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ISOLATION AND CHARACTERIZATION OF THE SURFACE MEMBRANES OF FAST AND SLOW MAMMALIAN SKELETAL MUSCLE

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Summary

Fast (extensor digitorum longus) and slow (soleus) rat skeletal muscles served as the source for isolation and biochemical comparison of two distinct surface membrane fractions with properties of the sarcolemma and transverse tubular system. Enriched sarcolemmal membrane from soleus demonstrated a lighter density after sucrose density centrifugation. Sialic acid content was 1.5-fold higher in soleus (62 nmol/mg) than extensor (40 nmol/mg). The specific activity of ($Na^+ + K^+ + Mg^{2+}$)-ATPase was similar (1.40 and 1.65 μmol P_i/mg per 5 min) with the soleus enzyme displaying a (1) greater resistance to inhibition by ouabain, and (2) broader ionic ratio (Na^+/K^+) requirement than extensor enzyme. The polypeptide and phospholipid composition showed no major differences between the two muscle types.

The second surface membrane fraction, tentatively identified as transverse tubule, differed in membrane composition. The major polypeptide of extensor was of 95 000 molecular weight whereas for soleus a M_r = 28 000 species was dominant. Total phospholipid content of soleus was 1.5-fold greater than extensor due mostly to increased levels of phosphatidylcholine and phosphatidylethanolamine. Endogenous membrane protein kinase for the 28 000 molecular weight polypeptide was found exclusively in this membrane. The reaction conditions were identical for extensor and soleus since both required divalent cations (Ca^{2+} and Mg^{2+}) and neither was affected by cyclic AMP. Soleus showed a 2-fold higher capacity for phosphate incorporation than extensor.

These studies show that surface membrane fractions derived from fast and slow muscles differ in terms of functional and compositional properties. These

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Abbreviation: EGTA, ethylene-bis(oxyethylenenitrilo)-tetraacetic acid.

differences are specific not only for the surface membrane but for the muscle type and may relate to the known physiological differences observed between fast and slow mammalian muscle.

Introduction

Previous studies from our laboratory have defined two distinct surface membrane fractions derived from mixed skeletal muscle [1]. Sarcolemma has been identified by the presence of high specific activities for the surface markers $(Na^+ + K^+ + Mg^{2+})$-ATPase, α-bungarotoxin binding and sialic acid-containing macromolecules [1,2]. The same membrane fraction was highly iodinated after intact muscle fibers were incubated with ^{125}I in the presence of lactoperoxidase. An additional membrane fraction also appeared to be derived from the surface membrane since it, too, was highly iodinated by the lactoperoxidase procedure. This fraction differed from the enriched sarcolemmal fraction by the absence of appreciable sarcolemmal marker activities and by the unique presence of endogenous membrane protein kinase activity for a 28 000 mol. wt. polypeptide substrate. We have tentatively designated this fraction as enriched transverse tubule membrane [1—3].

The availability of a scheme for the fractionation of muscle membranes prompted the present biochemical characterization of the surface membrane constituents of two different muscle types. Fast and slow mammalian muscles differ with respect to the magnitude of the resting membrane potential [4,5], cation permeability [6,7], sensitivity to acetylcholine [8,9], catecholamines [10,11], end-plate morphology [12—14], and types of neurons which form synapses on the muscle surface [15—17]. It, thus, seemed appropriate to compare biochemical features of these two surface membranes in an attempt to explain the distinctive morphological and physiological characteristics of fast and slow muscle.

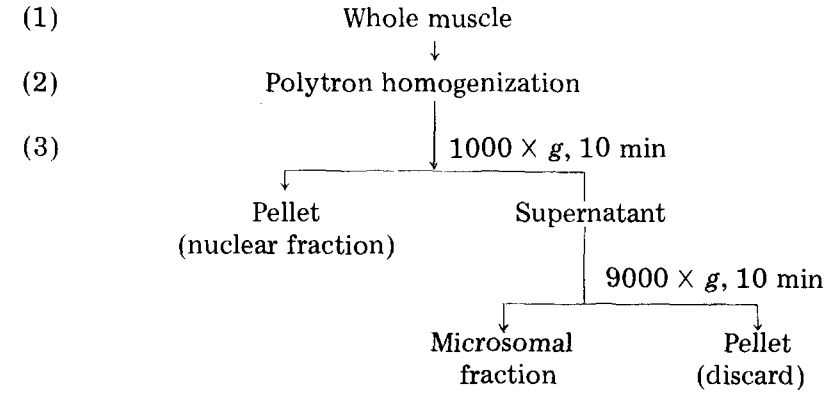
Methods

Membrane isolation. Adult female Wistar rats (200—250 g) were killed by decapitation and the extensor digitorum longus and soleus muscles removed to ice-cold sucrose buffer (0.25 M sucrose, 0.2 mM Na_2 EDTA, 0.2 mM Tris · HCl,

