ELECTROPHORETIC MOBILITY OF MICROSOMES FROM RAT LIVER

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SUMMARY

The electrophoretic mobilities of rough and smooth microsomes were studied using free electrophoresis in a sucrose gradient. Rough microsomes have a higher net negative surface charge but removal of the ribosomes decreases their mobility to that of smooth microsomes. Treatment with neuraminidase and phospholipases C and D does not affect the mobility of total smooth microsomes, but this mobility is increased by ~20% after trypsin and papain treatment and by ~12% after phospholipase A treatment. Further treatment of trypsin-digested smooth microsomes with phospholipase C re-establishes the original mobility. This effect is not caused by the removal of lipid phosphate groups, but by the liberation of negatively charged protein species that are normally buried under trypsin-sensitive proteins. Low concentrations of trypsin also solubilize enzyme proteins from smooth liver microsomes of phenobarbital-treated rats, but the electrophoretic mobility is not increased, indicating structural differences between induced and control membranes.

INTRODUCTION

The surface charge properties of biological membranes are important in determining surface characteristics and have therefore been the subject of a number of investigations. The surface charge of cells such as ascites or blood cells can be studied in simple cuvettes or cylindrical cells in various microelectrophoretic systems where direct observation of movement is possible. Many cell types have been studied in this way, including leucocytes, lymphocytes and leukaemic cells (Meharishi & Thomson, 1968), erythrocytes (Haydon & Seaman, 1967), Ehrlich ascites tumour cells (Ambrose, James & Lowick, 1956; Wallach & Eylar, 1961; Vassar, 1963; Mayhew, 1968), monocytes (Weiss, Mayhew & Ulrich, 1966), fibroblasts (Forrester, Ambrose & Macpherson, 1962), mammalian tissue cells (Simon-Reuss, Cook, Seaman & Heard, 1964), various tissue culture cells (Mayhew & O’Grady, 1965), and HeLa cells (Brent & Forrester, 1967). Cancer and transformed cells possess surface glycoproteins which are very important in determining the properties of the cell surface; this has been extensively studied using various types of hydrolytic enzymes (cf. Cook & Stoddart, 1973).

The system used for studying the electrophoretic mobility of whole cells is not suitable for subcellular particles. It has become clear that the most effective system for such particles is free electrophoresis which allows the use of a stabilizing gradient. The stabilization of liquid columns for electrophoresis by means of a density gradient was first reported in 1951 by Brakke, and various elaborations of this system have
since been described. Polson & Russell (1967) described a method for the separation of viruses by density gradient electrophoresis. The technique of zonal density gradient electrophoresis has been used to measure the surface charge of intracellular membranes of brain cortex (Sellinger, Borens & Nordrum, 1969; Sellinger & Borens, 1969). Vos, Kuriyama & Roberts (1968) applied the technique of Svensson (1960) in the further fractionation of brain subcellular particles prepared by centrifugation. Ryan, Kaland & Thomas (1971) described the electrophoretic separation of synaptosomes, mitochondria and synaptic vesicles by free-flow electrophoresis. Density gradient electrophoresis has been used to study the surface charge of liver mitochondria and microsomes (Davenport, 1964; Plummer, 1965). Hannig (1968) developed a carrier-free continuous electrophoretic apparatus for deflexion electrophoresis. This system was utilized for analyses of lysosomes, microsomes, and outer and inner mitochondrial membranes (Stahn, Maier & Hannig, 1970; Heidrich, Stahn & Hannig, 1970).

The object of this study was to arrive at an electrophoretic system which had enough stability, resolution and accuracy to allow analysis of isolated microsomes and their subfractions. Particle mobility was studied in a density-gradient U-tube system, and smooth microsomes were also subjected to enzyme treatments in order to remove specific groups before electrophoresis.

MATERIALS AND METHODS

Particle preparations

Adult male albino rats weighing 180 g were used. The animals were starved 20 h before sacrifice. In the case of phenobarbital treatment rats were injected intraperitoneally (16 mg/rat) daily for 5 days.

Radioactive labelling was carried out by injection of 100 μCi [3H]choline (10-1 Ci/mmol, Radiochemical Centre, Amersham, England) in sterile Ringer’s solution into the portal vein of a rat under Nembutal anaesthesia 0.5 h before decapitation.

