THE PROTEIN COMPOSITION OF SARCOPLASMIC RETICULUM MEMBRANES

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Summary: By the use of various polyacrylamide electrophoresis methods the proteins of sarcoplasmic reticulum membranes were resolved into a large number of distinct bands. A major band was tentatively identified with the ATPase enzyme implicated in the active Ca transport.

In a recent report Masoro and Yu (1969) concluded, primarily on the basis of polyacrylamide-gel electrophoresis, that the protein content of sarcotubular membranes consists mainly of a single species of polypeptide with a molecular weight of $\sim$ 17. 000.

As our earlier experiments (Martonosi, 1968a) on similar preparations indicated gross heterogeneity of protein composition, the problem was reinvestigated using the polyacrylamide electrophoresis methods of Takayama et al (1966) and of Masoro and Yu (1969) with minor modifications.

Sarcotubular membranes prepared by the method of Martonosi et al (1968) yielded on electrophoresis according to Takayama et al (1966) the complex electrophoretic patterns shown in Fig. 1A and 1B. Similar

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patterns were obtained on microsome samples prepared according to the procedure of Yu et al (1968) which includes sucrose density gradient centrifugation (Fig. 1C), or after repeated washing of microsomes with 0.6M KCl (Fig. 1D). Extraction of lipids with 90% acetone had only slight effect on the mobility of proteins (Fig. 1A and 1B).

In electrophorograms prepared according to Takayama et al (1966) the sharply resolved bands constituted about 30-40% of the protein; the rest of the protein was retained on the gel surface. Performic acid oxidation, or reduction and carboxymethylation produced only moderate improvement. Similar observations were made earlier (Martonosi, 1968a) using cholate-deoxycholate mixtures for solubilization of membrane proteins.

The characteristic sharp bands were absent or much reduced in intensity following trypsin treatment of microsomes (Fig. 1E) while the amount of protein retained on the gel surface remained unaltered. In the light of earlier electron microscopic evidence (Martonosi, 1968b), it is likely that the proteins hydrolyzed by trypsin are located on the outside surface of microsomes.

The proteins of sarcoplasmic reticulum membranes can be readily resolved into several distinct bands by disc-electrophoresis in a solution containing 0.1% sodium dodecylsulphate and 0.1M Na-phosphate buffer, pH 6.0 (Fig. 1F). This finding is in disagreement with the conclusions of Masoro and Yu (1969) concerning the relative homogeneity of the protein composition of sarcoplasmic reticulum membranes. Removal of phospholipids by extraction of microsomes with acetone and ether causes a distinct change in the mobility of the proteins (Fig. 1G) and permits the entry of nearly all protein material into the gel. The improved resolution

