We were able to confirm the above findings with PM preparations isolated from rat liver (both in 0.25 sucrose, 2 mM $CaCl_2$ [2] and in 1 mM $NaHCO_3$ [4]) and from rat hepatomas [1]. The fraction at the interface of two layers of sucrose (1.18 and 1.16 density) after ultracentrifugation for 75 min at 105,000 *g* (Spinco model, L-2 SW-39) was collected. The purity of the PM preparations was controlled using electron microscopy and enzyme markers. The final preparations were not significantly contaminated with fragments of endoplasmic reticulum and mitochondria since neither glucose-6-phosphatase nor succinic dehydrogenase activities were detectable. No ribosomes were seen in the electron micrographs. The ATPase (stimulated by Na^+ and K^+) in the final PM preparations was 3–4 times higher than in the original homogenate and the 5′-nucleotidase was 50 times higher.

The RNA content of rat liver PM was 2% and that of hepatomas PM was 3% on the basis of dry weight.

A question arises whether the RNA found in PM is integrated into lipoprotein material of these membranes or originates from other subcellular structures, for instance, endoplasmic reticulum (ER) or ribosomes and is adsorbed during the isolation procedure. Some authors [1] favour the latter possibility. In the present paper we investigated the matter experimentally and characterized the RNA isolated from PM.

PM RNA was prepared from a sample of PM containing 15–20 mg protein. It was suspended in 0.03 M tris-HCl buffer pH 7.6 in the presence of bentonite (1 mg/ml suspension) to inhibit RNAase activity with pH optima 5.4 and 7.6 found in the membranes. Sodium dodecylsulfate (SDS) in the same buffer was added to the suspension (final concentration 1%). The whole mixture was homogenized at 60°C for 1 min. The clear solution was deproteinized with water-saturated phenol pH 6 at 2–4°C in the usual way. The protein-free RNA obtained was repeatedly precipitated in ethanol, dissolved in 0.05 M NaCl and passed through a Sephadex G-100 column. RNA came off as a single peak in the first fraction. Its UV spectrum was typical of nucleic acids: $A_{260/280} = 1.98$; $A_{260/230} = 2.3$.

Several lines of evidence from the following experiments do not support the assumption that the PM RNA is an alien substance adsorbed during the isolation procedure:

(a) We solubilized 26–28% of the total PM protein by extraction with 0.14 M NaCl [cf. 1]. No RNA was found in this protein fraction by chemical analysis nor spectrophotometry.

On the other hand, the lipoprotein residue left after salt extraction, dissolved in 8 M urea or 0.2% SDS, revealed a distinct UV maximum at 260 nm (fig. 1), provided 0.5 mg of membrane protein was used.

(b) The RNA of intact PM preparations was relatively resistant to the action of RNAase. On incubating PM for 5 hr at 37°C in the presence of one absorbance unit of RNA plus 10 enzyme units of guanylic RNAase from *Actinomycetes* [5] (in 0.05 M tris-HCl buffer pH 7.6;

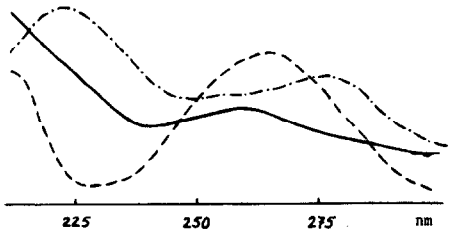

Fig. 1. Spectrophotometry of PM and some of their fractions. Plasma membranes were solubilized in 0.2% SDS (0.5 mg protein). Purified ribonucleoprotein was dissolved in water (1.4 mg protein). Water soluble protein contained no RNA (1.54 mg protein).
—— Plasma membranes. - - - - Purified ribonucleoprotein.
-.-.-.- Water soluble protein containing no RNA.
Unicam spectrophotometer, 1 cm light path.

0.1 M NaCl), less than 7% of acid soluble RNA fragments appeared whereas isolated RNA incubated under similar conditions formed 75% acid soluble fragments. The relative inaccessibility of the RNA integrated into PM might explain why Bosman could not detect RNA in rat liver PM using the RNAase test [6].

(c) Rat liver ribosomal RNA or transfer RNA labelled with ^{14}C-orotic acid were added to an unlabelled fraction of the rat liver homogenate which served as a source of PM for the isolation of RNA. The amount of ^{14}C-RNA added was 5–10 times as higher than the final yield of PM RNA. From the total radioactivity of purified PM RNA and the original specific radioactivity of the labelled RNA used, we calculated that contamination of the PM RNA with alien RNAs did not exceed 4–7%.

Similar results were obtained with rat liver microsomes or ribosomes labelled with ^{14}C-adenine. A sample of labelled microsomes was divided into two portions. One was treated with 0.9% deoxycholate to obtain ribosomes in the usual way. The microsomes and ribosomes were added to separate samples of the unlabelled homogenate. The results of one experiment were as follows: The microsomes added contained 11.6 mg RNA (for the special precautions needed for this analysis see [7]) with a specific activity of 142,000 cpm/mg. The final yield of the PM RNA from the mixed homogenate was 545 μg with only 5138 cpm/mg.