Microsomes were prepared from a liver homogenate in 0.25 M sucrose (Dallner, 1974). Rough and smooth microsomes were isolated according to Rothschild (1963) with certain modifications (Dallner, 1974). The smooth fraction was diluted to a sucrose concentration of 0.25 M and rough microsomes were rehomogenized in 0.25 M sucrose; both were resuspended at a final concentration of 5 mg protein/ml.

Treatments

Adsorbed proteins were removed by resuspending the smooth microsomes in 0.15 M Tris buffer, pH 8, and subsequently sedimenting them at 105,000 g for 60 min (Spinco-Beckman ultracentrifuge, model L2-65B). For EDTA treatment of rough and smooth microsomes, 50 mM EDTA, pH 7.4, was used (Eriksson, Svensson, Bergstrand & Dallner, 1972). After EDTA addition, the microsomal suspension was incubated at 0 °C for 10 min; 5 ml of this suspension was layered on to a discontinuous gradient consisting of 3 ml of 1.4 M and 2 ml of 0.35 M sucrose. Centrifugation was performed at 202,000 g for 60 min in the SW 40 rotor. The vesicle fraction at the 0.35/1.4 M sucrose interface was used for the experiments.

Enzyme treatment involved the incubation of particles with enzymes at 30 °C. In order to avoid aggregation, no buffer or cation was added. Smooth microsomes were incubated with 40 μg trypsin/mg protein, if not otherwise stated, in 0.25 M sucrose for 10 min at 30 °C. The reaction was stopped by adding trypsin inhibitor (twice as much as the amount of trypsin present). In those cases where incubation was continued with other enzymes, the appropriate enzyme was added in a concentration given below and the incubation was continued for a further 10 min at the same temperature.
Electrophoretic mobility of microsomes

The following enzyme concentrations were used: papain, 0.1 mg/mg protein; neuraminidase, 0.05 mg/mg protein; phospholipase (PLPase) A, 30 \( \mu \)g/mg protein; PLPase C, 50 \( \mu \)g/mg protein; and PLPase D, 80 \( \mu \)g/mg protein. The *Naja naja* snake venom was suspended in Tris-HCl, 0.05 M, pH 7.5, at a concentration of 1 mg/ml and treated at 90 °C for 8 min in order to inactivate proteolytic enzymes before use. The resulting precipitate was removed by centrifugation.

Particle incubation in the absence of enzyme was used as a control. Unless otherwise stated, all enzyme treatments were performed at 30 °C for 10 min.

**Electrophoresis**

The electrophoretic system constructed is shown in Fig. 1. Twenty millilitres of 30 % sucrose containing 10 mM Tris-acetate, pH 8.0, were placed on the bottom of the U-vessel. This amount was enough to cover the side tube utilized for removal of the gradient. The stopcock was then closed. The sucrose gradient was made in a model 570 ISCO gradient former and consisted of a continuous gradient between 0.44 and 0.73 M sucrose containing 10 mM Tris-acetate buffer, pH 8.0, throughout. The gradient, which had a volume of 16 ml, was introduced into the left-hand limb of the U-tube. The right-hand limb of the U-tube was filled with 17 ml of 20 % sucrose for balance and 0.12-0.17 ml of particle suspension (5 mg protein/ml in 7.5 % sucrose) was layered on to this density gradient. The particle suspension was the only solution in the system which did not contain buffer. The suspension was covered with 2.5 % buffered sucrose. The wide vessel was now attached to the top of the left limb and filled with 10 ml of 10 mM Tris-acetate buffer. Five millilitres Tris-acetate buffer were then layered on the right-hand side and a plastic holder with a long platinum electrode, reaching to the bottom of the right-hand tube, was introduced. The inside of this plastic holder was filled with 5 ml saturated KCl. The stopcock was then opened. The position of the sample was controlled, and any necessary correction was made by adding or removing buffer on the left side. Electrophoresis was routinely performed in a thermostatic bath (5 °C) at 1000-1150 V and 28-30 mA with the anode to the right. After a run of 50-70 min, the U-tube was removed from the bath and the stopcock of the left limb was closed; 40 % sucrose was then pumped in through the rubber inlet above the stopcock with
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the help of a synchronous pump. The wide vessel on the top of the left limb was replaced by a conical glass attachment, which was connected to an ISCO u.v. analyser.