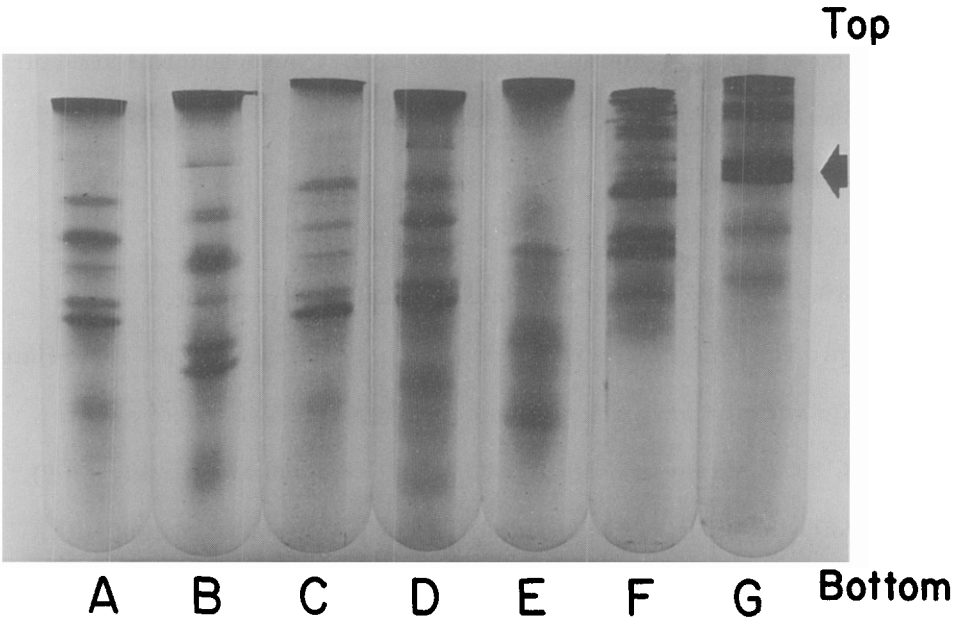

Fig. 1. Separation of solubilized proteins from sarcoplasmic reticulum membranes of rabbit skeletal muscle. Samples A-E were electrophoresed on polyacrylamide gel according to Takayama et al (1966) for 16 hours at 5 mA per tube. For samples F and G a modification of the method described by Masoro and Yu (1969, Fig. 4) was used in the form of disc electrophoresis carried out at pH 6.0 for 6 hrs at 5 mA per tube with 0.1% sodium dodecyl-sulphate and 0.1M Na-phosphate as buffer. Two types of fragmented sarcoplasmic reticulum (FSR) preparations were tested. FSR-M was prepared according to Martonosi et al (1968) and FSR-Y according to Yu et al (1968). Sample A: FSR-M extracted twice with 90% acetone. Sample B: FSR-M, without acetone extraction. Sample C: FSR-Y extracted twice with 90% acetone. Sample D: FSR-M was washed thrice with large volume of 0.6M KCl followed each time by centrifugation at 95.000 g for 1 hour. The final sediment was extracted twice with 90% acetone. Sample E: FSR-M (19 mg protein/ml) was digested with trypsin (1 mg/ml) at room temperature for 1 hour. After centrifugation at 95.000 g for one hour the sediment was extracted with acetone. Sample F: FSR-M without acetone extraction. Sample G: FSR-M was incubated with 3.3 mM ^{32}P-acetylphosphate in a medium of 10 mM histidine, 5 mM $CaCl_2$ for 1 min at 25°C temperature. The reaction was stopped and the membranes extensively washed as described earlier (Martonosi, 1969). The washed ^{32}P-labeled membranes were extracted twice with acetone, twice with ether and subjected to electrophoresis in quadruplicates. Duplicate samples were removed for staining with amido black, at intervals. The remaining gels were cut into 5 mm sections, dried and counted in liquid scintillation counter.

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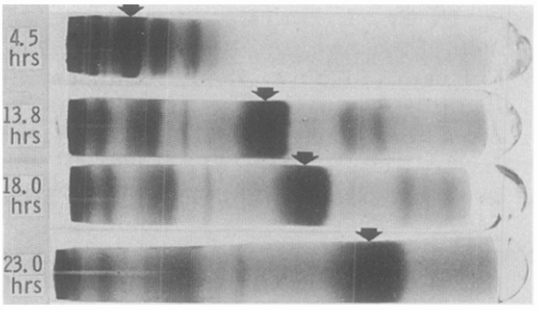
of our disc-electrophoresis system may explain the differences between the present data and those obtained by Masoro and Yu (1969).

The controversy concerning the protein composition of sarcoplasmic reticulum membranes is reminiscent to the case of mitochondrial structural protein (Lenaz, et al 1968; Schatz and Saltzgaber, 1969) and further emphasizes the need for caution in the interpretation of physical data obtained on solutions containing ionic detergents.

