Biochimica et Biophysica Acta, 556 (1979) 105—117

BBA 78477

SMOOTH MUSCLE CELL SARCOLEMMA

PURIFICATION AND PROPERTIES OF PLASMA MEMBRANES FROM THE RAT UTERUS

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(Received November 16th, 1978)
(Revised manuscript February 23rd, 1979)

Key words: Myometrium; Smooth muscle; Sarcolemma; Plasma membrane; (Equilibrium centrifugation)

Summary

A membrane fraction with sarcolemmal properties was purified from the smooth muscle layers (myometrium) of rat uterus by successive differential and equilibrium centrifugation in sucrose. The putative sarcolemmal fraction was identified by iodination with [^{125}I]iodosulfanilic acid, had an equilibrium density of 1.15, and was enriched in enzyme activities usually associated with the plasma membrane including 5′-nucleotidase (EC 3.1.3.5) and ($Na^+ + K^+$) ATPase (EC 3.6.1.3). These membranes were free of mitochondrial or nuclear membrane contamination, suggesting the relative enrichment of sarcolemmal membranes in the fraction. Proteins of the membranes were heterogeneous with respect to molecular weight, but only a few were labelled when intact muscle was radioiodinated. Uniform resistance of sarcolemmal proteins to trypsin digestion and salt extraction suggested many are tightly bound or intrinsic membrane proteins and was a further indication of the homogeneity of membranes in this fraction.

Introduction

Many of the agents which regulate uterine motility act at the level of the smooth muscle plasma membrane or sarcolemma [1]. Despite this fact, sarco-

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lemmal properties other than the regulation of electrolyte permeability have received little attention [2,3], even though procedures for purification have been developed [4]. Preparatory to investigating the effects of regulators of uterine motility on sarcolemmal properties, we have characterized some structural features of these membranes. Isolation of the uterine smooth muscle sarcolemma was accomplished by a method which was rapid and yielded plasma membranes free of major contaminants by several criteria.

Materials and Methods

Source of materials

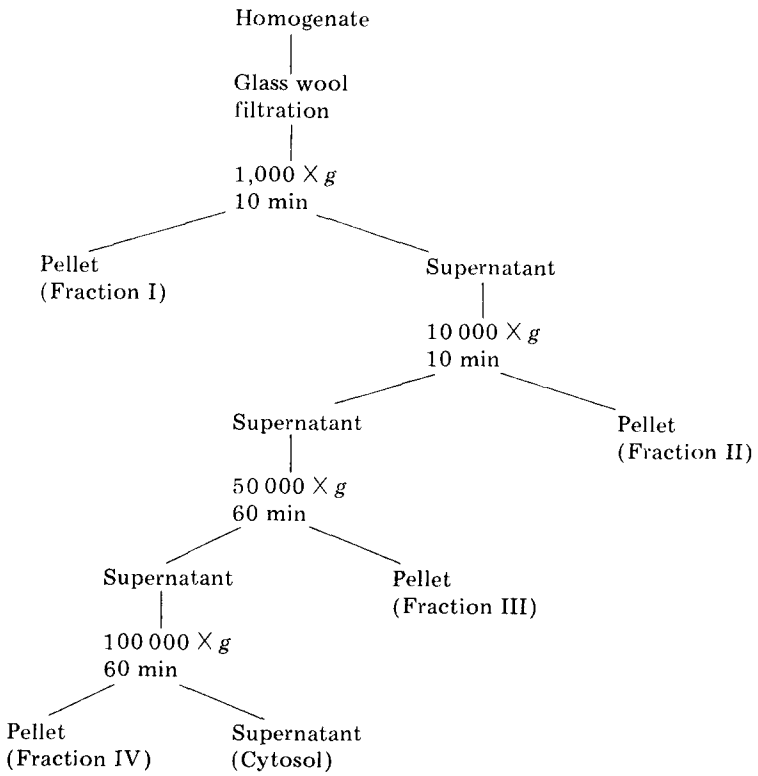
Sucrose was enzyme-grade, obtained from Schwartz-Mann (Orangeburg, NY). 5′-AMP (Na^+ salt), kinuramine, and 4-hydroxyquinoline were from Sigma (St. Louis, MO). ATP (Na^+ salt) was from Boehringer Mannheim (Indianapolis, IN), and α,β-methylene adenosine diphosphate was from BL Biochemicals, Inc. (Milwaukee, WI) and Calbiochem (San Diego, CA) respectively. Trichloroacetic acid was obtained from Fisher Scientific Co. (Pittsburg, PA).