TABLE I

Membrane designation (density)	Sucrose range (%)	
	Nuclear fraction	Microsomal fraction
L, light	15—16	15—16
M_L, middle light	17—21	17—21
M_M, middle middle	22—25	22—26
M_H, middle heavy	26—29	27—31
H, heavy	30—35	32—35
P, pellet	Pellet	Pellet

pH 7.6). A preparation employed 65 animals from which approx. 12 g wet weight of each muscle type were obtained. Membranes were isolated as previously described [1]. Whole muscle was homogenized and centrifuged (1000 $\times g$, 10 min) to separate the nuclear and microsomal fractions. Both fractions were extracted with LiBr. Crude membrane material was further resolved by sucrose density gradient centrifugation. The nuclear fraction was the source of sarcolemma and the microsomal fraction was the source of sarcoplasmic reticulum and transverse tubule. The fractionation scheme is summarized below:



(4) Extraction of each fraction with LiBr medium
(5) Differential centrifugation plus two 0.6 M KCl wash steps
(6) Two distilled water wash steps of crude membrane fractions
(7) Continuous (15—35%) sucrose gradient centrifugation of 100 000 $\times g$ pellets of each fraction from step 6. Membrane distributing throughout the gradient was pooled according to Table I and studies performed on each subfraction.

Because of a shift in density of membranes derived from the nuclear fraction of soleus muscle, the fractionation was changed as described in the legend of Fig. 1. Throughout the Results section membrane fractions will be referred to as either H, M_H, M_M, M_L, or L which, in turn, designate the sucrose range of membrane distribution.

Protein determination. Protein was determined by the Lowry et al. [18] method using bovine serum albumin as the standard.

Adenosine triphosphatase determination. ATPase activity was measured in 1.0-ml reaction volumes at 37°C. The reaction mixture contained the stimulating ions: (1) 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$; (2) 5 mM $MgCl_2$; or (3) 5 mM $CaCl_2$. In preliminary studies reactions also contained varying concentrations of Ca^{2+} plus Mg^{2+}. However, no Ca^{2+} added Mg^{2+}-ATPase could be demonstrated. Reactions were initiated by addition of 20 μg membrane protein and terminated by addition of 0.2 ml of 1.2 M $HClO_4$. Inorganic phosphate was determined by the Ames method [19]. Net($Na^+ + K^+$)-stimulated activity was determined by the difference between Mg^{2+}-ATPase and ($Na^+ + K^+ + Mg^{2+}$)-ATPase. Reaction rates were linear with respect to time and protein concentration.

Sialic acid determination. Sialic acid was isolated by a Dowex AG1-X8 (for-

mate form) chromatography of membrane acid hydrolysate. Sialic acid content was quantitated by the Aminoff method [20].

Phospholipid analysis. 500 μg of membrane protein were extracted with 20 volumes of chloroform/methanol (2 : 1, v/v) [21]. Phospholipid in the chloroform phase was resolved by silica gel G thin-layer chromatography. The solvent systems were: 1st dimension, chloroform/methanol/concentrated NH_4OH (65 : 35 : 5, v/v); 2nd dimension, chloroform/acetone/methanol/acetic acid/water (50 : 20 : 10 : 10 : 5, v/v). Phospholipid was quantitated by phosphate content [22].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Membrane protein was solubilized by boiling in a mixture containing 1% sodium dodecyl sulfate, 1% 2-mercaptoethanol, 0.5 M urea and 0.1 M Tris · HCl, pH 7.2. Samples were applied to sodium dodecyl sulfate-polyacrylamide gel columns (6 mm × 12 cm) prepared as previously described [1]. Electrophoresis was performed at 7 mA/gel at room temperature for 15 h with 0.1 M sodium phosphate, 0.1% sodium dodecyl sulfate, pH 7.2, as the chamber buffer.

Membrane protein phosphorylation determination. Membrane protein (25 μg) was incubated at 27°C in 24 mM Tris · HCl, 12 mM $MgCl_2$ buffer, pH 7.5, plus $2 \cdot 10^{-5}$ M ATP (spec. act. 1.0 Ci/mmol) in a final volume of 200 μl. The reaction was terminated by addition of 100 μl of solubilizing buffer. Membrane protein was resolved by sodium dodecyl sulfate gel electrophoresis and radioactive phosphate incorporation determined as previously described [2].

Materials

[γ-^{32}P]ATP was prepared by the method of Glynn and Chappel [23]. Sodium dodecyl sulfate was purchased from Sigma Chemical Co., St. Louis, Mo. Acrylamide, *N*, *N*, *N'*, *N'*-tetramethylene diamine, and methylenebisacrylamide were obtained from Eastman Organic Chemicals, Rochester, N.Y. Silica Gel G was purchased from Brinkman Instrument Co., Westbury, N.Y. Other chemicals used in these studies were of reagent grade and were purchased from the usual commercial sources.

