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SARCOPLASMIC RETICULUM VESICLES AND GLYCOGEN-PROTEIN PARTICLES IN MICROSOMAL FRACTION OF SKELETAL MUSCLE

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Dedicated to Prof. W. Niemierko on his 80th birthday

1. Heavy microsomal fraction (HM) of rabbit skeletal muscle obtained by differential centrifugation between 8,000 - 30,000 g and consisting of sarcoplasmic reticulum (SR) vesicles contains variable amounts of glycogen and reveals some activity of phosphorylase b. The monomer of this enzyme of mol. wt. about 100,000 co-migrates in SDS-polyacrylamide gel electrophoresis with the main SR protein — Ca²⁺,Mg²⁺-dependent ATPase.

2. The highest specific activity of phosphorylase and the highest content of glycogen is present in the light microsomal (LM) fraction (30,000 - 100,000 g).

3. Contrary to the ATPase, phosphorylase b is released from the microsomal fraction by treatment with EDTA and is resistant to trypsin.

4. Both HM and LM fractions can be further fractionated on continuous sucrose density gradient at high speed. Main fraction of HM consists of highly purified SR vesicles. The second, small fraction of HM is identical with the main fraction of LM and consists of two populations: vesicles of structure and properties different from those of SR vesicles, and the particles of a complex of glycogen with some glycolytic enzymes.

Sarcoplasmic reticulum is an elaborate membranous system surrounding myofibrils. Its main function is storage of calcium during relaxation of muscle (Hasselbach, 1964; Martonosi, 1971). After homogenization of muscle, vesicles formed from the fragmented sarcoplasmic reticulum (SR)¹ are present in the heavy microsomal fraction sedimenting between 8,000 and 30,000 g. The light microsomal fraction

¹ Abbreviations: SR, sarcoplasmic reticulum; HM, heavy microsomal fraction; LM, light microsomal fraction; PHM, purified heavy microsomal fraction; PLM, purified light microsomal fraction; SDS, sodium dodecylsulfate; DOC, sodium deoxycholate.

[105]
sedimenting above 30,000 g has no relaxing activity (Hasselbach, 1964; Seraydarian & Mommaerts, 1965) and has not been carefully studied.

The protein composition of the heavy microsomal fraction is rather simple. The main integral protein, accounting up to 70% total protein, is a Mg\(^{2+}\), Ca\(^{2+}\)-dependent ATPase of mol. wt. about 100,000. In addition, two peripheral proteins of mol. wt. about 55,000 and 45,000, involved in calcium storage, are present; they are referred to, respectively, as the high affinity Ca\(^{2+}\)-binding protein and calsequestrin. Other proteins are present in rather small amount. (For review see MacLennan & Holland, 1976).

It has been pointed out by some authors (MacLennan, 1974) that the commonly used preparations of SR are contaminated by phosphorylase b, but this problem was never studied in detail.

Since the monomer of phosphorylase b has a mol. wt. close to that for ATPase (Taylor et al., 1975) it is difficult to demonstrate the presence of this enzyme in the preparations of SR by polyacrylamide gel electrophoresis in the presence of SDS.

Meyer et al. (1970) and Wansca & Drochmans (1972) found that phosphorylase b and some other enzymes involved in glycogen metabolism are present in skeletal muscle in the form of a complex with glycogen. The particles of this complex, forming distinct structural units of the cell, are present in the postmitochondrial fraction of skeletal muscle sedimenting at 80,000 g. In general, however, the authors studying the structure and function of sarcoplasmic reticulum membrane have not paid much attention to the possible presence of glycogen-protein particles in the preparations.

The aim of the present work was to determine the distribution of glycogen-protein particles during the fractionation of muscle microsomes and to elaborate possibly conditions for their removal during purification of SR vesicles.