Animals and tissue preparation

Uteri from 200 to 250 g Sprague Dawley rats were excised, rinsed in ice cold nomal saline and then placed in homogenization buffer (0.25 M sucrose; 0.01 M Tris-HCl, pH 7.4; 0.01 M $MgCl_2$; 0.0025 M dithiothreitol) at 4°C. In some experiments, 0.05 M histidine (pH 7.4) replaced Tris in the homogenization buffer, because the latter has been reported to have deleterious effects on myometrial ($Na^+ + K^+$)-ATPase activity [5]. Uteri were trimmed of fat, cut open lengthwise and endometrium removed by scraping with a glass microscope slide. The resultant muscle strips were minced with scissors and homogenized at 4°C in 10 volumes of homogenization buffer for 30 s using a Polytron (Brinkman Instruments, Westbury, NY) with a PT-10ST generator at a pulse frequency of 7.3 kHz (22 000 rev./min). Homogenization time, temperature, and generator output were regulated by a Tekmar Time-Temperature Control Module (Tekmar Company, Cincinnati, OH). All further steps were performed at 4°C.

Homogenate was filtered through glass wool and the filtrate centrifuged at the centrifugal forces and for the times indicated in Scheme 1. The resultant pellets were rinsed and resuspended in 1 volume of homogenization buffer with a hand-driven teflon-glass homogenizer. For some applications, the differential centrifugation step was shortened by centrifuging the filtered homogenate at 10 000 × *g* for 10 min. The pellet was then discarded and the supernatant re-centrifuged at 50 000 × *g* for 60 min to yield the F-III particulate fraction.

Protein concentrations were determined by the method of [6]. Aliquots of homogenate, membrane suspension, or cytosol were precipitated in 0.4 N perchloric acid and washed 3 times by centrifugation with cold acid to remove sucrose. The washed insoluble precipitate suspended in 0.4 N perchlorate was heated for 30 min at 90°C to hydrolyze nucleic acids. After chilling on ice for 30 min, the insoluble proteins were collected by centrifugation and dissolved in 0.1 N NaOH for protein determination using bovine serum albumin as standard. The acid soluble supernatant was assayed for DNA content according to the method of [7].



Scheme 1

Sucrose gradient centrifugation

Sucrose was dissolved in 0.01 M Tris-HCl (pH 7.4), 0.001 M EDTA, and 0.001 M dithiothreitol at the desired concentration and density determined and adjusted by refractive index. Linear sucrose gradients were prepared with a Buchler Universal Gradient Former and Autodensiflow fractionator (Buchler Instruments, Fort Lee, NJ). Discontinuous gradients were pipetted by hand. Both types of gradients were allowed to stand for 16 h at 4°C prior to use except when the vertical rotor was used. Membranes suspended in 0.25 M sucrose dissolved in the gradient buffer were centrifuged to equilibrium at 100 000 × *g* on the gradients using a variety of rotors including: Beckman SW 41 and SW 27 swinging bucket rotors; Beckman fixed angle Type 40 and 60 Ti rotors; and the Sorvall TV-850 vertical rotor. Gradients were fractionated either with an ISCO Model 183 with continuous ultraviolet monitoring (Instrumentation Specialties Co., Lincoln, NB) or with the Buchler Autodensiflow fractionator and absorbance at 280 nm of each fraction determined spectrophotometrically. When quantitative recovery of a discontinuous sucrose gradient fraction was not a consideration, visable bands could be conveniently withdrawn from the sucrose interface with a Pasteur pipet. Sucrose density of fractions was determined from refractive index.

Membranes were concentrated from gradient fractions by 2-fold dilution with 0.01 M Tris-HCl (pH 7.4), 0.001 M EDTA, 0.01 M dithiothreitol and centrifugation for 30 min at 100 000 × *g* at 4°C.

Membrane enzyme determinations

5'-Nucleotidase was quantified according to an already published procedure [8], except that free phosphorous was determined by the method of Fiske and Subbarow [9]. Result were corrected for values obtained in the absence of added protein, and no increase above these backgrounds occurred when AMP (or other phosphate) was omitted from the assay mixture. Phosphorous release from 5'-AMP by myometrial fractions was suppressed greater than 90 percent by the specific competitive inhibitor of 5'-nucleotidase activity α,β-methylene adenosine diphosphate [10].

$(Na^+ + K^+)$-ATPase was determined according to the method of Beauge and Glynn [11]. 0.03 M histidine (pH 7.5), 0.06 M KCl, 0.06 M NaCl, 0.025 M $MgCl_2$, and 0.001 M EGTA in a total volume of 0.45 ml were incubated at 37°C. Assay tubes containing the same mixture but without KCl or NaCl were incubated in parallel. After 5 min, 0.01 ml of protein was added to each series of tubes and incubation continued at 37°C for 3 min longer. 1.0 ml of ice-cold trichloroacetic acid was added and free phosphorous determined as above. $(Na^+ + K^+)$-ATPase activity, the difference between tubes with and without 0.06 M KCl/0.06 N NaCl, was up to 55% greater than basal activity and was suppressed by more than 95% by the cardiac glycoside ouabain.

Glucose-6-phosphatase was assayed by the method of Aronson and Touster [12]. Results were corrected for non-enzymatic substrate hydrolysis by incubating and processing a mixture of all the reactants except protein in every experiment.

