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THE ENZYME ACTIVITY IN A DETERGENT-TREATED SARCOLEMMA OF SKELETAL MUSCLES

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SUMMARY

From rabbit skeletal muscles, a fraction of plasma membrane sarcolemma was isolated. The sarcolemma was treated with nonionic and anionic detergents. Investigations resulted in the following conclusions:

1. 0.1% solution of Triton X-100 separates off from the sarcolemma 25–33% of the protein, which is almost completely sedimented by ultracentrifugation at 105000 × *g*. 80–85% of the phospholipids remain in the ultracentrifugate. Thus, both in the sarcolemma and its fragments, following treatment with Triton X-100, the phospholipid content is greatly reduced.

The specific activity of ATPase (EC 3.6.1.3), acetylcholinesterase (EC 3.1.1.7) and AMP-aminohydrolase (EC 3.5.4.6), after treatment of sarcolemma with Triton X-100, undergoes no changes.

2. 0.5% solution of sodium deoxycholate separates off 60–70% of the sarcolemma protein, most of which is not sedimented by ultracentrifugation. Just as after treatment with Triton X-100, the main part of the phospholipids remains in the ultracentrifugate. 0.5% solution of sodium deoxycholate has an inactivating effect both on ATPase and AMP aminohydrolase. The activity of sarcolemma acetylcholinesterase after treatment with sodium deoxycholate becomes substantially increased.

3. Sodium dodecyl sulfate at a concentration of 0.001% separates off from the sarcolemma 30–40% of the protein, which is almost entirely sedimented by ultracentrifugation at 105000 × *g*. Corresponding to this amount a distribution of phospholipids takes place. Acetylcholinesterase and AMP aminohydrolase after treatment with sodium dodecyl sulfate are mainly accumulated in the fraction sedimented at 5000 × *g*. A part of the ATPase activity becomes transferred to the fraction sedimented by ultracentrifugation, with an increase in its specific activity.

INTRODUCTION

An important question of membrane molecular organization is one concerned with the arrangement of principal membrane components, proteins and lipids, and also their interaction forces. A certain role in solving this problem belongs to studies on membrane fragmentation with detergents. Such work enables the investigator to study the nature of interaction between membrane components, as well as the

properties of obtained enzymes and the importance of phospholipids for an active conformation of the membranous proteins.

The majority of authors in their work on membrane fragmentation have tried to achieve a complete solubilization of the membranes, whereby no care has been taken to preserve the enzyme activity in the obtained fragments[1].

In our studies, we selected an appropriate detergent concentration which permitted us to preserve the activity of enzymes. We studied ratios of proteins and phospholipids, both in solubilized and insoluble parts of sarcolemma. We investigated the activities of ATPase (EC 3.6.1.3), acetylcholinesterase (EC 3.1.1.7) and AMP aminohydrolase (EC 3.5.4.6) in skeletal muscle sarcolemma and the dependence of those enzymes on the amount phospholipids. The treatment of sarcolemma membranes with Triton X-100 (a nonionic detergent), sodium deoxycholate and sodium dodecyl sulfate (anionic detergent) enables us to evaluate the selectivity of these detergent actions.

METHODS

Isolation of the plasma membrane (*sarcolemma*) from rabbit skeletal muscle was essentially by the method of ROSENTHAL *et al.*[2] except that liberation of the isolated sarcolemmal tubules from residues of actomyosin gel was achieved by treatment of the suspension with an equal volume of a cooled 4 mM solution of sodium pyrophosphate (instead of an ATP solution according to ROSENTHAL *et al.*[2]).

A typical electron micrograph of sarcolemma isolated by this method is presented in Fig. 1. The suspension of isolated sarcolemmal tubules was fixed in 2% OsO_4 in 0.1 M barbital buffer at pH 7.4 dehydrated in a graded series of ethanol and embedded in butyl- and methylmethacrylate (1:1, by vol.).