MATERIAL AND METHODS

Chemicals. ATP, ethyleneglycol-bis (β-aminoethyl ether)-N,N'-tetraacetic acid, phosphorylase b, shellfish glycogen and trypsin were purchased from Sigma Chemical Company (St. Louis, Mo., U.S.A.), and SDS from Serva (Heidelberg, G.F.R.); \(^{42}\)CaCl\(_2\) was obtained from the Institute of Nuclear Research (Świerk, Poland).

All other reagents were of analytical grade.

Preparation of microsomal fraction. The microsomal fraction was isolated from skeletal muscle of adult rabbits. The trimmed muscle was minced and homogenized 2 x 30 sec with a Polytron PT-20 homogenizer in a solution containing 20 mM-imidazole, pH 7.2, and 100 mM-KCl, and subjected to differential centrifugation as described by Drabikowski et al. (1972). The pellets sedimented at 8,000-30,000 g and 30,000-100,000 g were suspended in the homogenization buffer and purified by centrifugation in continuous sucrose density gradient (0.25 - 2.0 m) at low speed (2600 g) for 60 min (Sreter, 1969; Drabikowski et al., 1972). Layers not penetrating into the gradient were collected, diluted with the same buffer solution, centrifuged
at 100,000 g, and the sediments used as the heavy (HM) and light (LM) microsomal fractions, respectively.

_Determination of enzymatic activity._ Calcium uptake and ATPase activities were determined as described by Sarzala _et al._ (1975). The $\text{Ca}^{2+}$-$\text{Mg}^{2+}$-dependent ATPase was calculated as the difference between total and $\text{Mg}^{2+}$-dependent ATPase. The activity of phosphorylase $b$ (EC 2.4.1.1) was determined as described by Cori _et al._ (1955).

_SDS-gel electrophoresis._ Samples were dissolved in a medium containing 1.2% SDS, 0.33% 2-mercaptoethanol, 20% glycerol and sodium phosphate buffer, pH 7.0, by heating for 5 min at 100°C. Bromophenol blue was used as tracking dye. The electrophoresis was carried out on 7.5% polyacrylamide gel essentially according to Weber & Osborn (1969) as described previously (Sarzala _et al._, 1974).

_Electron microscopy._ A droplet of the microsomal suspension in 100 mm-KCl and 20 mm-imidazole, pH 7.2 (about 1 mg/ml) was placed on collodion and carbon-coated grids and stained with 2% ammonium molybdate or 1% potassium phosphotungstate. To obtain thin sections, samples of the microsomal suspension were treated as described by Drabikowski _et al._ (1972). The grids were examined in a JEM 100B electron microscope (accelerating voltage 80 kV).

_Chemical analysis._ Protein concentration was determined by the procedure of Lowry _et al._ (1951), inorganic phosphate according to Fiske & SubbaRow (1925), and glycogen content by the procedure of Krisman (1962). Protein, which interfered with the glycogen-iodine reaction, was removed by the addition of 5% trichloroacetic acid.

**RESULTS**

The protein pattern of the heavy microsomal fraction, HM, is shown in Fig. 1, gel 1. In agreement with the previous work of several authors, the main protein band is 100,000 dalton one. Next in amount are 55,000 and 45,000 dalton proteins. Other proteins are present in minute and variable amounts in the faint bands of molecular weight higher as well as lower than that of ATPase. Virtually all of the latter bands, a part of the 100,000 dalton protein, as well as 55,000 and 45,000 dalton proteins, were released by EDTA (Fig. 1, gel 5). Unlike $\text{Mg}^{2+}$-$\text{Ca}^{2+}$-dependent ATPase (gel 2), which is easily degraded by trypsin (Sarzala _et al._, 1974; Stewart & MacLennan, 1974) the portion of 100,000 dalton material which was released by EDTA (gel 6), was trypsin-resistant. This behaviour corresponds to that of phosphorylase $b$, which is not split by trypsin under these conditions (gels 7 and 8).