Cytochrome c oxidase activity was determined by the method of Wharton and Tzagoloff [13]. If necessary, the protein concentration of the myometrial fraction was diluted so the reaction rate was linear during the entire course of incubation. Using these procedures, the cytochrome *c* oxidase specific activity of the most active myometrial fraction was more than 140% that of a mitochondrial fraction prepared from rat liver according to the procedure of Guerra [14].

Monoamine oxidase activity was measured as the rate of kynuramine conversion to 4-hydroxyquinoline according to the method of Krajl [15] as modified by Century and Rupp [16]. Enzymatic 4-hydroxyquinoneline production, corrected by subtraction for background fluorescence of a blank, was determined from a standard curve. The most active myometrial fraction had monoamine oxidase specific activity which was 125% that of rat liver mitochondria prepared according to the procedure of Guerra [14].

^{125}I iodination of sarcolemmal proteins

Myometrial cell surface proteins were radioiodinated using a New England Nuclear (Boston, MA) [^{125}I]iodosulfanilic acid labeling kit. [^{125}I]Iodosulfanilic acid (>1000Ci/mmol) was diazotized just before use by incubation with 0.05 M sodium nitrite under acidic condition at 4°C for 15 min, according to directions in the kit. Membranes (1.5 mg) from the S-1 sucrose gradient fraction or pieces of myometrium 0.5 X 3.0 mm (0.003 g) were washed and suspended in 0.475 ml of 0.02 M sodium phosphate buffer (pH 7.4) with 0.15 M NaCl. 1 mCi, 0.025 ml, of the diazotized [^{125}I]iodosulfanilate was added and the suspension was incubated on ice. Under these conditions, iodina-

tion was complete after 20 min. Radioiodinated muscle pieces were rinsed with unlabelled 0.001 M NaI in 0.02 M sodium phosphate(pH 7.4), 0.15 M NaCl and combined with the muscle strips from which they were initially cut for subsequent homogenization and fractionation. The [^{125}I]iodosulfanilic acid: S-1 membrane suspension was diluted to 1 ml with 0.02 N sodium phosphate buffered saline with 0.001 M NaI and the membranes concentrated by centrifugation. The pellet was washed twice more by centrifugation with buffered unlabelled NaI and finally dissolved in electrophoresis sample buffer.

SDS-polyacrylamide gel electrophoresis

The protein composition of the myometrial sarcolemma was electrophoretically analyzed using the SDS-polyacrylamide gel system of Laemmli [17] according to our previously published methods [18]. Membranes were dissolved in sample buffer (0.06 M Tris-HCl, pH 6.8; 3% SDS; 0.7 M 2-mercaptoethanol, 10% glycerol) by heating 10 min at 90°C. 30 to 50 μg of the solubilized membrane protein was electrophoresed in 8.75% polyacrylamide slab-gels with 0.1% SDS at 20 mA for 4 h. After electrophoresis, gels were fixed by soaking in 3.5% sulfosalicylic acid, 11.5% trichloroacetic acid, and 45% methanol, stained with Coomassie brilliant blue [19] and destained either electrophoretically or by diffusion. For subsequent autoradiography, destained gels were dried onto filter paper backing and exposed to DuPont Cronex mamography film for 3—8 days.

Apparent molecular weights of membrane proteins were estimated by migration rate relative to 5 standard proteins electrophoresed in parallel and covering the molecular weight range 12 600 to 212 000 [18].

Results

Myometrial homogenate, filtered through glass wool, was centrifuged to yield four particulate fractions and cytosol. The distribution of 5 membrane associated enzyme activities within the particulate fractions is shown in Fig. 1. Fraction III (F-III), sedimenting between 10 000 and 50 000 $\times g$, was enriched for 5′-nucleotidase (EC 3.1.3.5) and ($Na^+ + K^+$)-ATPase (EC 3.6.1.3), activities generally associated with the plasma membrane (sarcolemma). Substantial ($Na^+ + K^+$)-ATPase activity was also recovered in the 1000 $\times g$ sediment (F-I) which contained most of the smooth muscle cell nuclei by DNA analysis (Fig. 1). Electron microscopic examination of thin sections of F-III did not, however, reveal any membrane-bound ribosomes (not shown), so glucose-6-phosphate activity (EC 3.1.3.9) was not associated with elements of the rough endoplasmic reticulum in that fraction. Myometrial cytosol contained no detectable ($Na^+ + K^+$)-ATPase or mitochondrial enzyme activity, less than 20% of the 5′-nucleotidase activity, and 80% of the glucose-6-phosphatase activity (not shown).