When electrophoresis was performed in the presence of 5 mM MgCl₂ or 10 mM CsCl, all solutions in the electrophoretic system contained the appropriate cation with the exception of the particle suspension and the saturated KCl.

Analytical procedures

Protein was measured by the Biuret method, using bovine serum albumin as standard (Gornall, Bardawill & David, 1949). NADPH-cytochrome c reductase activity and the content of cytochrome b₅ were assayed as described earlier (Dallman, Dallner, Bergstrand & Ernster, 1969).

Chemicals

Twice-crystallized trypsin (activity approx. 9000 units/mg) was purchased from Boehringer (Mannheim). Twice-crystallized trypsin inhibitor from soybean; phospholipase C, type I from Clostridium welchii; phospholipase D, type II from cabbage; phospholipase A from Vipera russelli; neuraminidase, type VI from Clostridium perfringens; and twice-crystallized papain from Papaya latex were obtained from the Sigma Chemical Co. (St Louis).

RESULTS

Effect of aggregation and adsorbed protein

In order to test the possible influence of aggregation on mobility in the system used, smooth microsomes were subjected to treatments known to change vesicles prior to electrophoresis (Table 1). When vesicles were aggregated with Mg²⁺, the electrophoretic mobility was not changed to any appreciable extent. On the other hand, sedimentation, which is known to increase the average particle size several-fold, decreases particle movement in this electrophoretic system. When adsorbed proteins were removed by washing with alkaline buffer, a treatment requiring sedimentation and thereby causing aggregation, electrophoretic mobility of the vesicles was decreased by 8%. These experiments demonstrate that sedimentation of smooth microsomes should be avoided in order to obtain reproducible results.

Table 1. Effect of aggregation and adsorbed protein on the electrophoretic mobility of smooth microsomes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mobility µm s⁻¹ V⁻¹ cm</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.55 ± 0.05</td>
<td>100</td>
</tr>
<tr>
<td>MgCl₂, 1.5 mM*</td>
<td>0.56 ± 0.03</td>
<td>98</td>
</tr>
<tr>
<td>Sedimentation†</td>
<td>0.48 ± 0.04</td>
<td>88</td>
</tr>
<tr>
<td>Washing with Tris buffer‡ (0.15 M; pH 8.0)</td>
<td>0.51 ± 0.04</td>
<td>92</td>
</tr>
</tbody>
</table>

• Only the sample was supplemented with MgCl₂.
† The smooth microsomes were sedimented by centrifugation at 105,000 g for 90 min followed by resuspension in 0.25 M sucrose.
‡ The washing procedure is given in Materials and methods. The values represent the means ± S.E.M. of 5 experiments.
Electrophoretic mobility of microsomes

Fig. 2. Electrophoretic mobilities of rough (A) and smooth (B) microsomes and ribosomes (C). Electrophoresis was performed at 1120 V, 31 mA for 55 min. After the run, the gradient was pumped through a recording u.v. analyser. One unit on the abscissa equals 1 cm in the gradient. The distance between the electrodes was 37.5 cm.

Microsomal subfractions

Isolated microsomal subfractions displayed different electrophoretic mobilities. The recording chart is shown in Fig. 2 for rough and smooth microsomes as well as detergent-isolated ribosomes. The fractions appeared as single symmetrical peaks. These peaks were sufficiently narrow to discern differences in mobility of only a few per cent. The mobility values, in μm s⁻¹ V⁻¹ cm (Table 2), were 0.68 for rough, 0.55
for smooth microsomes and 0.80 for ribosomes. Since mono- and divalent cations influence rough and smooth microsomes differently, the effects of Cs\(^+\) and Mg\(^{2+}\) are also shown in Table 2. The appropriate cation was incorporated in both sucrose solutions of the gradient mixer in order to obtain the same concentration throughout the gradient. There was a general decrease in mobility as a result of the increased ionic strength of the medium. Furthermore, Cs\(^+\) influenced mainly rough microsomes while Mg\(^{2+}\) affected the smooth subfraction to a larger extent in agreement with the behaviour of these fractions upon gradient centrifugation in the presence of these cations (Dallner & Nilsson, 1966).