Results

Membrane distribution

In these experiments membrane fractions with the highest specific activities of sarcolemmal markers were isolated in the broad middle density fraction derived from the nuclear pellet (Fig. 1A). Soleus showed a slight density shift when compared to extensor (17—22% vs. 17—25% sucrose). Soleus also displayed a relatively greater content of light density membrane (15—16% sucrose) than extensor.

The microsomal fraction contained two distinct membrane fractions identified as sarcoplasmic reticulum (20—35% sucrose) and transverse tubule (15—16% sucrose). Extensor showed a greater relative content of the heavier density material than soleus. Light density membrane appeared enriched in soleus over extensor by absorbance measurements, but protein yields were the same for the two muscle types. The reason for this discrepancy is due to the greater turbi-

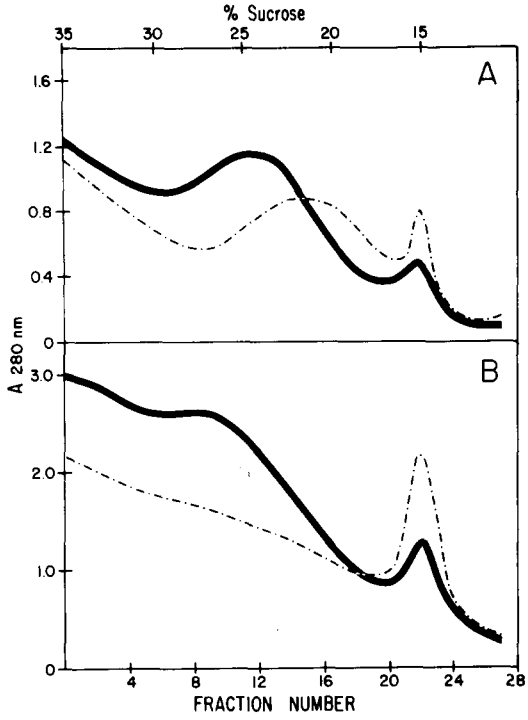


Fig. 1. Membrane distribution. Crude membrane material was applied to continuous sucrose gradients (15—35%) and centrifugation performed as described in Methods. Membrane material derived from the nuclear fraction (A) and the microsomal fraction (B) of extensor (———) and soleus (- - - - - -) was collected in 0.5-ml fractions. Absorbance was measured at 280 nM and membrane material pooled by the following sucrose concentration ranges: Nuclear fraction, pellet (P), heavy (H, 30—35%), middle heavy (M_H, 26—29%), middle middle (M_M, 22—25%), middle light (M_L, 17—21%), and light (L, 15—16%). Because of the shift of middle density membranes of soleus (A), the sucrose concentration was changed for subfractionation to: M_H (23—26%), M_M (20—22%), and M_L (17—19%). The microsomal fraction was subfractionated as described in Methods for both muscle types.

dity of soleus membranes than that of extensor.

For biochemical studies membrane recovered after gradient centrifugation was subfractionated according to the scheme used for mixed muscle studies. Because of the density shift of soleus sarcolemmal membrane, the sucrose concentration range for subfractionation was changed (see legend, Fig. 1).

Adenosine triphosphatase distribution

The distribution and specific activities of $(Na^+ + K^+ + Mg^{2+})$-ATPase and Ca^{2+}-ATPase are compared in Figs. 2 and 3. The peak specific activity of $(Na^+ + K^+ + Mg^{2+})$-ATPase was found in the nuclear fraction membrane, M_M M_L (Fig. 2), for both extensor and soleus. There was no significant difference in activity between the two muscle types (1.40—1.65 μmol P_i/mg per 5 min). $(Na^+ + K^+ + Mg^{2+})$-ATPase activity was considerably less in the microsomal fraction (Fig. 3). Approx. 80.5 and 74.4% of the total $(Na^+ + K^+ + Mg^{2+})$-ATPase activity was located in the nuclear fraction membrane of extensor and soleus, respectively.

The highest specific and total activity of Ca^{2+}-ATPase was found in the microsomal membranes (Fig. 3). 65% of the total activity was contained in the