To purify uterine smooth muscle cell sarcolemma further, the differential centrifugation fraction most enriched in plasma membrane enzyme activity (F-III) was centrifuged to equilibrium on linear sucrose gradients (Fig. 2). After 4 h at 100 000 $\times g$, a major peak with a light shoulder was resolved in the gradient, along with a pellet fraction. When membranes from the light shoulder

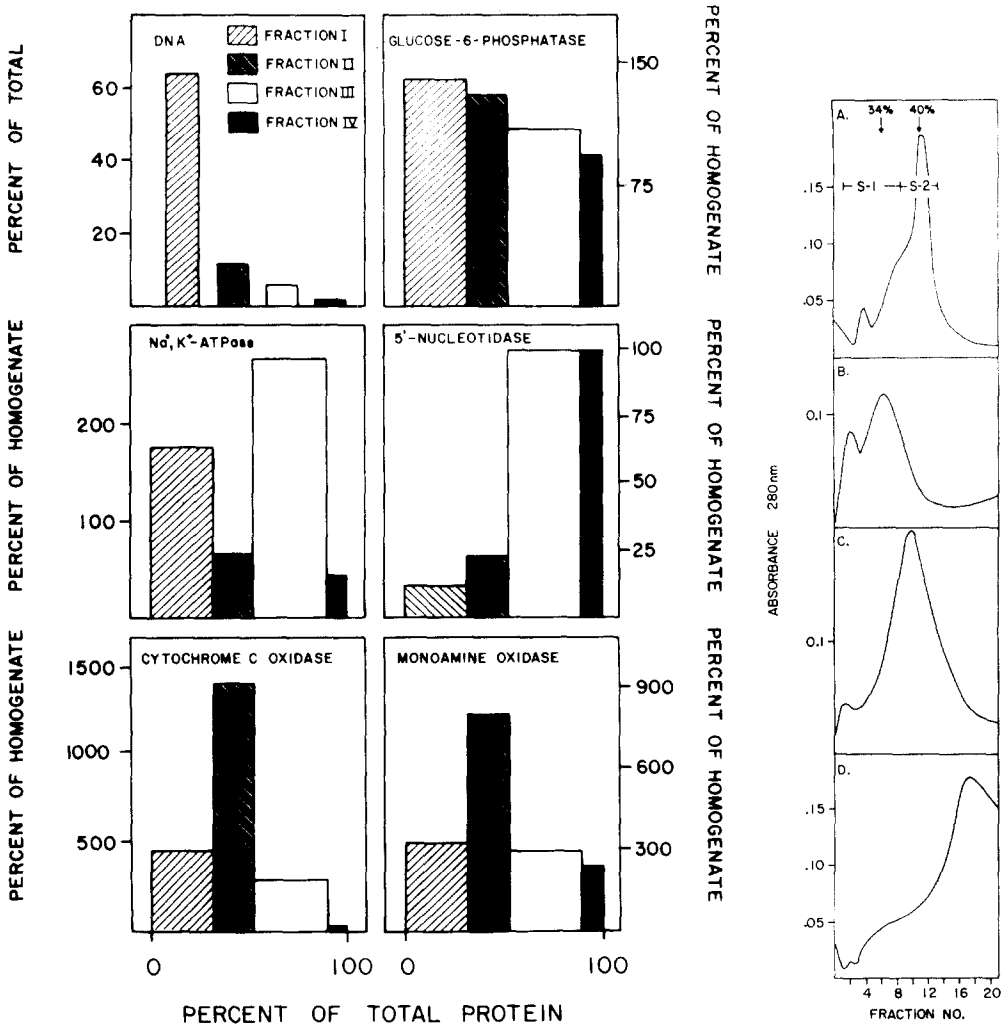


Fig. 1. Analysis of the distribution of myometrial DNA and membrane enzyme activities as a function of sedimentation coefficient as determined by differential centrifugation in 0.25 M sucrose. Myometrial homogenate was centrifuged at the centrifugal forces and the times indicated in Materials and Methods. The resultant pellets were resuspended and the indicated determinations made. Upper left: DNA content of fractions F-I to IV; ordinate, percent of total DNA in fractions F-I through F-IV. The remainder of the histograms are the distributions of the indicated enzyme activities expressed according to the method of deDuve et al. [39]; Ordinates, enzyme specific activity as a percent of the specific activity of the homogenate; abscissa, protein content of each particulate fraction as a percent of the total particulate protein so that the width of all four bars (F-I through F-IV) equals 100%. Values are the mean of 5 to 8 separate experiments with standard deviations less than 20% of the mean (ordinate) and 10% of the mean (abscissa) respectively.