Table 2. Electrophoretic mobility of microsomal subfractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mobility, (\mu\text{m s}^{-1} \text{V}^{-1} \text{cm})</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rough</td>
<td>0.68 ± 0.03</td>
<td>60</td>
</tr>
<tr>
<td>Smooth</td>
<td>0.55 ± 0.04</td>
<td>98</td>
</tr>
<tr>
<td>Ribosomes</td>
<td>0.80 ± 0.08</td>
<td>82</td>
</tr>
</tbody>
</table>

\begin{itemize}
  \item In gradients containing CsCl the final concentration of cation was 10 mM and for MgCl\(_2\), the concentration was 5 mM.
  \item Details of the experiments are described in Materials and methods. The values are means ± S.E.M. (n = 10).
\end{itemize}

Table 3. Effect of EDTA treatment on rough and smooth microsomes

<table>
<thead>
<tr>
<th>Microsomes</th>
<th>Mobility, (\mu\text{m s}^{-1} \text{V}^{-1} \text{cm})</th>
<th>% of smooth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth</td>
<td>0.55 ± 0.05</td>
<td>100</td>
</tr>
<tr>
<td>EDTA-smooth</td>
<td>0.54 ± 0.06</td>
<td>99</td>
</tr>
<tr>
<td>Rough</td>
<td>0.68 ± 0.06</td>
<td>123</td>
</tr>
<tr>
<td>EDTA-rough</td>
<td>0.58 ± 0.05</td>
<td>104</td>
</tr>
</tbody>
</table>

The data are given as mean values ± S.E.M. (n = 7).

**EDTA treatment**

In order to find out whether the higher mobility observed for rough microsomes is a property of the rough membrane itself or arises from the presence of bound ribosomes, rough microsomes were treated with EDTA. Under appropriate conditions, EDTA removes all of the bound ribosomes, thereby creating 'smooth' vesicles (Eriksson, Svensson, Bergstrand & Dallner, 1972). The electrophoretic mobility of rough microsomes was 0.68, while EDTA-treated rough and smooth as well as untreated smooth microsomes had about the same mobilities, or 0.58, 0.54 and 0.55, respectively (Table 3). Thus, the high electrophoretic mobility of rough microsomes depends on the presence of bound ribosomes.
Effects of various enzymes

It has been established that under controlled conditions various proteolytic and lipolytic enzymes remove only components located on the outer surface of the microsomal vesicle (Ito & Sato, 1969; Nilsson & Dallner, 1975). To study the influence of phospholipids on electrophoretic mobility, smooth microsomes were incubated with various enzymes.

Table 4. Effect of enzyme treatment on the electrophoretic mobility of smooth microsomes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mobility</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.55 ± 0.06</td>
<td>100</td>
</tr>
<tr>
<td>PLPase A</td>
<td>0.62 ± 0.04</td>
<td>112</td>
</tr>
<tr>
<td>PLPase C</td>
<td>0.54 ± 0.05</td>
<td>98</td>
</tr>
<tr>
<td>PLPase D</td>
<td>0.54 ± 0.03</td>
<td>98</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>0.56 ± 0.03</td>
<td>102</td>
</tr>
<tr>
<td>Trypsin</td>
<td>0.65 ± 0.08</td>
<td>119</td>
</tr>
<tr>
<td>Papain</td>
<td>0.68 ± 0.07</td>
<td>123</td>
</tr>
<tr>
<td>Trypsin + PLPase A</td>
<td>0.64 ± 0.03</td>
<td>117</td>
</tr>
<tr>
<td>Trypsin + PLPase C</td>
<td>0.56 ± 0.04</td>
<td>102</td>
</tr>
<tr>
<td>Trypsin + PLPase D</td>
<td>0.66 ± 0.04</td>
<td>120</td>
</tr>
</tbody>
</table>

The values are given as mean values ± s.e.m. (n = 8).

3 phospholipases: PLPase A, prepared from Naja naja, which removed fatty acids from the β position; PLPase C, which mainly attacks lecithin and removes phosphorylcholine; and PLPase D, an enzyme which removes only the choline moiety. The first part of Table 4 shows the results of PLPase treatments on smooth microsomes. The electrophoretic mobility was unchanged after treatment with PLPase C or D but a significant increase in mobility occurred after only short exposure to PLPase A.

Smooth microsomes have only a limited amount of sialic acid available on the outer surface; only about 10% of the total sialic acid content can be removed by neuraminidase treatment (Winqvist, Eriksson, Dallner & Ersson, 1976). Contrary to the findings with individual cells (Ward & Ambrose, 1969), neuraminidase did not influence the mobility of smooth microsomes.