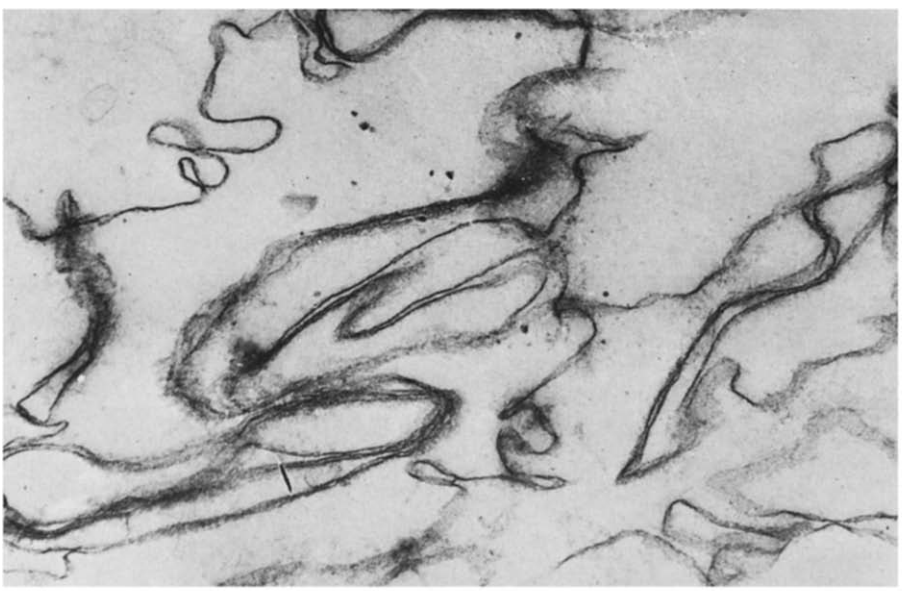

Fig. 1. Fraction of plasma membrane of skeletal muscle sarcolemma, magnification 35 000 ×.

Protein concentration was determined by the method of LOWRY *et al.*[3] with bovine serum albumin as the standard.

Phospholipids were extracted after having precipitated sarcolemma protein in the cold using 5% trichloroacetic acid in chloroform–methanol (2:1, by vol.) by the method of FOLCH *et al.*[4]. Total lipid phosphorus values were determined by the method of CHEN *et al.*[5], following digestion with $HClO_4$ and H_2SO_4.

Conditions of treatment with detergents. The sarcolemma suspension in double-distilled water was incubated in the presence of a detergent for 30 min at 0°. The suspension was centrifuged at 5000 × *g* for 10 min, and the sediment was washed twice with a detergent solution of initial concentration, with an additional washing using cooled double-distilled water. The centrifugates were combined and subjected to ultracentrifugation at 105000 × *g* for 1 h. The following results were obtained: the first fraction, the detergent-treated sarcolemma, sedimented at 5000 × *g* for 10 min; the second fraction, sarcolemma fragments, sedimented at 105000 × *g* for 1 h; and the third fraction did not sediment at 105000 × *g*.

ATPase activity was determined using Ca^{2+} and Mg^{2+} as activators. Incubation samples contained 25 mM Tris–HCl buffer (pH 7.6), 3 mM $CaCl_2$ or $MgCl_2$, and 3 mM ATP (as Tris salt). The activity was evaluated by the amount of the separated inorganic phosphorus which was determined by the method of FISKE AND SUBBAROW[6].

Acetylcholinesterase activity was measured by the hydroxamate assay of HESTRIN[7]. Incubation medium contained 3 mM acetylcholine chloride, 20 mM Tris–HCl buffer (pH 7.8), 100 mM NaCl and 50 mM $MgCl_2$.

AMP-aminohydrolase activity was determined spectrophotometrically, recording the formation of inosinic acid at 265 mμ (ref. 8). The incubation mixture for determining enzyme activity contained 6 μmoles adenylic acid and 50 mM Tris–HCl buffer (pH 7.0) at a final volume of 1 ml.

RESULTS

Influence of detergents

Triton X-100, sodium deoxycholate and sodium dodecyl sulfate were added to the membrane suspension (containing 1 mg membrane protein per ml) to a final concentration of 0.1, 0.5 and 0.001% (w/v), respectively.

The amount of protein separated off from sarcolemma by 0.1% Triton X-100 was found to vary between 25 and 35% in different preparations. These proteins were almost completely sedimented by centrifugation at 105000 × *g* for 1 h (Table I). The percentage of separated phospholipids equalled 80–85%, the major part of them remaining in the ultracentrifugate (Table I).

Thus, the treatment of sarcolemma by 0.1% Triton X-100 enabled us to eliminate 80–85% of phospholipids from sarcolemma and to study the effect of that elimination upon the enzyme activity.

Sodium deoxycholate in a final concentration of 0.5% (w/v) separated off 60–70% of sarcolemma protein, the main part of which failed to sediment during ultracentrifugation for 1 h at 105000 × *g*. Just as after treatment with Triton X-100, the main part of phospholipids remained in solution, *i.e.* a reduction of both the first and second fractions with phospholipids had taken place (Table I).