Fig. 2. Analysis of F-III by density equilibrium centrifugation. (A), F-III resuspended in 0.25 M sucrose was applied to 20 to 50% linear sucrose gradients and centrifuged in a Beckman SW 41 rotor at 2°C for 4 h at 100 000 × *g*. Gradients were fractinated and the indicated fractions (S-1 and S-2) concentrated according to the procedure in Material and Methods. S-1 and S-2 were resuspended and applied to linear sucrose gradients of the same composition as in A and centrifuged under identical conditions but for 16 h (B, S-1; C, S-2). (D), F-II suspended in 0.25 M sucrose and centrifuged in linear density gradients as for A above. Sucrose concentration at the equilibrium density of the peak S-1 and S-2 fractions was determined by refractive index.

and main peaks (S-1 and S-2, respectively) were separated, concentrated, and re-centrifuged on similar gradients, each migrated as a largely homogeneous band whose position in the gradient was unaltered by continued centrifugation for up to 16 h. The equilibrium density of S-1 was determined to be 1.15 (34% sucrose) under these conditions and that of S-2 1.18 (40% sucrose). The sucrose density gradient pellet fraction (S-3) was similar in density (>1.24, 50% sucrose) to differential centrifugation fraction F-II, enriched in mitochondrial enzymes (Fig. 1).

The equilibrium densities of S-1 and S-2, determined by centrifugation on linear sucrose gradients, were used to construct discontinuous gradients which would adequately separate fractions S-1, S-2 and S-3 for subsequent characterization. F-III resuspended in buffered 0.25 M sucrose was layered over a discontinuous gradient of approximately equal volumes of 20, 35 and 40% sucrose and centrifuged to equilibrium (4 h, 100 000 × *g*). S-1 banded at the 20/35% interface, S-2 at the 35/40% interface, and S-3 pelleted (Fig. 3, upper). Protein and plasma membrane marker enzyme activity were about equally distributed between the three fractions, but only S-1 was consistantly free of mitochondrial enzyme activity (Tables I and II). Greater than 80% of the protein and enzyme activity loaded was recovered in gradient fractions S1, S2 and S3.

Time to reach equilibrium could be reduced by 75% without sacrificing resolution of S-1 and S-2 through use of the vertical ultracentrifuge rotor (Fig. 3, lower). Equilibrium centrifugation using the vertical rotor required a 60% sucrose pad at the bottom of the centrifuge tube to avoid trailing by the high density fraction (S-3) upon reorientation of the gradient during deceleration. Similar results with a comparable saving in time were obtained, however, by centrifuging the discontinuous sucrose gradients in a fixed angle rotor (Fig. 4). S-3 formed a firm pellet at the bottom of the tube in these rotors, precluding the need for a dense sucrose pad.

Table I contains results from a single fractionation and is representative of those obtained from 5 such complete purifications. The extent of S-1 enrichment in $(Na^+ + K^+)$-ATPase, 5′-nucleotidase, and glucose-6-phosphatase as well as monoamine oxidase and cytochrome *c* oxidase activity is shown in Table II. 5′-Nucleotidase, the membrane enzyme least widely distributed in differential centrifugation fractions F-I to F-IV (Fig. 1) showed a ten-fold increase in specific activity upon purification of S-1. S-1 contained negligable monoamine oxidase and no cytochrome *c* oxidase activity.

In addition to enzyme activity, the protein composition of S-1 membranes was further characterized by electrophoresis on SDS-polyacrylamide gels (Fig. 5). A few sarcolemmal proteins were definitively identified by incubating small pieces of myometrium with diazotized [^{125}I]iodosulfanilic acid, shown to exclusively label cell surface proteins [20,21]. The majority of the radioactivity was associated with the S-1 fraction and with the pellet, S-3 (not shown). Recovery of radioiodinated sarcolemma with S-3, which also contained mitochondrial enzyme activity (Table I), further suggested this dense fraction was composed primarily of an aggregated mixture of membranes. Membranes prepared from ^{125}I-iodinated myometrium analyzed by electrophoresis and autoradiography disclosed distinct labelling of four principal sarcolemmal peptides (Fig. 6). A more heterogeneous population of membrane protein was

TABLE I

ENZYMATIC CHARACTERIZATION OF FRACTIONS OF MYOMETRIAL HOMOGENATE

This single experiment is representative of the results obtained in five separate experiments. N.D., none detectable.

Fraction	$(Na^+ + K^+)$-ATPase * ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	5′-Nucleotidase * ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	Glucose-6-phosphatase * ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	Monoamine oxidase ** ($nmol \cdot mg^{-1} \cdot min^{-1}$)	Cytochrome *c* oxidase ** ($nmol \cdot mg^{-1} \cdot min^{-1}$)
Homogenate	0.063 ± 0.007	0.572 ± 0.141	0.037 ± 0.001	0.056	18.0
F-I	0.058 ± 0.036	0.000 ± 0.040	0.053 ± 0.022	0.189	30.9
F-II	0.000 ± 0.020	0.130 ± 0.085	0.049 ± 0.004	0.459	92.1
F-III	0.143 ± 0.048	0.476 ± 0.100	0.041 ± 0.006	0.133	14.7
S-1	0.210 ± 0.079	4.425 ± 0.316	0.171 ± 0.003	N.D.	N.D.
S-2	0.206 ± 0.019	2.965 ± 0.546	0.147 ± 0.006	N.D.	24.1
S-3	0.127 ± 0.033	0.538 ± 0.026	0.033 ± 0.000	4.0	23.2
F-IV	0.024 ± 0.016	0.473 ± 0.105	0.038 ± 0.002	N.D.	7.9
Cytosol	N.D.	0.080 ± 0.065	0.038 ± 0.002	N.D.	N.D.