Two proteases, trypsin and papain, were also tested (Table 4). Both increased the mobility of smooth microsomes by about 20%. The trypsin-treated microsomes were also subjected to further enzyme treatment. This was possible since the action of trypsin can be stopped with trypsin inhibitor, so that removal of trypsin by sedimentation of the microsomes is unnecessary. The increased mobility of the trypsin-treated membranes remained unchanged after PLPase D treatment. PLPase A, which increases the mobility of smooth microsomes, had no effect on the high-mobility, trypsin-treated smooth membranes. On the other hand, PLPase C treatment of trypsinized smooth microsomes decreased their mobility back to that of the untreated control.
the concentration to 50 \( \mu \text{g} \) trypsin/mg protein in order to solubilize 70\% of the cytochrome \( b_6 \). The mobility of control microsomes increased about 13\% after treatment with a trypsin concentration of 10 \( \mu \text{g} \)/mg protein. Trypsin-treated microsomes from phenobarbital-treated rats differed significantly from the vesicles of untreated rats. In this case, a low concentration of trypsin (10 \( \mu \text{g} \)/mg protein) also solubilized all of

### Table 5. Influence of trypsin on smooth microsomes from phenobarbital-treated rat liver

<table>
<thead>
<tr>
<th>Trypsin concentration, ( \mu \text{g}/\text{mg} ) protein</th>
<th>NADPH-cyt. c red., % in pellet</th>
<th>Cyt. ( b_6 ), % in pellet</th>
<th>Mobility, % increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Pb</td>
<td>Control</td>
<td>Pb</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>3</td>
<td>81</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>3</td>
<td>25</td>
</tr>
</tbody>
</table>

Various concentrations of trypsin in a final volume of 2 ml smooth microsomes (5 mg microsomal protein per ml) were used. Incubations proceeded for 10 min at 30 °C. The reaction was stopped by addition of trypsin inhibitor (twice the amount of trypsin present). The values are the means of 3 experiments. cyt. = cytochrome; Pb = phenobarbital-treated.

### Table 6. Effect of combined enzyme treatments on smooth microsomes

<table>
<thead>
<tr>
<th>Enzyme treatment</th>
<th>Released cpm, % of total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td>None</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Trypsin</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>Phospholipase A</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>Phospholipase C</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Trypsin + phospholipase A</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>Trypsin + phospholipase C</td>
<td>42 ± 5</td>
</tr>
</tbody>
</table>

Smooth microsomes were prepared from rats injected intraperitoneally with \(^{3}\text{H}\)choline (100 \( \mu \text{Ci} \)/rat) 30 min before decapitation and the isolated fraction was subjected to the Tris-water–Tris washing procedure. The concentrations of enzymes used are given in Materials and methods. After incubation (30 °C, 10 min) the suspensions were centrifuged (105,000 g, 90 min) and released protein and choline radioactivity were determined. The values are means ± S.E.M. (n = 5).

the NADPH-cytochrome c reductase and 20\% of the cytochrome \( b_6 \), but the mobility was not increased. Thus, it is necessary to incubate phenobarbital microsomes with a larger amount of trypsin to obtain the same increase in mobility as for the control microsomes.

**Effect of phospholipases on membrane composition**

In order to investigate the mechanism of PLPase C action after trypsin treatment, smooth microsomes prepared from rats labelled \textit{in vivo} with \(^{3}\text{H}\)choline and washed free from adsorbed and luminal proteins were treated with various hydrolytic enzymes
and released protein and released labelled choline were measured (Table 6). As expected, trypsin removed about 30% of the protein and a lesser amount of the choline label. On the other hand, phospholipase C released only a small amount of protein, but practically all of the choline label. Clearly, phospholipase C exerts its full effect on phospholipids even without pretreatment with trypsin, while the 2 enzymes together seem to solubilize more membrane protein.

DISCUSSION

This paper describes some of the surface charge properties of microsomal membranes as determined using an electrophoretic system based on free electrophoresis in a stabilizing sucrose gradient. Enzymic modification of the membrane surface also gave information about the nature of the groups determining the surface charge.

There are a number of reports describing determination of the electrophoretic mobility of various individual cells using a simple chamber system and direct optical recording of the movements. The situation is much more difficult with subcellular particles, which are too large to move in most supporting media and too small to be observed in the experimental system used for whole cells. The system described here uses free electrophoresis in which stabilization is obtained by a suitable continuous sucrose gradient in a U-tube and where the ionic strength is kept low to prevent aggregation. The analytical system is also automated, which gives relatively high resolution.