Determination of phosphorylase $b$ activity in HM fraction (Table 1) showed that this enzyme was quantitatively released into the soluble fraction by treatment of microsomes with 1 mm-EDTA. Also 1 m-KCl in the presence of low concentration of deoxycholate (but not KCl or deoxycholate alone) led to the release of phosphorylase $b$. These results clearly indicated that conventionally prepared vesicles of sarcoplasmic reticulum contained activity of phosphorylase $b$. 
Fig. 1. SDS-polyacrylamide gel electrophoresis of proteins of heavy microsomal (HM) fraction. For preparation see Material and Methods. Untreated HM fraction—A. HM fraction after treatment with 1 mM-EDTA, pH 8.0: pellet, B, supernatant, C, pure preparation of phosphorylase b, D. Gels nos. 1, 3, 5, 7 represent samples not submitted to trypsin treatment, and gels 2, 4, 6, 8, the samples digested with trypsin. For trypic digestion, the samples (about 5 mg protein/ml) were incubated in the medium containing 100 mM-KCl, 20 mM-imidazole, pH 7.2, and 4 mM-CaCl₂, at 25°C for 10 min, at the trypsin to microsomal protein ratio of 1:300 (w/w). The reaction was stopped by addition of the SDS solution used for solubilization of the samples for gel electrophoresis (see Material and Methods).

Table 1

Distribution of phosphorylase b activity after extraction of heavy microsomal fraction

Samples of HM fraction (5 mg protein/ml) were treated at 4°C for 30 min either with 1 mM-EDTA at pH 8.0, 1 mM-KCl, deoxycholate Na salts (DOC) (0.25 mg/mg protein) or with 1 mM-KCl and DOC (0.25 mg/mg protein). The suspensions were then centrifuged at 100,000 g for 1 h and phosphorylase b activity was determined in the pellets and supernatants.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Phosphorylase b activity ( % of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extraction medium</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
</tr>
<tr>
<td>Pellet</td>
<td>3</td>
</tr>
<tr>
<td>Supernatant</td>
<td>97</td>
</tr>
</tbody>
</table>

The highest specific activity of phosphorylase b was found in the light microsomal fraction, i.e. the fraction sedimenting between 30,000 and 100,000 g, whereas the 100,000 g supernatant had the lowest specific activity. The distribution of glycogen was very similar (Table 2).
Table 2

Distribution of phosphorylase b activity and glycogen content in the subcellular fractions of skeletal muscle

Limit values are given, with the number of determinations in parentheses.

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>Glycogen (mg/g muscle)</th>
<th>Phosphorylase b (μmoles P_i/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy microsomes, 8,000 - 30,000 g</td>
<td>0.5 - 0.6 (3)</td>
<td>2.5 - 5.5 (8)</td>
</tr>
<tr>
<td>Light microsomes, 30,000 - 100,000 g</td>
<td>0.8 - 1.0 (3)</td>
<td>5.0 - 9.5 (5)</td>
</tr>
<tr>
<td>100,000 g supernatant</td>
<td>0.4 - 0.6 (3)</td>
<td>1.0 - 2.5 (5)</td>
</tr>
</tbody>
</table>

In the search for a method of separation of glycogen-protein particles from the vesicles, both heavy and light microsomal fractions were further fractionated by centrifugation on sucrose density gradient (0.25 - 2m) at high speed (Fig. 2). The heavy microsomal fraction (HM) was separated into two layers. The lighter one (denoted 1), accounting for about 10 - 15% of the total protein, was found at a density of 1.07 - 1.09. The main one (denoted 2) was found at a density of 1.11 - 1.20. Light microsomal fraction (LM) consisted essentially of one broad layer present at a density corresponding to that of layer 1. Control determinations have proved that layer 1 in HM fraction was in fact a contamination originating from LM fraction. Sometimes, the light microsomal fraction contained a small amount of material present at the level of layer 2 and originating from HM fraction.