* Mean of triplicate determinations ± S.D.
** Mean of duplicate determinations.

TABLE II

SUMMARY OF THE EFFECT OF ISOLATION OF S-1 MEMBRANES ON THE SPECIFIC ACTIVITY OF 5 MEMBRANE ENZYMES

Results are the mean ± S.E. of 8 separate preparations.

Fraction	$(Na^+ + K^+)$-ATPase * ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	5′-Nucleotidase * ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	Glucose-6-phosphatase * ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	Monoamine oxidase ** ($nmol \cdot mg^{-1} \cdot min^{-1}$)	Cytochrome *c* oxidase ** ($nmol \cdot mg^{-1} \cdot min^{-1}$)
Homogenate	0.053 0.011	0.468 0.019	0.042 0.003	0.069 0.017	5.8 2.8
S-1	0.314 0.022	4.481 0.100	0.213 0.020	0.007 0.003	0.0 0.0
$\frac{\text{S-1}}{\text{Homogenate}}$	6.0	9.6	5.1	0.1	0

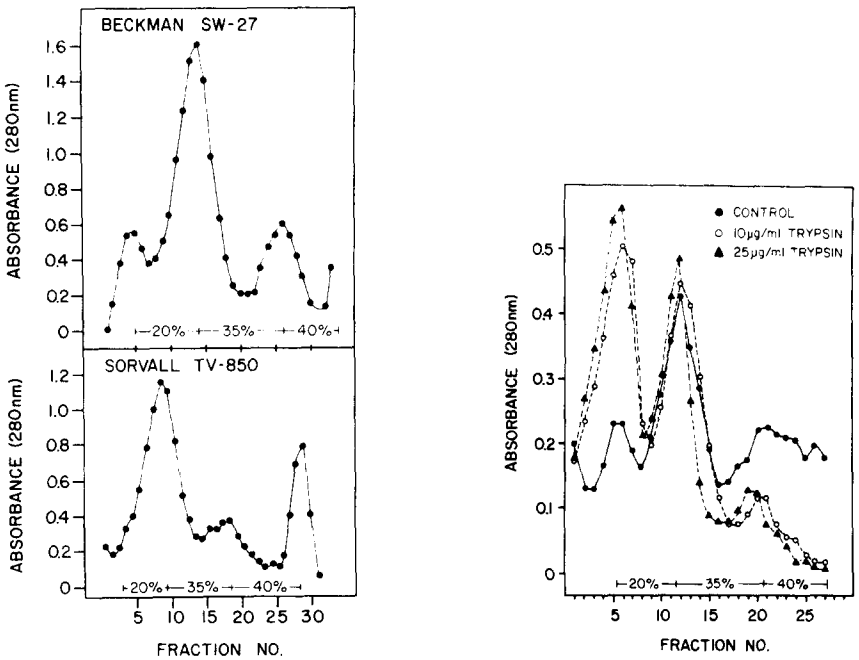


Fig. 3. Centrifugation of F-II to equilibrium on discontinuous sucrose gradients using swinging bucket (upper) and vertical (lower) rotors. F-III suspended in 0.25 M sucrose was applied to discontinuous sucrose gradients of the indicated composition. With the vertical rotor, it was necessary to construct the gradient on top of a 60% sucrose pad. Tubes were centrifuged at 96 000 to 100 000 × *g* for 60 min and fractionated according to the procedure in Materials and Methods.

Fig. 4. Resistance to trypsin digestion of the S-1 membrane fraction. Fraction F-III obtained by differential centrifugation according to the procedure in Materials and Methods, was suspended in 0.15 M histidine (pH 7.5) with 0.005 M CaCl and incubated with the indicated concentrations of trypsin for 15 min at 30°C. The mixtures were chilled and applied directly to discontinuous sucrose gradients and centrifuged to equilibrium at 100 000 × *g* for 60 min in a Beckman 60 T_i (fixed angle) rotor.

labelled when purified S-1 was incubated with diazotized [^{125}]iodosulfanilic acid, but the four principal sarcolemmal peptides were still prominent (Fig. 6).

All of the Coomassie brilliant blue-stained membrane proteins were resistant to extraction with 0.6 M LiBr or by sonication in 0.6 M LiBr with 0.1% deoxycholate (Fig. 5b and c). S-1 prepared from myometrium homogenized in isotonic buffer (0.15 M NaCl, not shown) had electrophoretic patterns identical with those in Fig. 6. S-1 proteins showed marked resistance to digestion with trypsin (Fig. 5d) and enzyme concentrations as high as 25 μg/ml did not change the distribution of the membrane fraction in discontinuous sucrose gradients (Fig. 4). Trypsin treatment of F-III did, however, convert at least a portion of S-2 and S-3 to elements which did not enter the gradients.

