ISOLATION AND PROPERTIES OF ROUGH AND SMOOTH VESICLES FROM RAT LIVER

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The endoplasmic reticulum of the liver cell appears in thin sections as a system of channels, composed of membranes, the majority of which have ribonucleoprotein (RNP) particles attached to their outer surfaces. The microsomal fraction, isolated after homogenization and differential centrifugation, contains free RNP particles and two kinds of vesicles: rough, with RNP particles attached to the outer surface, and smooth, which lack these particles (1). Recently many efforts have been made to separate these two structures and to characterize them (2-5). Long centrifugation times were necessary to obtain relatively pure fractions. In the following, a rapid and simple method for isolation of rough and smooth vesicles from rat liver homogenate will be described together with some of the main enzymic characteristics of these structures.

MATERIALS AND METHODS

All fractionations were performed with a commercial Christ ultracentrifuge, model Omega. An angle-head rotor was used which can take eight plastic centrifuge tubes, each with a capacity of 11.5 ml. The tube angle with the rotor axis is 20°. At the maximal speed, 60,000 rpm, the centrifugal force is 250,000 g, as calculated for the middle of the tube.

In all experiments, rats of the inbred homozygote strain R/StDa (strain R, subline Stock-holm-Dallner) were used. All animals fasted overnight. The livers were homogenized in enough 0.3 m sucrose to give a 25 per cent homogenate, which was centrifuged at 20,000 g for 30 minutes to eliminate cell debris, nuclei, and mitochondria. The supernatant, containing most of the microsomes, was decanted. To 9.9 ml of this supernatant, 0.1 ml of 1 M MgCl₂ was added. This will be referred to as “supernatant A.”

To obtain the total microsomal fraction, 7 ml of the supernatant A was diluted with 4.5 ml of a 0.3 m sucrose-0.01 m MgCl₂ solution and centrifuged at 250,000 g for 60 minutes. The pellet was rinsed three times and resuspended by homogenization in 0.3 m sucrose.

For separation of smooth (a) and rough (b) vesicles, 7 ml of the supernatant A was layered over 4.5 ml of a 1.5 m sucrose-0.01 m MgCl₂ solution. After centrifugation at 250,000 g for 30 minutes, a tight, light brown pellet was obtained and a red fluffy layer was found at the boundary of the two phases.

(a) The upper phase (7 ml) including the fluffy layer was aspirated, diluted with 4.5 ml 0.3 m sucrose, and recentrifuged at 250,000 g for 60 minutes. The pellet obtained, called “subfraction I,” was rinsed three times and used either for investigation by electron microscopy or resuspended by homogenization in 0.3 m sucrose for biochemical analysis.

(b) The lower 1.5 m sucrose layer was discarded and the tight bottom pellet, labeled “subfraction II,” was rinsed three times. This pellet was treated in the same way as subfraction I.

In order to maintain the structural and func-

Figure 1
Subfraction I. Smooth vesicles and numerous particles presumably ferritin. × 60,000.

Figure 2
Subfraction II. Essentially rough vesicles and free RNP particles. Some of the smallest vesicles may be of the smooth type. × 60,000.
tional integrity of the free RNP particles, MgCl₂ was used during the procedure (6). This caused these particles to sediment along with the rough vesicles.

All pellets were so hard that they could easily be removed from the tubes with a small spatula. For investigation at the ultrastructural level they were placed in 1 per cent buffered OsO₄ (pH 7.2) (7) and cut into small pieces. After fixation for 1 hour, the specimens were embedded in Epon (8).

RESULTS AND COMMENTS

In all experiments, electron microscopical investigation showed striking similarity of pellets from the same subfraction at all levels examined. Subfraction I contained smooth vesicles and small particles, presumably ferritin (Fig. 1). Rough vesicles or free RNP particles could not be detected. The sections of subfraction II showed predominantly larger vesicles with RNP particles attached to the outer surface of the membranes and many free RNP particles between the vesicles (Fig. 2). It cannot be excluded that some of the smaller vesicles are of the smooth type.