![Fig. 2. Scheme of distribution of layers after high speed centrifugation of microsomal fractions on sucrose density gradient. Centrifugation was carried out in SW 27 rotor operated in Beckman L5-50 centrifuge at 131,000 g for 1 h. A, heavy microsomal fraction; B, light microsomal fraction, both after purification at low speed (see Material and Methods); C, fraction of muscle homogenate sedimenting between 8,000 - 100,000 g and not purified on sucrose gradient at low speed. The position of the layers was established visually, by their turbidities. After removal, each layer was diluted with 100 mM-KCl and 20 mM-imidazole, pH 7.2 and centrifuged at 100,000 g for 1 h. The obtained pellets were suspended in the same KClimidazole solution.](image-url)
When the fraction of muscle homogenate sedimenting between 8 000 and 100 000 g was fractionated directly under the same conditions, four layers were obtained: two of these corresponded to the layers 1 and 2; layer 3 was brownish in colour and contained mitochondria and layer 4 was the pellet consisting of myofibrillar material. The latter two layers were removed from microsomal fractions during purification on the sucrose gradient at low speed.

The layers 1 and 2 were used for further studies and are designated as the purified, light and heavy microsomal fractions (PLM and PHM), respectively. In Table 3 the enzymatic activities of both fractions are presented. The PHM fraction had a lower Mg\(^{2+}\)-dependent ATPase activity, but a much higher activity of Ca\(^{2+},\text{Mg}\(^{2+}\)-dependent ATPase than the light microsomal fraction. Only the heavy microsomal fraction was able to accumulate calcium. On the other hand, the entire phosphorylase b activity and the total glycogen content was present in the light microsomal fraction.

<table>
<thead>
<tr>
<th>Assay</th>
<th>PHM</th>
<th>PLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg(^{2+})-dependent ATPase</td>
<td>0.13 - 0.15</td>
<td>0.18 - 0.20</td>
</tr>
<tr>
<td>((\mu)moles P(_i)/mg protein/min)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>Mg(^{2+},\text{Ca}(^{2+})-dependent ATPase</td>
<td>0.6 - 0.8</td>
<td>0.4 - 0.5</td>
</tr>
<tr>
<td>((\mu)moles P(_i)/mg protein/min)</td>
<td>(15)</td>
<td>(9)</td>
</tr>
<tr>
<td>Ca(^{2+}) accumulation</td>
<td>3.5 - 4.0</td>
<td>0.02 - 0.05</td>
</tr>
<tr>
<td>((\mu)moles Ca(^{2+})/mg protein/5 min)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>Phosphorylase b</td>
<td>0.0</td>
<td>7.0 - 9.5</td>
</tr>
<tr>
<td>((\mu)moles P(_i)/mg protein/min)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td>Glycogen</td>
<td>0.0</td>
<td>0.8 - 1.5</td>
</tr>
<tr>
<td>(mg/g muscle)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

A comparison of protein pattern of the heavy microsomal fraction before (Fig. 1, gel 1) and after purification on a sucrose gradient at high speed (Fig. 3) revealed that all minor protein bands disappeared, so that the PHM fraction contained only three proteins of mol. wt. about 100 000, 55 000 and 45 000. Treatment with EDTA removed only the 55 000 and 45 000 dalton bands from PHM fraction, and did not remove any trace of the 100 000 dalton band (Fig. 3, gel 3).

The 100 000 dalton band was also present in the PLM fraction, which contained, moreover, a 160 000 dalton band and the other faint bands. On the other hand, the 55 000 and 45 000 dalton bands were only very faint. The supernatant of the EDTA-treated PLM fraction contained, in addition to the 55 000 and 45 000 dalton
bands, most of the 160 000 dalton band and a large part of the 100 000 dalton band (Fig. 3, gel s), accounting up to 40% of the total protein of the PLM fraction. In the pellet two proteins bands at the level of 100 000 daltons were clearly visible, the upper, corresponding to the ATPase and the lower, present in small amounts, corresponding to phosphorylase b (Fig. 3, gel p). Almost all of the activity of phosphorylase b was present in the supernatant.