Discussion

We have used a modification of the sucrose gradient method of smooth muscle cell sarcolemma purification [4] to characterize these membranes

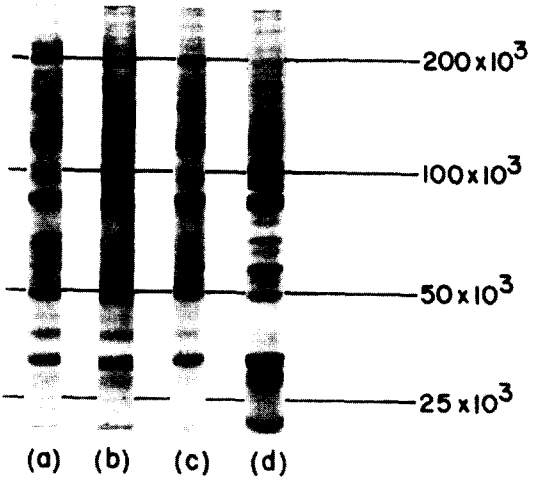


Fig. 5. Analysis of S-1 membrane proteins by SDS-polyacrylamide gel electrophoresis performed according to the procedure in Materials and Methods. a, control; b, stirred 16 h at 4°C in 0.6 M LiBr, 0.01 M Tris-HCl (pH 7.2), and concentrated by centrifugation for 60 min at 100 000 × *g* prior to solubilization in electrophoresis sample buffer; c, same treatment as b, but after stirring 16 h in LiBr the suspension was made 0.1% with respect to deoxycholate and sonicated for 2 min (12 × 10-s bursts interspersed with 10-s cooling periods) with a Branson Sonifier (Branson Sonic Power Co., Danbury, CT) at a setting of 4 using the microtip. The membranes were concentrated by centrifugation (100 000 × *g* for 60 min), rinsed and suspended in distilled water and sonicated as before. The resistant membranes were collected by centrifugation prior to being solubilized in electrophoresis sample buffer; d, F-III membranes suspended in 0.15 M histidine (pH 7.5) containing 0.005 M $CaCl_2$ were incubated with 25 μg/ml trypsin for 15 min at 30°C, then chilled and applied to discontinuous sucrose gradients and centrifuged to equilibrium at 100 000 × *g*. The trypsin-resistant S-1 fraction was collected and concentrated as described in Materials and Methods before being solubilized in electrophoresis buffer.

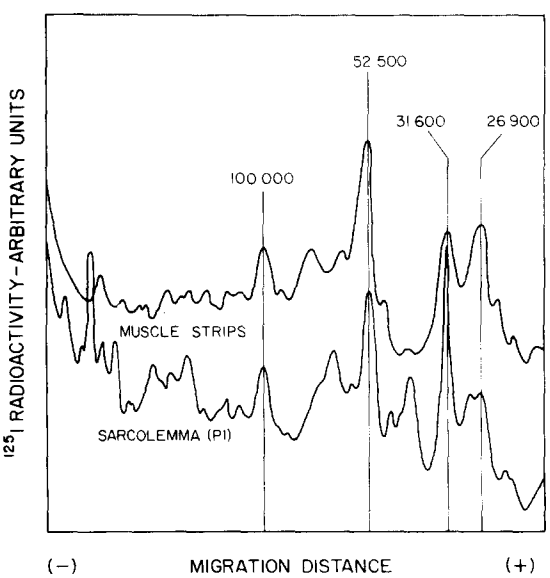


Fig. 6. Iodination of myometrial sarcolemmal and S-1 membrane proteins. Pieces of intact uterine smooth muscle were incubated with diazotized [^{125}I]iodosulfanilic acid and the F-III membranes prepared by differential centrifugation according to the procedure in Materials and Methods. Previously purified S-1 membranes were incubated under the same conditions and the radioiodinated sarcolemmal proteins from the two sources were compared by SDS-polyacrylamide gel electrophoresis and subsequent autoradiography. Developed autoradiographs were scanned using a Gilford recording spectrometer equipped with a mechanical linear transport. Apparent molecular weights of the prominent radioactive bands were obtained by co-electrophoresis of purified standards.

further. Using discontinuous sucrose gradients and the vertical or fixed angle roters, preparatory time is rapid and the purity of the S-1 fraction is comparable or better than previously characterized smooth muscle sarcolemmal preparations. Only the mitochondrial enzymes and 5′-nucleotidase were restricted adequately in their distribution in differential centrifugation fractions F-I to F-IV to conclude that membranes associated with these fractions comprised any specific subcellular compartment. Preferential recovery of 5′-nucleotidase, regarded as a specific plasma membrane enzyme [22,24], with F-III suggested this fraction contained most of the smooth muscle sarcolemma.