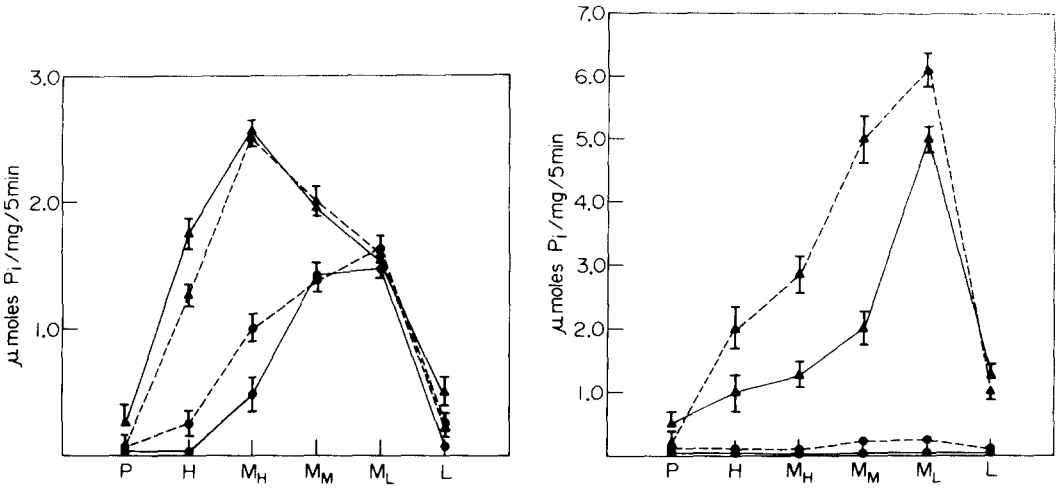


Fig. 2. ATPase distribution of nuclear fraction membranes. Membranes were subfractionated as described in the legend of Fig. 1. $(Na^+ + K^+ + Mg^{2+})$-ATPase and Ca^{2+}-ATPase were measured as described in Methods. ———, extensor; - - - - - -, soleus. •, $(Na^+ + K^+ + Mg^{2+})$-ATPase; ▲, Ca^{2+}-ATPase. The values represent the means (±S.E.) of duplicate determinations on three separate membrane preparations.

Fig. 3. ATPase distribution of microsomal fraction membrane. Membranes were subfractionated as described in the legend of Fig. 1. $(Na^+ + K^+ + Mg^{2+})$- and Ca^{2+}-ATPase activities were measured as described in Methods. ———, extensor; - - - - - -, soleus. •, $(Na^+ + K^+ + Mg^{2+})$-ATPase; ▲, Ca^{2+}-ATPase. The values represent the means (±S.E.) of duplicate determinations on three separate membrane preparations.

heavier density membranes (25—35% sucrose). The highest specific activity for Ca^{2+}-ATPase was found in the M_L region of the gradient. Soleus showed a greater specific activity than extensor throughout the gradient. The nuclear fraction membranes also demonstrated Ca^{2+}-ATPase activity. In this case, however, the activity was equivalent for soleus and extensor and was localized in heavier density membrane material.

TABLE II

CHARACTERIZATION OF $(Na^+ + K^+ + Mg^{2+})$-ATPase

Properties of the $(Na^+ + K^+ + Mg^{2+})$-ATPase of extensor and soleus. $(Na^+ + K^+ + Mg^{2+})$-ATPase activity was measured in nuclear fraction M_M M_L membrane as described in Methods. For substrate specificity studies, membrane material was incubated simultaneously with each indicated nucleotide (10 mM) plus ATP (1 mM). Ouabain inhibition was measured by preincubating membrane material for 10 min with ouabain over a concentration range of $1 \cdot 10^{-7}$—$1 \cdot 10^{-3}$ M. At the end of this period the reaction was initiated by addition of reaction buffer and stimulating ions and terminated after 5 min. Ionic ratio requirement was determined for ratios of Na^+/K^+ from 10 : 1 to 1 : 10 with the total stimulating ion concentration ($[Na^+] + [K^+] + [Mg^{2+}]$) being held constant. All reaction rates were determined over a 5 min period at 27°C. Mg^{2+}-ATPase activity was subtracted from total $(Na^+ + K^+ + Mg^{2+})$-ATPase activity to calculate the net $(Na^+ + K^+ + Mg^{2+})$-stimulated ATPase activity.

Property	Extensor	Soleus
Rate I	1.38	1.65
Na^+/K^+ ratio	5 : 1	5 : 1—2 : 1
K_m (ATP)	0.2—0.4 mM	0.2—0.4 mM
Substrate	ATP ≫ GTP > CTP, UTP	
Ouabain I_{50}	$2 \cdot 10^{-5}$—$4 \cdot 10^{-5}$ M	$1 \cdot 10^{-4}$ M

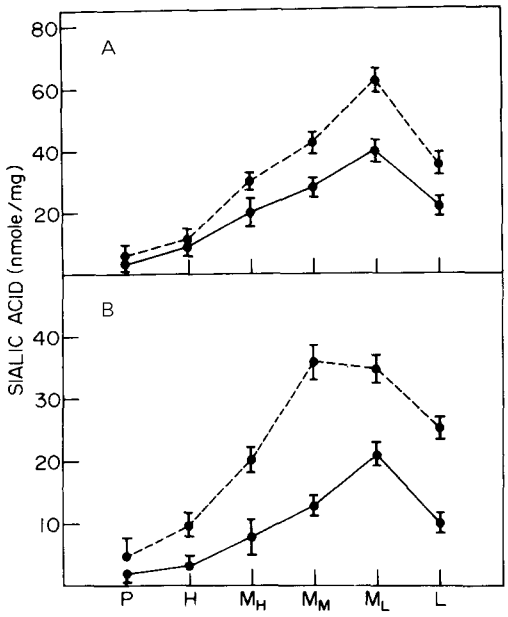


Fig. 4. Sialic acid distribution. Membrane hydrolysis, sialic acid isolation and quantitation were performed as described in Methods. Upper panel, nuclear fraction; lower panel, microsomal fraction. ———, extensor; - - - - - -, soleus. The values represent the means (±S.E.) of duplicate determinations on three separate membrane preparations.