Rough microsomes appear to possess a high negative surface charge, greatly exceeding that of the smooth microsomes. Removal of the ribosomes from rough microsomes with EDTA results in a surface charge similar to that of smooth microsomes. These facts agree well with previous findings regarding particle surface properties (DePierre & Dallner, 1975). Rough microsomes are easily influenced by low concentrations of monovalent cations, which have much less effect on smooth microsomes.

The chemical nature of the charged surface groups was studied by analysing the electrokinetic behaviour of particles after treatment with enzymes known to attack specific groups. No particles display any change after PLPase C and D treatment, indicating that neither the choline nitrogen nor the phosphate group of lecithin make any substantial contribution to the net surface charge. The possibility has been raised that the C-3 carbon of cholesterol is bound to the choline-N of lecithin, which in the isolated form is an amphoteric compound. In this bound form the negatively charged phosphate group could be near or on the surface, thereby contributing substantially to the negative charge of the membrane surface (Finean, 1953; Vandenheuvel, 1963). The experiment with PLPase C described here speaks against this possibility.

PLPase A increases the surface charge of smooth microsomes. It is not quite clear why PLPase A has this effect on microsomes, since the hydrolysis of glycerol-fatty acid ester bonds in the hydrophobic portion of the membrane cannot have a direct effect on surface charge. The liberation of fatty acids with or without liberation of glycerol-phosphoryl-base may cause conformational changes in surface proteins. Another possibility is that the solubilization of certain proteins (Imai & Sato, 1960) unmask negative groups previously buried beneath these proteins.
Trypsin and papain increase the surface charge of smooth microsomes and this may be due to removal of some positively charged moieties. Trypsin affects microsomes in a selective fashion: it solubilizes about 30% of the protein including, among others, 2 constitutive enzymes, NADPH-cytochrome c reductase and cytochrome b₅. Amino groups are major candidates for positive free groups. Since microsomal glycoproteins have only N-acylated amino sugars, these amino groups cannot contribute to the surface charge (Miyajima, Tomikawa, Kawasaki & Yamashina, 1969). Free amino groups are found as N-termini of peptide chains or on lysine and arginine and, therefore, proteins with such groups should be responsible for a part of the surface charge in intact microsomes. Regions of negative surface charge do not appear to be caused by the presence of lipid as demonstrated by the PLPase treatments, but probably originate from the carboxyl groups of surface proteins. These carboxyl groups may be in the C-terminal position or in other positions as part of glutamic and aspartic acids. The increased mobility observed after trypsin treatment is opposite to what one would expect if the enzyme remained adsorbed to the surface of the vesicles after treatment. The isoelectric point of trypsin is 9.3 and, consequently, such adsorption would diminish the negative surface charge substantially.

It appears that the removal of membrane proteins by trypsin increases the amount of anionic charges on the surface. For this reason, the nature of these 'unmasked' groups was studied by enzyme treatments. In smooth microsomes PLPase C alone was effective in restoring low mobility. The phosphate group of lecithin cannot be responsible for this decrease in mobility, since it is removed by PLPase C alone. It appears likely that after trypsin pretreatment PLPase C liberates a few specific proteins characterized by high negative charge. It is possible that at least a part of this negative charge is related to sialic acid residues. Previous investigations demonstrated that the surface of smooth microsomes does not contain sizeable amounts of freely accessible sialic acid residues (Winqvist, Eriksson, Dallner & Ersson, 1976). However, after proteolytic treatment one third of the sialic acid is liberated by neuraminidase. According to this idea some of the microsomal sialoproteins have their sialic acids directed towards the cytoplasmic side of the membrane, but these residues are masked by peripheral or loosely bound integral proteins.

The new membranes arising in phenobarbital-treated rats are chemically very similar to control membranes, but it is possible that they differ in structure. Low trypsin concentrations are effective in removing the same amount of electron-transport enzymes from both control microsomes and microsomes from induced rats, but treatment of the latter with trypsin does not remove enough protein to increase net negative surface charge density. This finding requires further investigation, since a better understanding of the structure of microsomes isolated from phenobarbital-treated rats could explain some of the functional modifications occurring during membrane biosynthesis.

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