The ribosome sample contained 12.7 mg RNA with specific activity of 97,000 cpm/mg. 945 μg of

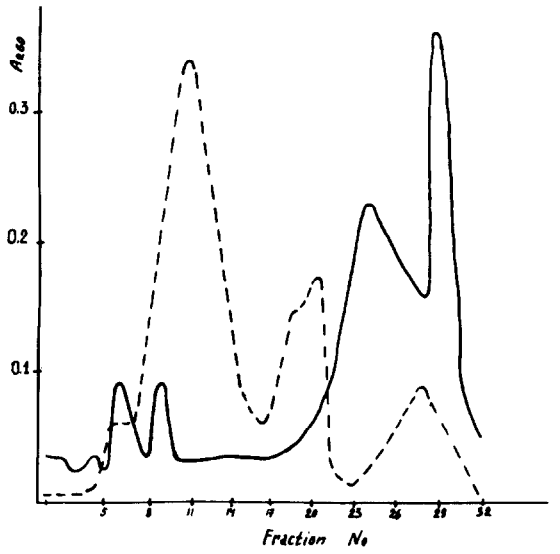

Fig. 2. Sucrose gradient ultracentrifugation of PM RNA. Spinco L-2, SW-39, 200 min. 300 μg RNA in 0.01 M acetate buffer pH 5 applied. The sucrose gradient was 5–20% with a 50% sucrose cushion. Sucrose solution had been treated previously with bentonite and contained 0.01 M EDTA.
—— PM RNA. -.-.-.- ribosomal RNA and tRNA.

the PM RNA preparation (4444 cpm/mg) was isolated from the mixed homogenate in this case. Thus, the contamination of the PM RNA with microsomal and ribosomal RNA was estimated as 6.6 and 4.8% respectively.

(d) PM RNA preparations were subjected to sucrose gradient ultracentrifugation using cytoplasmic and ribosomal RNA fractions as markers. Fig. 2 shows that the PM RNA was heterogeneous and the positions of the components did not coincide with those of ribosomal RNA. The two main peaks correspond to 10–12 S and 3–3.5 S (63–66% and 20% respectively). A minor, heavy 28–30 S component, which sometimes (but not always) appears as a double peak, is also found. These molecular characteristics resemble those of endoplasmic reticulum RNA which has been previously studied in detail in our laboratory [8], but the 20–22 S component present in ER RNA was missing from PM RNA. This makes it unlikely that PM RNA represents RNA from the ER membranes contaminating our PM preparations. The same distribution of the RNA components was found whether or not potent RNAase inhibitors were added to the original homo-

genate. Thus the assumption that PM RNA might be a product of enzymic degradation of other kinds of RNA also seems unlikely.

No differences in PM RNA from rat liver and Zajdela hepatoma cells were observed using the sucrose gradient centrifugation procedure.

The nucleotide composition of the total liver PM RNA preparation resembles that of the RNA from membranes of ER but differs from that of ribosomal RNA (table 1).

The next step in the experiments was designed to determine in what form RNA is integrated into PM. A detailed fractionation procedure was developed for this purpose. The PM preparation was exhaustively extracted with 0.14 M NaCl at 3–4°C. The residual lipoprotein complex was homogenized and subjected to mild dissociation in the presence of 0.1% dodecylsulfate for 15 min at 37°C. The total protein was precipitated with $(NH_4)_2SO_4$ (0.7 saturation). Dissociation did not proceed completely and a portion of the total lipid (determined according to Folch et al. [10]) remained in the protein precipitate. No protein was found [11] in the lipid fraction in the supernatant. The dissociation procedure used was relatively mild since the original 5′-nucleotidase activity was preserved in the protein fraction.

From the protein precipitate treated with acidified (pH 5) water, a ribonucleoprotein (RNP) (38% of the total protein) could readily be extracted with vigorous stirring within the first 5–10 min of treatment. Repeated extraction with acidified water solubilized another 40% of the protein containing no RNA.

The RNP isolated was subjected to two successive purification procedures: (1) Gel filtration through a Sephadex G-100 column (1.5 × 90 cm) and (2) ultracentrifugation of the concentrated partially purified fractions through a sucrose layer (1.25 density) in a SW-39 rotor for 4 hr. Contaminating proteins were at the top, polysaccharides sedimented to the bottom and RNP moved to the middle of the tube. RNP fractions collected showed both $A_{260/280}$ and $A_{260/230}$ coefficients of 1.8. The relative amounts of protein and RNA components in the purified ribonucleoprotein were 20–30% and 80–70%, respectively. The sensitivity of the RNA moiety of the RNP to the action of guanylic RNAase was intermediate between that of the RNA in the intact PM and the isolated RNA preparation. Spectrophotometry of the RNP and of the water soluble fraction from the PM lipoprotein complex which contained no RNA is shown in fig. 1.

Table 1
Base composition* of total RNA preparations.

Source of RNA	G	C	A	U	Note
Plasma membranes	40.4	29.8	18.9	11.0	The mean of three measurements
Endoplasmic reticulum	40.0	30.0	17.0	12.0	[8]

* measured as free bases by method of Wyatt [9].

The data described in this paper support the view that the RNA found in rat liver and rat hepatoma PM do belong to this structure and are integrated in a triple liporibonucleoprotein complex.

Experiments are underway to determine whether all PM lipoprotein complexes contain RNA.

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