Basic enzymological properties of the $(Na^+ + K^+ + Mg^{2+})$-ATPase of extensor and soleus are compared in Table II. The inhibition of enzyme activity by ouabain and the ionic ratio requirement were different in the two muscle types. Soleus was more resistant to inhibition by ouabain than extensor with 50% inhibition occurring at concentrations of $1 \cdot 10^{-4}$ and $2 \cdot 10^{-5}$—$4 \cdot 10^{-5}$ M, respectively. Maximum activation of the extensor $(Na^+ + K^+ + Mg^{2+})$-ATPase was achieved with Na^+/K^+ ratio of 5 : 1. Soleus enzyme demonstrated equivalent activities at 5 : 1 and 2 : 1 ratios of Na^+/K^+.

Sialic acid content

The distribution of sialic acid is shown in Fig. 4. The highest content of sialic acid was found in the nuclear fraction membrane (panel A, M_M, M_L) corresponding to the peak activity of $(Na^+ + K^+ + Mg^{2+})$-ATPase. Soleus showed a 1.5-fold greater content of sialic acid than extensor (62 vs. 40 nmol/mg). Microsomal membrane displayed one half the sialic acid content of the nuclear fraction (panel B).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The polypeptide composition of each major membrane fraction was assessed by sodium dodecyl sulfate gel electrophoresis.

Microsomal membrane (20—35% sucrose range) showed a polypeptide of 95 000 mol. wt. to comprise between 70 and 80% of the total Coomassie Blue-

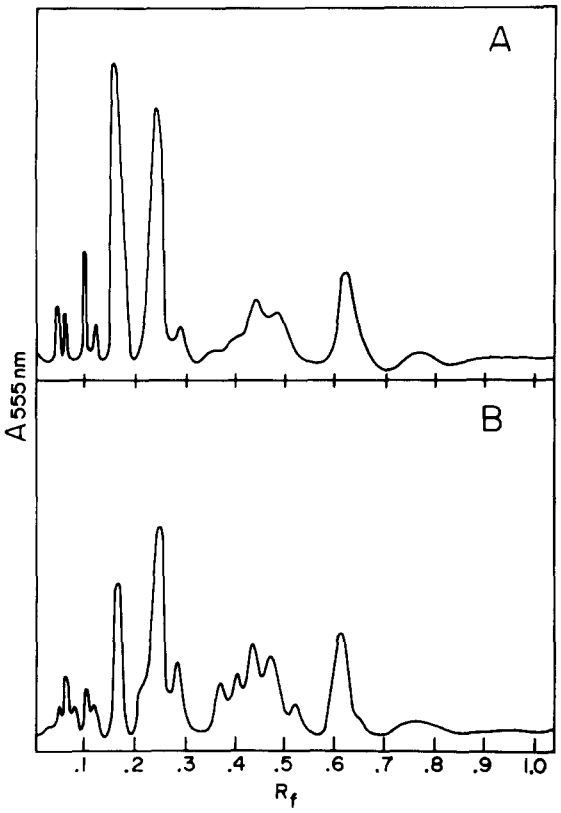


Fig. 5. Polypeptide composition of nuclear fraction, M_M M_L membrane of extensor and soleus. Membrane material (35 μg protein) was solubilized by boiling for 3 min in solubilizing buffer containing 1% sodium dodecyl sulfate. Solubilized membranes were applied to sodium dodecyl sulfate gels (7.5% acrylamide) and resolved as described in Methods. (A) Extensor. (B) Soleus.

staining material. This polypeptide was in relatively greater amounts in extensor than soleus. No differences were observed in the other minor 15 polypeptides.

The polypeptide profiles of nuclear fraction M_M M_L are shown in Fig. 5 for extensor (A) and soleus (B). Polypeptides of molecular weights 109 000, 95 000 and 34 000, were the dominant species. The major difference between extensor and soleus was the greater relative content of the 109 000 mol. wt. polypeptide in extensor sarcolemma.

Fig. 6 shows the polypeptide composition of microsomal light (15—16% sucrose) membranes of extensor (A) and soleus (B). The major component of extensor was of molecular weight 95 000, whereas for soleus the 28 000 mol. wt. component was dominant. Soleus differed from extensor in the number and proportion of polypeptides in the 60 000—40 000 molecular weight range. Soleus showed three distinct polypeptides and extensor demonstrated two polypeptides in that region of the gel. Polypeptides of 60 000 and 95 000 mol. wt. were relatively increased in extensor over soleus.