Glucose-6-phosphatase activity has been difficult to demonstrate in several types of smooth muscle [25,4], but the presence of free glucose intracellularly could be derived from glycogen, predicting the presence of the enzyme. Since F-III was free of rough endoplasmic reticulum, glucose-6-phosphatase could have been associated with the smooth endoplasmic reticulum (sarcoplasmic reticulum). The sarcoplasmic reticulum of the smooth muscle cell is a convoluted system of tubular membranes located beneath the sarcolemma [26], and some of these membranes might readily co-purify as a contaminant of the sarcolemma. Sarcoplasmic reticulum fractions have been prepared from uterine smooth muscle [27,28], but these were characterized only with respect to their calcium binding and transport activity. Janis and Daniel [29] have characterized calcium uptake by sarcolemmal fractions (prepared according to the method of Kidwai et al. [4]), so calcium transport may not, in itself, adequately distinguish between membaranes of the sarcolemma and sarcoplasmic reticulum.

Previous methods of plasma membrane preparation from myometrium yielded sarcolemmal preparations with appreciable mitochondrial contamination reflected by a nearly 3-fold increase in cytochrome *c* oxidase specific activity over that of the homogenate [4]. We, therefore, used both cytochrome *c* oxidase and monoamine oxidase activity to follow the distribution of the inner [30,31] and outer mitochondrial membranes [32,31] respectively. The concentration of both activities in F-II identify it as predominantly mitochondrial. F-III was also substantially enriched in these activities compared to the homogenate. S-1, the putative purified sarcolemmal fraction, was free of contamination by inner mitochondrial membranes and had no to barely detectable levels of outer mitochondrial membrane enzyme activity (Table I).

Our results suggest that equilibrium density fraction S-1 represented purified myometrical sarcolemma, free of nuclear or mitochondrial membrane contamination but perhaps containing elements of the sarcoplasmic reticulum. Kidwai et al. [4] resolved the particulate component of rat myometrial homogenate into 3 density fractions by centrifugation to equilibrium on sucrose density gradients. These fractions, with equilibrium densities of approximately 1.14, 1.16 and 1.20 were characterized as, respectively, sarcolemmal, mitochondrial, and nuclear, based on membrane enzyme activities and DNA content. The sarcolemmal fraction we have prepared (S-1) had an equilibrium density of 1.15, greater than previously reported for uterine smooth muscle but nearer that reported for other tissues [22]. Our results also suggest a substantial mitochondrial component in denser sucrose gradient fractions (S-2 ans S-3).

As discussed above, the purified sarcolemmal fraction may be contaminated

with elements of the smooth muscle cell sarcoplasmic reticulum. Several structural features of S-1 membrane proteins suggest that if this was the case, there are profound differences between the sarcoplasmic reticulum of the smooth muscle cell and that of skeletal and cardiac muscle. A variety of striated muscle sarcoplasmic reticulum proteins can be extracted by moderate concentrations of salt (0.6 M KCl or 0.5 M LiBr) and by deoxycholate [33,34,35]. In addition, few of these proteins are resistant to digestion with trypsin [33,36]. Finally, striated muscle sarcoplasmic reticulum has been characterized as having low glucose-6-phosphatase activity [37]. None of these was a property of the S-1 fraction, and the resistance of these proteins to LiBr and deoxycholate extraction as well as to trypsin digestion would be an unanticipated property of a mixture of different membrane types, further suggesting the homogeneity of the sarcolemmal fraction.

The protein composition of the purified sarcolemma was complex and comprised a wide spectrum of apparent molecular weights. The resistance of these to extraction with the chaotropic salt LiBr and deoxycholate suggest most were intrinsic or integral membrane proteins and that few of the uterine smooth muscle cell sarcolemmal proteins were either loosely associated 'extrinsic' proteins or contaminants of the cytosol [33,34,38]. The integral nature of the sarcolemmal proteins was further reflected by their marked resistance to trypsin digestion. This interpretation of sarcolemmal structure was further substantiated by the observation that few, if any, of the membrane proteins were exposed enough to be labeled by [^{125}I]iodosulfanilic acid, whether iodination was carried out on intact muscle strips or the purified sarcolemma.

Diazotized [^{125}I]iodosulfanilic acid, which does not penetrate the cell surface to the interior of intact cells [20,21,39], iodinated a greater variety of sarcolemmal proteins in the S-1 fraction compared to the intact muscle strips. Since few proteins of the sarcolemma could be accounted for by cytoplasmic contamination, the increased iodination of S-1 proteins indicates labeling of membrane proteins which became accessible to diazotized [^{125}I]iodosulfanilic acid as a result of homogenization and purification.

Acknowledgements

These investigations were supported by USPHS grant number HD-09285 and by research and education funds from the Veterans Adminstration. The authors gratefully acknowledge the technical contributions of Mrs. Janet L. Miller.

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