![Image of SDS-gel electrophoresis patterns](image)

Fig. 3. SDS-gel electrophoretic pattern of proteins of EDTA-treated and trypsin-digested purified light (PLM) and heavy (PHM) microsomal fractions. Samples of PLM and PHM fractions were extracted with 1 mM-EDTA. After subsequent centrifugation, protein pattern was analysed in the pellets (p) and supernatants (s). Both fractions were digested at ratio of 1:300 of trypsin to microsomal protein for the time indicated in the Figure.

The digestion of the PHM fraction with trypsin led to a rapid disappearance of all of the 100 000 dalton band (Fig. 3). First the fragments of about 55 000 and 45 000 daltons appeared, followed by 30 000 and 20 000 dalton peptides, in agreement with the observations of Stewart et al. (1976). On the other hand, most
of 100 000 dalton band in the light microsomal fraction was resistant to trypsin (Fig. 3) and only a part, probably corresponding to the ATPase, was digested. It seems, moreover, that its rate of splitting was much slower than that in the PHM fraction and that the pattern of peptides formed during the digestion of the ATPase from the PLM fraction differed somewhat from that formed during the digestion of heavy microsomes. The protein of 160 000 mol. wt. disappeared during digestion, being split first to the 130 000 dalton protein.

In Fig. 4 the ultrastructure of the vesicles present in the two purified microsomal fractions is shown. Vesicles in the PHM fraction revealed in negative staining 4 nm surface particles typical of fragmented sarcoplasmic reticulum (Ikemoto et al., 1968). In the PLM fraction, vesicles of a similar size were present. Their surface, however, seemed to differ from that of vesicles in heavy microsomes. On the other hand, no difference in the thickness of the membrane of the vesicles from both preparations could be found in the thin sectioned material.

The light microsomal fraction contained, in addition, particles of glycogen-protein complex. These were seen both in negatively stained preparations and in thin sections of embedded material. These particles disappeared after EDTA-treatment, in agreement with the biochemical observations.

DISCUSSION

The existence in muscle of a complex of glycogen with several enzymes involved in its metabolism such as phosphorylase, phosphorylase kinase, phosphorylase phosphatase and glycogen debranching enzyme has been demonstrated by Meyer et al. (1970), Nelson et al. (1972) and Wanson & Dorchans (1972). It is not widely recognized, however, that preparations of SR obtained by differential centrifugation can be contaminated by the particles of this complex. Only few authors noticed that such preparations contain phosphorylase b activity. It is difficult to detect the presence of this protein by SDS-polyacrylamide gel electrophoresis of SR. Because of very similar molecular weights of the monomer of phosphorylase b and Mg²⁺,Ca²⁺-dependent ATPase, both proteins usually co-migrate under these conditions. However, Louis et al. (1974) obtained two protein bands in the region of 100 000 daltons under certain conditions of electrophoresis and reported that one of these bands was resistant to trypsin. The authors used the preparation of sarcoplasmic reticulum vesicles obtained by differential centrifugation according to Martonosi (1968) and washed with 0.6 M-KCl. As has been shown in the present work, this procedure does not lead to the removal of phosphorylase b.

The presence in SR preparations of the other proteins of glycogen metabolism can be also assumed by examination of the protein pattern of SR preparations published by other authors (Migala et al., 1973; Meisner et al., 1973; MacLennan, 1975). The minor protein bands found in SDS-polyacrylamide gels may correspond to glycogen synthetase of mol. wt. 88 000, the subunits of phosphorylase kinase of mol. wt. 128 000 and 145 000, and to the debranching
Fig. 4. Ultrastructure of PHM and PLM fractions. A. Negative staining of PLM fraction. *Insert*: high magnification of a typical vesicle. In addition to the vesicles, the particles with the shape and size of glycogen particles are often visible (arrows). B. PLM fraction examined in thin sections. Agglomerations of granular densely lead-stained material, corresponding to glycogen-protein complex, can be seen (arrows). C. Negative staining of vesicles of PHM fraction. Only spherical, thin-walled vesicles with 4 nm surface particles are present. *Insert*: high magnification of a typical sarcoplasmic reticulum vesicle. D. PHM fraction examined in thin sections. The majority of vesicles appear empty. Bars indicate 0.5 μm, and for inserts 0.1 μm.