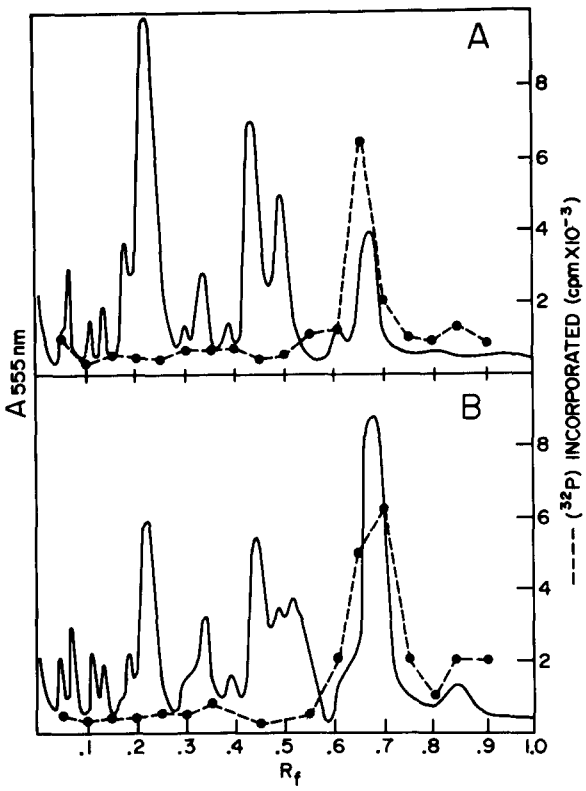


Fig. 6. Polypeptide composition and endogenous protein kinase activity of microsomal light membranes of extensor and soleus. Membrane material (50 μg protein) was incubated with 24 mM Tris · HCl, 12 mM $MgCl_2$, [γ-^{32}P]ATP (specific activity, 1 Ci/mmol), pH 7.5, in a volume of 200 μl at 27°C. The reaction was terminated after 1 min by addition of 100 μl of solubilizing buffer. Solubilized membrane was resolved and assessed for radioactive phosphate incorporation as described in Methods. (A) Extensor. (B) Soleus.

Protein kinase activity

Microsomal light membranes incorporated radioactive phosphate into the 28 000 mol. wt. polypeptide after incubation of membranes with [γ-^{32}P]-ATP (Fig. 6, dashed line). This activity was not observed in any other membrane fractions.

The time dependence of the phosphorylation reaction is shown in Fig. 7. Initially, extensor and soleus membranes had identical rates of phosphorylation (60 pmol phosphate/mg per min). At time periods after 3 min extensor no longer showed net incorporation, but soleus had an attenuated rate approximately one-third that of the 10—120 s interval. At 10 min soleus membranes (163 pmol phosphate/mg) incorporated about 2-fold greater phosphate than extensor (92 pmol phosphate/mg). The K_m for ATP was identical at $1 \cdot 10^{-5}$ M for both muscle types.

Table III summarizes the effect of various factors on the phosphorylation reaction. The reaction was dependent on divalent cations since omission of Mg^{2+} or treatment with EGTA abolished activity. The inhibition by EGTA was reversed with 1 mM Ca^{2+} but not Mg^{2+}. Cyclic AMP at 10^{-5} and 10^{-6} M had no

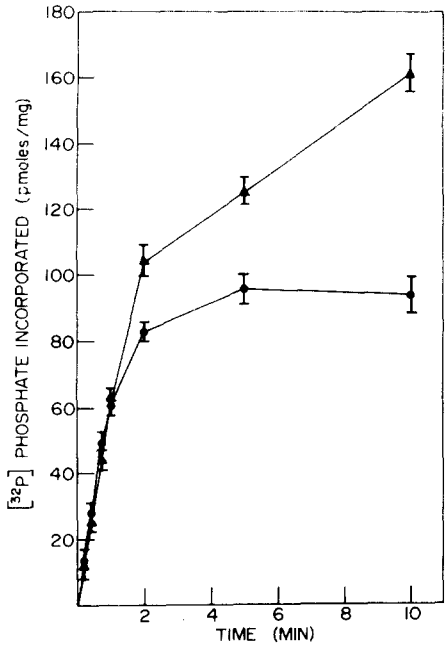


Fig. 7. Time course of endogenous protein kinase activity of extensor and soleus. Microsomal light membrane (125 μg/ml) was incubated with 24 mM Tris · HCl, 12 mM $MgCl_2$ and [γ-^{32}P]ATP (specific activity, 1 Ci/mmol), pH 7.5, at 27°C. At the indicated time intervals, 200 μl of the reaction mixture were removed and rapidly pipetted into 100 μl of solubilizing buffer and radioactive phosphate incorporation into the 28 000 mol.wt. polypeptide assessed as described in Methods. ●——●, extensor; ▲——▲, soleus. The values represent the means (±S.E.) of duplicate determinations on three separate membrane preparations.