Sodium dodecyl sulfate in a final concentration of 0.001% (w/v) separated off

TABLE I

DISTRIBUTION OF PROTEIN AND PHOSPHOLIPIDS (%) IN FRACTIONS FOLLOWING SARCOLEMMA TREATMENT WITH DETERGENTS

Triton X-100, sodium deoxycholate and sodium dodecyl sulfate were added to the membrane suspension (containing 1 mg membrane protein per ml) to a final concentration of 0.1, 0.5 and 0.001%, respectively. The mixture was incubated on ice for 30 min. The first, second and third fractions were obtained as described in METHODS. This table as well as the next ones present results of one characteristic experiment selected out of ten carried out.

Fraction	*Detergents*		
	Triton	*Sodium deoxycholate*	*Sodium dodecyl sulfate*
The first fraction			
Protein	70.0	37.0	62.5
Phospholipids	19.0	12.0	65.0
The second fraction			
Protein	25.0	18.0	35.5
Phospholipids	7.0	9.0	31.0
The third fraction			
Protein	5.0	45.0	2.0
Phospholipids	74.0	79.0	4.0

from sarcolemma 30–40% of its protein which was almost competely sedimented by ultracentrifugation at 105000 × *g* for 1 h. A phospholipid content per 1 mg of protein in the first and second fractions remained equal to its content in the native sarcolemma.

Effects of detergents upon the enzyme activity

The low level of phospholipid content in the first and second fractions after sarcolemma treatment with Triton X-100 did not cause significant reduction of specific ATPase activity. Contrary to that, in the second fraction the specific ATPase activity is, as a rule, twice as high as in the initial native material.

Sodium dodecyl sulfate, exerting a quite different action upon the sarcolemma phospholipids (Table I) in comparison with Triton X-100, produced upon ATPase a similar effect to that of the latter: a considerable increase of the specific ATPase activity in the second fraction. Evidently, the observed increase of ATPase activity is explained by sarcolemma fragmentation which results in a greater accessibility of active sites to the substrate.

Sodium deoxycholate resulted in inactivation of ATPase. In sarcolemma fragments the ATPase activity could be revealed only after the elimination of detergent by ultracentrifugation. The enzyme activity in the presence of Ca^{2+} showed a greater stability to sodium deoxycholate than in the presence of Mg^{2+}. Presumably, the inactivation resulted from conformational changes of the enzyme molecule caused by detergent (Table II).

As may be seen from Table III, a marked decrease in phospholipid content, both in the first and second fractions after treatment with Triton X-100, did not alter the specific activity of acetylcholinesterase.

The treatment of sarcolemma with sodium deoxycholate resulted in a substantial increase of acetylcholinesterase specific activity in the first and second fractions. At the same time the determination of phospholipid content of these fractions

TABLE II

EFFECT OF DETERGENTS UPON THE SARCOLEMMA ATPASE ACTIVITY

The ATPase activity was measured at 37°. Reaction mixture contained 50 μg sarcolemma protein, 25 mM Tris–HCl buffer (pH 7.6), 3 mM $MgCl_2$ or 3 mM $CaCl_2$ and 3 mM ATP (as Tris salt). The activity of all studied enzymes in "Original sarcolemma" was assayed without added detergents. The first and second fractions were washed free of detergents.

Detergent		*ATPase activity (μmoles P_i/mg protein per h)*		
		Original sarcolemma	*Fractions*	
			First	*Second*
Triton X-100	Ca^{2+}	60.7	50.7	145.3
	Mg^{2+}	31.1	26.3	66.6
Sodium dodecyl sulfate	Ca^{2+}	88.9	67.0	122.0
	Mg^{2+}	34.0	28.1	64.5
Sodium deoxycholate	Ca^{2+}	66.1	12.9	44.0
	Mg^{2+}	31.4	0.0	9.5

TABLE III

EFFECTS OF DETERGENTS UPON THE SARCOLEMMA ACETYLCHOLINESTERASE ACTIVITY

The acetylcholinesterase activity was measured at 37°. Reaction mixture contained 0.2–0.5 mg protein, 3 mM acetylcholine chloride, 20 mM Tris–HCl buffer (pH 7.8), 100 mM NaCl and 50 mM $MgCl_2$.