M. Michalak *et al.* (facing p. 112).
enzyme of mol. wt. 160 000 (Taylor et al., 1975). The 160 000 dalton protein was reported by Yu et al. (1975) to be the only SR protein laying on the surface of the membrane. Our results strongly suggest that this protein belongs not to the SR membrane but to the glycogen particles, and may correspond to debranching enzyme.

Commonly used differential centrifugation procedures for preparation of SR do not fully remove glycogen-protein particles. Often SR vesicles obtained by differential centrifugation are treated with 0.6 M-KCl in order to get rid of contamination by myofibrils (Martonosi, 1968). The results of this paper show that under these conditions phosphorylase is not removed. Ostwald & MacLennan (1974) reported that most of phosphorylase b and phosphorylase kinase activities can be removed from sarcoplasmic reticulum vesicles by repeating washing and centrifugation, leaving the peripheral 55 000 and 45 000 dalton proteins bound to the membrane.

In our previous work (Drabikowski et al., 1972; Sarzala et al., 1974) we have used preparations of SR purified by continuous sucrose gradient centrifugation at low speed, the procedure developed by Sreret (1969). By this method mitochondria, myofibrils and aggregated vesicles are completely removed. However, since the SR fraction does not enter the gradient under these conditions, the possible contamination by material lighter than SR vesicles is not eliminated.

The fractionation of muscle microsomes by sucrose density gradient centrifugation at high speed seems to be the best separation of intact glycogen-protein particles from SR vesicles. SR vesicles obtained by this procedure are not contaminated by other material and revealed high activity of Mg²⁺,Ca²⁺-dependent ATPase and ability for calcium uptake.

On the other hand, the PLM fraction consists of two distinct populations: glycogen-protein particles, and vesicles, whose ultrastructure and biochemical properties seem to differ from those of vesicles present in PHM fraction. Thus, the postulate of Wanson & Drochmans (1972), that the binding occurs between glycogen-protein particles and sarcoplasmic reticulum, does not receive support from the results of this work. However, it should be noted that Entman et al. (1976) reported that similar glycogen-protein particles are present in the microsomal fraction of heart muscle. In contrast to skeletal muscle, they are associated with sarcoplasmic reticulum vesicles.

Extraction of the material present in PLM fraction with EDTA results in the release of about 40% of total protein. Since EDTA causes dissociation of glycogen-protein particles (Gergely et al., 1975), all glycolytic enzymes are released into solution.

The treatment with deoxycholate alone does not release phosphorylase (Table 1), unless high concentrations of KCl are also present. Thus, the procedure of isolation of ATPase developed by MacLennan (1974), which includes a treatment of sarcoplasmic reticulum vesicles with KCl and deoxycholate, enables removal of all proteins present in glycogen-protein particles.

The question arises what is the origin of the vesicles present in the light microsomal fraction. The sarcoplasmic reticulum membrane is very asymmetrical one (Packer et al.,
1974) and the intramembranous particles originating from the ATPase are concentrated on the concave fracture face (Baskin, 1971). Chevallier et al. (1975) separated SR vesicles into two fractions and have shown that the density of intramembranous particles in the lighter fractions is greater on convex fracture face, which suggests that the vesicles in this fraction are inside-out. Several observations in this work seem to indicate that the vesicles present in PLM fraction are also inside-out. First, negative staining shows that the surface of the vesicles of this fraction differs from that of the vesicles present in the PM fraction. Second, the protein in the PLM fraction, corresponding to the ATPase seems to be split much more slowly by trypsin than the ATPase present in PM fraction. This might be explained by the accessibility of different part of the ATPase molecule to trypsin in the inside-out vesicles. Another observation is connected with the low content of 55,000 and 45,000 dalton proteins in the vesicles of light microsomal fraction. It has been shown by MacLennan (1975) that these proteins are located inside the SR vesicles. One might expect that in the inside-out vesicles the 55,000 and 45,000 dalton peripheral proteins, being loosely bound to the membrane, are released during fractionation and purification of SR membrane. The possibility cannot be excluded, however, that the vesicles present in the PLM fraction originate from other membranous system present in muscle cell.