The results of biochemical analysis (Table I) indicate a low RNA/protein ratio in subfraction I and a high ratio in subfraction II. This is in agreement with the values previously presented by other authors (1, 2) for smooth and rough vesicles.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Protein mg/gm liver</th>
<th>RNA mg/gm liver</th>
<th>Phospholipids mg/gm liver</th>
<th>RNA Protein</th>
<th>Phospholipids Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total microsomal fraction</td>
<td>32.6</td>
<td>6.72</td>
<td>13.6</td>
<td>0.21</td>
<td>0.42</td>
</tr>
<tr>
<td>Subfraction I (smooth vesicles)</td>
<td>6.6</td>
<td>0.31</td>
<td>3.6</td>
<td>0.05</td>
<td>0.55</td>
</tr>
<tr>
<td>Subfraction II (rough vesicles + free RNP particles)</td>
<td>23.0</td>
<td>6.08</td>
<td>8.1</td>
<td>0.26</td>
<td>0.35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fractions</th>
<th>DPNH per gm liver</th>
<th>DPNH per mg protein</th>
<th>DPNH + DIC per gm liver</th>
<th>DPNH + DIC per mg protein</th>
<th>TPNH per gm liver</th>
<th>TPNH per mg protein</th>
<th>TPNH + DIC per gm liver</th>
<th>TPNH + DIC per mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total microsomal fraction</td>
<td>13.70</td>
<td>0.42</td>
<td>12.70</td>
<td>0.39</td>
<td>2.29</td>
<td>0.07</td>
<td>1.63</td>
<td>0.05</td>
</tr>
<tr>
<td>Subfraction I</td>
<td>0.66</td>
<td>0.10</td>
<td>0.53</td>
<td>0.08</td>
<td>0.26</td>
<td>0.04</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>Subfraction II</td>
<td>12.70</td>
<td>0.55</td>
<td>13.30</td>
<td>0.58</td>
<td>2.53</td>
<td>0.11</td>
<td>2.53</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Sections were cut at different levels of the pellets and examined in a Siemens Elmiskop I at magnifications ranging from 18,000 to 30,000.

Protein was measured by the method of Lowry et al. (9). Ribonucleic acid was estimated according to Ceriotti (10). Lipids were extracted (11) and the phosphorus content estimated (12). The value obtained was multiplied by 25 to get the total amount of phospholipids (13). Enzyme assays were performed according to previously described methods (13-15).
membranes. The same is true for the phospholipid/protein ratio (1, 2).

An interesting finding in the study of the electron-transporting enzymes was the total lack of DT diaphorase activity in subfraction II (Table II). DT diaphorase, which was purified by Ernster et al. (16, 15), reacts at equal rates with both DPNH and TPNH and is highly sensitive to dicoumarol. Dicoumarol-sensitive DPNH and TPNH (non-specific) activity was found exclusively in subfraction I. On the contrary, more than 90 per cent of the specific DPNH- and TPNH-oxidizing activities were found in subfraction II. As electron acceptor, 2,6-dichlorophenol indo-

Recently Fouts reported (4) a higher “TPNH oxidase” in smooth-surfaced vesicles from rabbit liver in comparison with rough vesicles. Using our method, we also performed subfractionations of the rabbit liver microsomal fraction, and a concentration of TPNH diaphorase and cytochrome c reductase activities was found in subfraction I, which contained smooth vesicles. A comparison of the enzymic pattern of the two microsomal subfractions in different species will be published subsequently. A direct comparison between our results and those of Chauveau, Moulé, Roulier, and Schneebeli (5), and Ernster, Siekevitz, and Palade (13) cannot be made since

<table>
<thead>
<tr>
<th>ADDITIONS</th>
<th>TOTAL MICROSOMAL FRACTION</th>
<th>SUBFRACTION I</th>
<th>SUBFRACTION II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles P/s 20 min. / gm liver / per mg protein</td>
<td>µmoles P/s 20 min. / gm liver / per mg protein</td>
<td>µmoles P/s 20 min. / gm liver / per mg protein</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>96.4</td>
<td>2.96</td>
<td>4.3</td>
</tr>
<tr>
<td>ATP</td>
<td>90.6</td>
<td>2.78</td>
<td>14.3</td>
</tr>
<tr>
<td>ADP</td>
<td>51.8</td>
<td>1.59</td>
<td>7.3</td>
</tr>
<tr>
<td>ADP + DOC</td>
<td>32.3</td>
<td>0.99</td>
<td>3.5</td>
</tr>
<tr>
<td>IDP</td>
<td>98.5</td>
<td>3.02</td>
<td>17.4</td>
</tr>
<tr>
<td>IDP + DOC</td>
<td>188.7</td>
<td>5.79</td>
<td>26.7</td>
</tr>
</tbody>
</table>

Their fractionation procedures differ greatly from ours.

Glucose-6-phosphatase activity was present almost exclusively in subfraction II (Table III). This finding is of special interest, as this enzyme has a well defined function in cell metabolism (21). Nucleoside diphosphatase activities, with and without addition of DOC, and nucleoside triphosphatase activity of the two subfractions were also investigated. The results are similar to those described by Ernster and Jones (14) for total liver microsomes. These enzyme activities were somewhat higher in subfraction II, although the specific activity in this fraction was never greater than twice that of subfraction I.

The conclusions are summarized in the following points: 1. The preparation method described is rapid and simple and permits a separation of smooth and rough vesicles from the microsomal fraction of rat liver. 2. The fraction consisting of
smooth vesicles contains all the microsomal DT diaphorase. This finding provides a suitable test for this fraction, as measurement of DT diaphorase activity is very simple. 3. The majority of specific pyridine nucleotide-oxidizing enzymes is located in the fraction containing mainly the rough vesicles. 4. Glucose-6-phosphatase activity is almost exclusively limited to the latter fraction. 5. These results indicate that the two kinds of microsomal vesicles from rat liver differ not only ultrastructurally but also functionally, and should perhaps be regarded as two different organelles.

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REFERENCES