Detergent	*Acetylcholinesterase activity (μg acetylcholinesterase/mg protein per h)*		
	Original sarcolemma	*Fractions*	
		First	*Second*
Triton X-100	883	845	880
Sodium dodecyl sulfate	740	1600	0
Sodium deoxycholate	543	1725	1000

showed a decrease in phospholipids, as after treatment with Triton X-100 (Table I). It should be noted that the protein which had remained in solution after ultracentrifugation (up to 50% of the native sarcolemma content) failed to show any presence of acetylcholinesterase activity. Evidently, this accounts for the increase of the acetylcholinesterase activity both in the first and second fractions. Thus, the almost equal effects of Triton X-100 and sodium deoxycholate upon the phospholipid distribution in the fractions were accompanied by entirely different actions of these detergents upon the fractional distribution of protein in particular, acetylcholinesterase. The enzyme specific activity in a fraction of the sarcolemma treated by sodium dodecyl sulfate increased 2- or 3-fold. In that fraction 90–100% of the initial acetylcholinesterase activity was concentrated.

Triton X-100 exerted the same effect upon AMP-aminohydrolase as upon acetylcholinesterase (Table IV). Sodium deoxycholate resulted in a decrease of AMP aminohydrolase activity in the first fraction. Both the second and third fractions

showed no enzyme activity. Sodium dodecyl sulfate separated off from sarcolemma proteins, which possessed no AMP aminohydrolase activity. At the same time a marked elevation of the AMP aminohydrolase activity was noted in the first fraction.

TABLE IV

EFFECT OF DETERGENTS UPON THE SARCOLEMMA AMP AMINOHYDROLASE ACTIVITY

The AMP aminohydrolase activity was measured at 37°. The sample contained 50–100 μg protein, 50 mM Tris–HCl buffer (pH 7.0) and 6 μmoles AMP.

Detergent	*AMP aminohydrolase activity (μmoles AMP/mg protein per 10 min)*		
	Original sarcolemma	*Fraction*	
		First	*Second*
Triton X-100	26.6	21.6	20.0
Sodium dodecyl sulfate	22.3	32.9	0.0
Sodium deoxycholate	33.0	7.3	0.0

DISCUSSION

The majority of authors, having studied the effects of detergents upon cellular membranes, mainly paid attention to physicochemical characteristics of the obtained fragments (particle size, homogenity, molecular weight, chemical composition)[9–12]. Therefore the problem of enzyme distribution and the preservation of their activity was almost not considered at all.

We studied the distribution of protein and phospholipids in obtained sarcolemma fragments and the effect of phospholipid elimination, under the action of detergents, upon the activity of enzymes. The results obtained in this study show that the treatment of sarcolemma by the non-ionic detergent Triton X-100 (which causes the elimination of 80–85% phospholipids) did not diminish the activity of any of the enzymes under study.

Evidently, the phospholipids eliminated by this treatment were unnecessary for the studied enzymes of the sarcolemma. However, we can not draw the conclusion that phospholipids were at all unessential for the activity of these enzymes. It is possible that the phospholipids not removed by Triton X-100 played a significant role in the maintenance of enzyme activity.

In comparing the effect of Triton X-100 upon the ATPase of sarcolemma and other intracellular structures (mitochondria and microsomes)[13], it should be noted that a full inactivation of this enzyme in microsomes was presumably caused by a different structure in the membrane cell components.

The anion detergents (sodium deoxycholate and sodium dodecyl sulfate) exerted a different effect upon the activity of sarcolemma enzymes. Sodium deoxycholate in a concentration of 0.5% (inactivating ATPase and AMP aminohydrolase) substantially increased the specific activity of acetylcholinesterase in the treated sarcolemma. Sodium dodecyl sulfate in a concentration of 0.001% transferred into the second fraction up to 40% of the protein, with an ATPase specific activity almost

2-fold that of native sarcolemma. However, the protein of this fraction did not possess any acetylcholinesterase or AMP aminohydrolase activity; these two enzymes remained in the treated sarcolemma. Hence dissociation between the membrane components, induced by the anion detergents, caused a different effect up on the studied enzymes.

The interaction of anion detergents with sarcoplasmic membrane proteins has a complicated character, because it may result from changes both in hydrophobic and electrostatic bonds. However, the different direction in the action of anion detergents, as compared with the non-ionic detergent Triton X-100, suggests in the present case a disturbance of electrostatic interaction to be more substantial. This is proved by the action of sodium dodecyl sulfate: this detergent separates sarcolemma proteins and phospholipids at the same ratio as in the native sarcolemma, suggesting the destruction of protein–protein bonds but not phospholipid–protein bonds. Preferential separation of phospholipids from sarcolemma with Triton X-100 suggests this detergent mainly destroys hydrophobic bonds.

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