TABLE III

EFFECT OF FACTORS ON PROTEIN KINASE ACTIVITY

Microsomal light membrane (25 μg protein) was preincubated with the various factors at the given concentrations for 10 min at 27°C. At the end of this period reaction buffer (24 mM Tris · HCl, 12 mM $MgCl_2$, [γ-^{32}P]ATP) (specific activity, 1 Ci/mmol), pH 7.5, was added and the incubation continued for 1 min. The reaction was terminated by addition of 100 μl of solubilizing buffer and radioactive phosphate incorporation into the 28 000 mol. wt. polypeptide assessed as described in Methods.

Factor	Percent total activity	
	Extensor digitorum longus	Soleus
Control	100.0	100.0
$-Mg^{2+}$	0	0
EGTA (0.5 mM)	9.5	14.7
+ 1 mM Ca^{2+}	92.8	96.4
+ 1 mM Mg^{2+}	4.5	16.0
10^{-5} M cyclic AMP	100.0	96.2
10^{-6} M cyclic AMP	80.9	86.4
Caffeine (1 mM)	87.0	85.8
Theophylline (1 mM)	90.8	78.4

TABLE IV

PHOSPHOLIPID COMPOSITION OF SURFACE MEMBRANES

Phospholipid composition of nuclear fraction membranes migrating at 17—25% sucrose (NF_{17-25}) and microsomal fraction membrane migrating at 15—16% (MF_{15-16}) of extensor and soleus. Phospholipid was extracted from 500 μg membrane protein with chloroform/methanol (2 : 1, v/v). Two-dimensional chromatography and isolation and quantitation of individual phospholipids were performed as described in Methods. Values expressed as μg phospholipid phosphorus/mg membrane protein. Results are given ±S.E.M.

Fraction	Phospohlipid						Ratio
	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Phosphatidyl-serine	Phosphatidyl-inositol	Sphingo-myelin	Total	
Nuclear fraction (17—25%)							
Extensor	10.08 ± 0.95	4.34 ± 0.38	1.55 ± 0.21	2.04 ± 0.18	0.99 ± 0.20	19.00 ± 1.87	1.03
Soleus	10.83 ± 1.20	3.71 ± 0.33	2.34 ± 0.21	2.35 ± 0.17	1.40 ± 0.31	19.63 ± 1.98	
Microsomal fraction (15—16%)							
Extensor	7.50 ± 0.66	5.36 ± 0.47	3.16 ± 0.32	1.90 ± 0.33	1.49 ± 0.12	19.41 ± 0.12	1.50
Soleus	11.04 ± 1.23	8.81 ± 0.80	3.10 ± 02.3	2.55 ± 0.25	3.66 ± 0.35	29.16 ± 3.12	

stimulatory effect, and phosphodiesterase inhibitors theophylline and caffeine had no effect on the reaction.

Phospholipid composition

The phospholipid composition of nuclear fraction membranes migrating at 17—25% sucrose (NF_{17-25}) and microsomal light membranes (MF_{15-16}) are compared for extensor and soleus in Table IV. Phosphatidylcholine and phosphatidylethanolamine were the dominant phospholipid constituents. Phosphatidylcholine represented 53—55% of the total phopholipid in nuclear fraction membranes of extensor but only 38—39% of microsomal light membranes. There were no appreciable differences in the individual or total amounts of phospholipids in the nuclear fraction membranes between extensor and soleus. Microsomal light membranes differed markedly with respect to amount of phospholipids but not with respect to their percent composition. Soleus membrane material (29.16 μg lipid P_i/mg) had 1.5-fold greater phospholipid content than extensor (19.41 μg lipid P_i/mg). The increased phospholipid was found primarily in phosphatidylcholine, phosphatidylethanolamine and sphingomyelin.

Discussion

The criteria employed for identifying muscle membrane fractions were identical to those previously employed for mixed muscle studies [1]. Sarcolemma was identified on the basis of (1) highest specific and total activity of ($Na^+ + K^+ + Mg^{2+}$)-ATPase, (2) sialic acid content, (3) their identification as surface membranes by their high specific radioactivity following lactoperoxidase iodination of whole muscle fibers and subsequent membrane fractionation [1], and (4) unique polypeptide profile. This fraction was also shown to contain the highest specific activity of adenyl cyclase and acetylcholine receptor [3]. Light density membranes isolated from the microsomal fractions were tentatively identified as transverse tubules on the basis of (1) their identification as surface membranes by the lactoperoxidase iodination technique [1—3], (2) the absence of sarcolemmal markers, and (3) the presence of endogenous membrane protein kinase. Sarcoplasmic reticulum was identified by the following characteristics: (1) polypeptide of 95 000 mol. wt. comprising 70—80% of the total membrane protein, (2) isolation from the microsomal fraction, and (3) highest specific activity of Ca^{2+}-ATPase and virtually absence of surface membrane markers including both enzyme markers and high specific iodination. The Ca^{2+}-ATPase studies do not monitor the Ca^{2+} transport ATPase since little activity is observed for the ($Ca^{2+} + Mg^{2+}$)-ATPase. This discrepancy in our experiments is due to the use of LiBr for surface membrane isolation. This procedure has been shown to inactivate the "Ca^{2+}-pump" and phosphoprotein phosphorylation activity and appears to uncouple the Ca^{2+}- and Mg^{2+}-dependent components of the Ca^{2+} transport ATPase [24,25]. No ($Ca^{2+} + Mg^{2+}$)-ATPase activity was present in any fraction.