The hydrolysis of ATP or acetylphosphate by fragmented sarcoplasmic reticulum membranes involves a protein-bound phosphorylated intermediate with the properties of an acylphosphate (Yamamoto and Tonomura, 1967; Martonosi, 1967 and 1969; Pucell and Martonosi, 1969) which permits the labeling of the active site of the ATPase enzyme with ^{32}P. Using the protein-bound radioactivity as indicator, the position of the ATPase enzyme on electrophorograms was identified. On electrophoresis of ^{32}P-labeled and acetone-ether extracted membranes of sarcoplasmic reticulum in a system of 0.1% sodium dodecylsulphate and 0.1M Na-phosphate pH 6.0, a large portion (44-66%) of the protein-bound radioactivity was recovered in a single protein band (Fig. 1G, marked with arrow). A more detailed analysis of the relationship between the location of radioactivity and proteins on gel columns is illustrated in Fig. 2. After electrophoresis of increasing duration (4.5 - 23 hours) the peak of protein-bound radioactivity, marked with arrow, precisely coincides with a distinct protein band. The peak of radioactivity in the sample electrophoresed for 4.5 hours, which is located at 3.5 - 5.0 cm. distance from the top of the gel is inorganic phosphate, formed during the preparation of the sample for electrophoresis. Due to slow hydrolysis of the phosphorylated intermediate the peaks of protein-bound radioactivity become gradually smaller as electrophoresis proceeds. Only a relatively



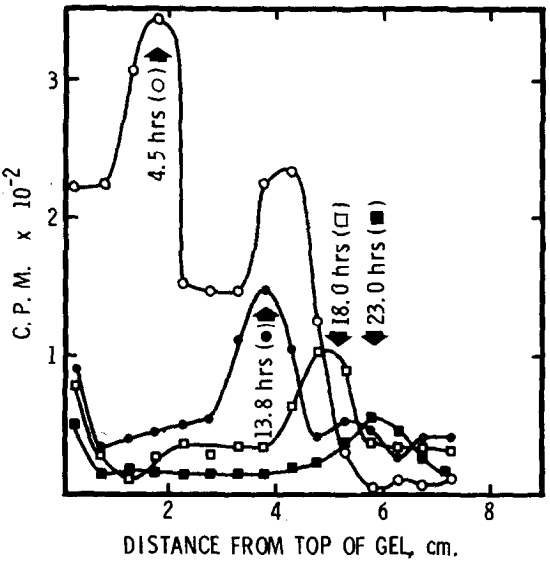


Fig. 2. Tentative identification of the microsomal ATPase on polyacrylamide gel electrophorograms. Samples were prepared and electrophoresis performed as described under Legend to Fig. 1, sample G. After electrophoresis for 4.5, 13.8, 18.0 and 23.0 hours duplicate samples were removed for staining of proteins and measurement of protein-bound radioactivity. For other details see text.

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small portion of the total radioactivity is retained on the surface of the gel. No significant protein-bound radioactivity entered the gel on electrophoresis of ^{32}P labeled membranes according to Takayama et al (1966).

References

1. Lenaz, G., Haard, N. F., Lauwers, A., Allmann, D. W., and Green, D. E., Arch. Biochem. and Biophys. 126, 746 (1968).
2. Martonosi, A., Biochem. Biophys. Res. Communs. 29, 753 (1967).
3. Martonosi, A., J. Biol. Chem. 243, 71 (1968a).
4. Martonosi, A., Biochim. Biophys. Acta 150, 694 (1968b).
5. Martonosi, A., J. Biol. Chem. 244, 613 (1969).
6. Martonosi, A., Donley, J. and Halpin, R. A., J. Biol. Chem. 243, 61 (1968).
7. Masoro, E. J. and Yu, B. P., Biochem. Biophys. Res. Communs. 34, 686 (1969).
8. Pucell, A. G. and Martonosi, Abstracts 3rd International Biophysics Congress, Cambridge, Massachusetts, 1969.
9. Schatz, G. and Saltzgaber, J., Biochim. Biophys. Acta 180, 186 (1969).
10. Takayama, K., MacLennan, D. H., Tzagoloff, E., and Stoner, C. D., Arch. Biochem. Biophys. 114, 223 (1966).
11. Yu, B. P., DeMartinis, F. D., and Masoro, E. J., Anal. Biochem. 24, 523 (1968).