Separation of sarcoplasmic reticulum vesicles by sucrose density gradient centrifugation was also achieved by Heuson-Stennson et al. (1972), Malam et al. (1975) and Meissner (1975). Since the lowest concentration of sucrose in the gradient used in their experiments was about 26 - 28%, it is probable that our light microsomal fraction, obtained at 18 - 23%, did not enter the gradient. Judging from the density, both fractions obtained by those authors originated from the material corresponding to our heavy microsomal fraction, which on prolonged centrifugation might resolve into two layers.

We wish to thank Miss A. Pisarek and Mr. S. Stachowski for excellent technical assistance.

REFERENCES

PĘCHERZYKI SARKOPLAZMATYCZNEGO RETIKULUM I CZĄSTKI KOMPLEKSU GLIKOGEN-ENZYMY GLIKOLITYCZNE WE FRAKCJI MIKROZOMALNEJ MIĘŚNI SZEKLETOWYCH

Stryższenie

1. Ciężka frakcja mikrozomalna (HM) z mięśni szkleowych królika otrzymana przez różnicowe wirowanie pomiędzy 8 000 - 30 000 g i składająca się z fragmentów sarkoplazmatycznego retikulum (SR) zawiera zmienne ilości glikogenu oraz aktywności fosforlaz b. Monomer tego enzymu o c. cząst. około 100 000 migruje w elektroforezie na żel poliakrylamidowym w obecności siarczanu dodecydy w jednym prążku z głównym białkiem błon SR – ATP-azą aktywowaną jonami wapnia i magnezu. 2. Najwyższa specyficzna aktywność fosforlazy i największa zawartość glikogenu jest obecna w frakcji lekkich mikrozomów (LM) sedimentującej pomiędzy 30 000 - 100 000 g.
3. Fosforylaza b, w przeciwieństwie do ATP-azy, ulega uwolnieniu z frakcji mikrosomalnej pod wpływem EDTA oraz, w przeciwieństwie do ATP-azy, jest odporna na działanie trypsyny.

4. Frakcje HM i LM poddano dalszemu rozdzielowi w gradiencie stężeń sacharydy stosując wysoką siłę odsieczową. Główna frakcja obecna w HM składa się z wysokiej czystości pęcherzyków SR. Druga, niewielka frakcja z HM jest identyczna z główną frakcją z LM i składa się z dwóch populacji: pęcherzyków o strukturze i właściwościach różnych od pęcherzyków SR oraz z cząstek kompleksu glikozenu z niektórymi enzymami glikolitycznymi.

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Note added in the proof.

We have been recently able to obtain the PLM fraction virtually free of glycogen-protein particles. This has been achieved by washing the crude LM fraction with 100 mM-KCl and 20 mM-imidazole solution and centrifugation at 100,000 g, as well as by prolongation of the time of fractionation on sucrose gradient. The PLM fraction obtained in that way contained, in addition to the main 100,000 dalton band, two bands corresponding to 55,000 dalton high affinity Ca\(^{2+}\) binding protein and 45,000 dalton calsequestrin. Other bands were present only in minute amount. Contrary to the observations presented in this paper (Fig. 3, p. 111), the peptide pattern obtained by the digestion of this PLM fraction was found to be virtually the same as that obtained in the case of PHM fraction. However, an essential difference between both fractions was observed: the 55,000 dalton fragment accumulated during digestion of PLM fraction, whereas in the case of PHM fraction an accumulation of 45,000 dalton fragment took place. These observations, as well as the results of other experiments supporting the view that the PLM fraction contains "inside-out" vesicles of sarcoplasmic reticulum, will be published elsewhere.