The surface membranes (sarcolemma and transverse tubules) of fast and slow muscles showed a number of differences in functional and compositional properties. Sarcolemma (nuclear fraction, M_M M_L) differed with respect to mem-

brane migration on sucrose density gradients, properties of the $(Na^+ + K^+ + Mg^{2+})$-ATPase, and sialic acid content. The phospholipid and polypeptide constituents were similar. Transverse tubule membranes (microsomal fraction, L) of soleus showed increased phospholipid content and qualitative and quantitative differences in polypeptide composition. Endogenous protein kinase activity was increased in soleus compared to extensor. In addition, the kinetics of protein phosphorylation showed soleus to possess a second phosphorylation stage not present in transverse tubule membranes of extensor muscle.

A comparison of membrane fractions between different muscle types could be complicated by differences in the density gradient distribution of these membranes. However, this situation does not apply to the present studies. In both soleus and extensor, $(Na^+ + K^+ + Mg^{2+})$-ATPase and sialic acid markers are distributed primarily in the broad middle density region of the gradient. Both muscle types showed little $(Na^+ + K^+ + Mg^{2+})$-ATPase in the microsomal fraction. Endogenous protein kinase activity was found exclusively in light density membranes identified as transverse tubule. If redistribution had occurred, these markers would have had a less homogeneous distribution.

The present fractionation scheme does not yield completely pure fractions, but the fractions are markedly enriched. For example, sarcolemmal markers are found in low specific activity in the microsomal fraction. Mixed muscle preparations have low activities of the sarcolemmal markers adenyl cyclase and acetylcholine receptor in fractions designated as sarcoplasmic reticulum and transverse tubule. These results are not unexpected since the initial separation of the nuclear and microsomal fraction depends on a $1000 \times g$ centrifugation step. Since the absolute specific activity of the various markers are not known for the membrane fractions, the degree of cross-contamination cannot be accurately estimated. However, the concordance of both total and specific activity in the same fraction permits the deduction that the fractions are markedly enriched.

The biochemical differences observed between the sarcolemma and transverse tubule membranes of extensor and soleus may relate to certain physiological characteristics observed for these two muscle types. The similarity in $(Na^+ + K^+ + Mg^{2+})$-ATPase suggests that the difference in resting membrane potential between extensor and soleus is due primarily to the difference in the ratio of Na^+ and K^+ permeability. This has been confirmed by the demonstration that soleus possesses a lower K^+ permeability than extensor [4,5].

The increased content of sialic acid reflects an increase in glycoprotein content in soleus since only 10% of membrane-bound carbohydrate is in the glycolipid fraction of muscle [26]. The reason for the higher sialic content is unknown, but it is of interest that denervated muscles also have a higher membrane sialic acid than innervated muscles [1]. Glycoproteins are known to participate in cell-cell recognition and adhesion [27,28], and the sialic acid differences in the varying physiological states of muscle may represent different capacities for the formation of neuromuscular junctions and/or muscle fusion. Sarcolemma does not show significant differences in bulk polypeptides or phospholipid constituents, and it would seem that functional membrane differences between fast and slow muscles do not depend on gross membrane constituents. Differences in receptors, ion conductance channels, and enzyme com-

ponents appear to confer the individual character of fast and slow muscle membrane function and are obviously not reflected in gross membrane analyses.

Membrane material identified as transverse tubule was characterized by the presence of endogenous protein kinase activity for a 28 000 mol. wt. polypeptide substrate. Soleus showed a two-phase reaction which indicates heterogeneity either in the protein kinase and/or the kinase substrate. Sodium dodecyl sulfate gel electrophoresis does not resolve polypeptides of similar molecular weight. As a result, there is a distinct possibility that the 28 000 mol. wt. band from soleus membranes contains several different phosphorylated species which may explain the different kinetics of phosphorylation.

The function of membrane protein phosphorylation is unknown. It is possible that this reaction participates in the membrane binding and/or transport of calcium and thus could be related to the transfer of information from transverse tubule to sarcoplasmic reticulum [29—31]. The extent of phosphorylation of this membrane system may thus contribute to the different excitation-contraction coupling properties of fast and slow skeletal muscles.

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