# TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS OF RABBIT SKELETAL MUSCLE MICROSOMAL PROTEINS

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## 1. Introduction

Since the application of polyacrylamide gel electrophoresis to the study of membrane proteins, there have been a number of reports on the protein composition of skeletal muscle microsomes prepared by differential pelleting [1–7] and by density gradient centrifugation [8–10]. These electrophoretic separations of the solubilised membrane proteins have been in one-dimension. There is general agreement that the principal protein component of these preparations is a protein of mol. wt 100 000. Furthermore this component has been shown to be the $Ca^{++}$ transport ATPase of the sarcoplasmic reticulum [1,2,8,9]. Estimates of the amount of this protein present have varied from 35-70%. In addition to this major protein five other proteins and a proteolipid have been separated [4,11]. These other proteins have been postulated to play roles associated with the calcium transport function of the sarcoplasmic reticulum [3,4,11,12].

This report describes the use of two-dimensional polyacrylamide gel electrophoresis in the study of membrane proteins from four microsomal fractions prepared by zonal centrifugation [10,13]. The two-dimensional separation consists of electrophoresis in the first dimension in 5.2% gels followed by electrophoresis in the second dimension in gels having a 6–27% gel gradient. This method is especially useful in the macromolecular mapping of multicomponent mixtures [14,15] and is a novel approach in the study of membrane proteins.

The present work is part of a continuing study of rabbit skeletal muscle microsomes. The results reported here show that muscle microsomes contain many more proteins than was previously believed.

## 2. Materials and methods

Skeletal muscle microsomes, prepared in the form of a concentrated suspension, were collected in four fractions as previously described [13,16]. Analyses for protein, phospholipid and enzyme activities were carried out by methods previously published [10,17]. Microsomal pellets, prepared by centrifugation of diluted microsomal suspensions at 100 000 *g* for 75 min and twice washed by sedimentation and resuspension in 0.25 M sucrose–5 mM imidazole–HCl pH 7.4, were used for electrophoretic analysis. After the final washing step the pellets were resuspended in the solubilisation medium containing 0.6% w/v sodium dodecyl sulphate, 40 mM dithioerythritol and 10% w/v sucrose in Tris–disodium EDTA – boric acid buffer, pH 8.3, to a final protein concentration of 3 mg/ml. Proteolysis did not occur during the solubilisation procedure as the samples were heated at 100°C in the presence of EDTA which conditions are known to prevent proteolytic activity. Molecular weight markers were treated in an identical manner to the resuspended microsomal pellets.

All electrophoretic separations were carried out in polyacrylamide gel slabs formed using the Uniscil/Gradipore gel casting set. The first dimension separations were carried out in 5.2% gel slabs at 75 V until the tracker dye (pyronin-y) reached the end of the slabs. The second dimension separation were carried out in 6–27% gradient gel slabs at 75 V for 12 hr. The buffer used throughout consisted of 88.7 mM Tris, 2.5 mM disodium EDTA, 81.5 mM boric acid and 0.1% w/v sodium dodecyl sulphate and had a pH of 8.3. All gels were fixed and stained according to the procedure of Fairbanks et al. [18]. A review of the procedures

used in the solubilisation and characterisation of membrane proteins has been published by Maddy [19]. Phospholipids have been shown not to interfere with the separation or visualisation of protein components in sodium dodecyl sulphate polyacrylamide gel electrophoresis [20]. Densitometer tracings of the first dimension separations were obtained using a Joyce-Lobel Chromoscan.

## 3. Results and discussion

The sucrose densities, protein concentrations and calcium uptake activities of the four fractions are listed in table 1. Calcium uptake activity peaks in fraction 3 at a density of 1.151. The specific activity of 2.39 $\mu$mol· mg protein $^{-1}$ · min$^{-1}$ is greater than that reported by other workers [21]. There is negligible contamination of this fraction by protein of the inner mitochondrial membrane as measured by succinate dehydrogenase activity. When allowances are made for the presence of nonsedimentable or soluble protein the phospholipid to protein ratio of these four fractions is approximately 0.55. A more detailed discussion and evaluation of the method of preparation has already been given [13,16].

An examination of the electrophoretic separations obtained (fig.1 and 2) shows that the four fractions differ in their protein composition. The protein component of molecular weight 100 000 reported as being the $Ca^{++}$ transport ATPase [1,2,8,9] and marked with an asterisk on the densitometer tracings migrates as one species in both dimensions. Its distribution parallels that of calcium uptake activity and while it is the principal component of fractions 2,3 and 4 it occurs in greatest quantity in fraction 3. Material sedimenting to a density greater than that of fraction 4 has also been examined. This material is characterised by a very low calcium uptake activity and a low content of the 100 000 dalton component. The first dimension separation of fraction 1 is characterised by two dense bands in the mol. wt range 70 000–80 000 and 25 000–30 000 followed by a weaker 100 000 dalton band. Bands corresponding to intermediate molecular weights are of low intensity and are further resolved into a number of components in the second dimension. This fraction 1 is the first reported case of a muscle microsomal membrane preparation not having the 100 000 dalton component as its major protein. The 25 000–30 000 and 70 000–80 000 dalton bands are significantly reduced in fraction 2. From fractions 2 to 4 there is an increase in the intensity of bands in the intermediate and low molecular weight ranges. All of these bands are heterogenous and each is resolved into at least three components in the second dimension separation. In fractions 1 to 4 there are 26, 18, 33 and 37 protein spots respectively. It appears that many of the minor protein components associated with the high calcium transport of fraction 3 are not detected in fraction 2 due to their lower concentration there.

These results show that the protein composition of muscle microsomal preparations having high calcium uptake activities is more complicated than was previously thought.

Table 1
Density, protein concentration and calcium uptake activity of muscle microsomal fractions

| Fraction number | Sucrose density at 5°C | Protein concentration mg/ml | Calcium uptake activity μmol/mg protein per min |
|---|---|---|---|
| 1 | 1.124 | 0.972 | 0.309 |
| 2 | 1.136 | 0.440 | 1.202 |
| 3 | 1.151 | 0.264 | 2.386 |
| 4 | 1.192 | 0.169 | 0.556 |

The preparation of the four microsomal suspensions and the determination of sucrose density, protein concentration and calcium uptake activity were carried out as previously reported [10,17].

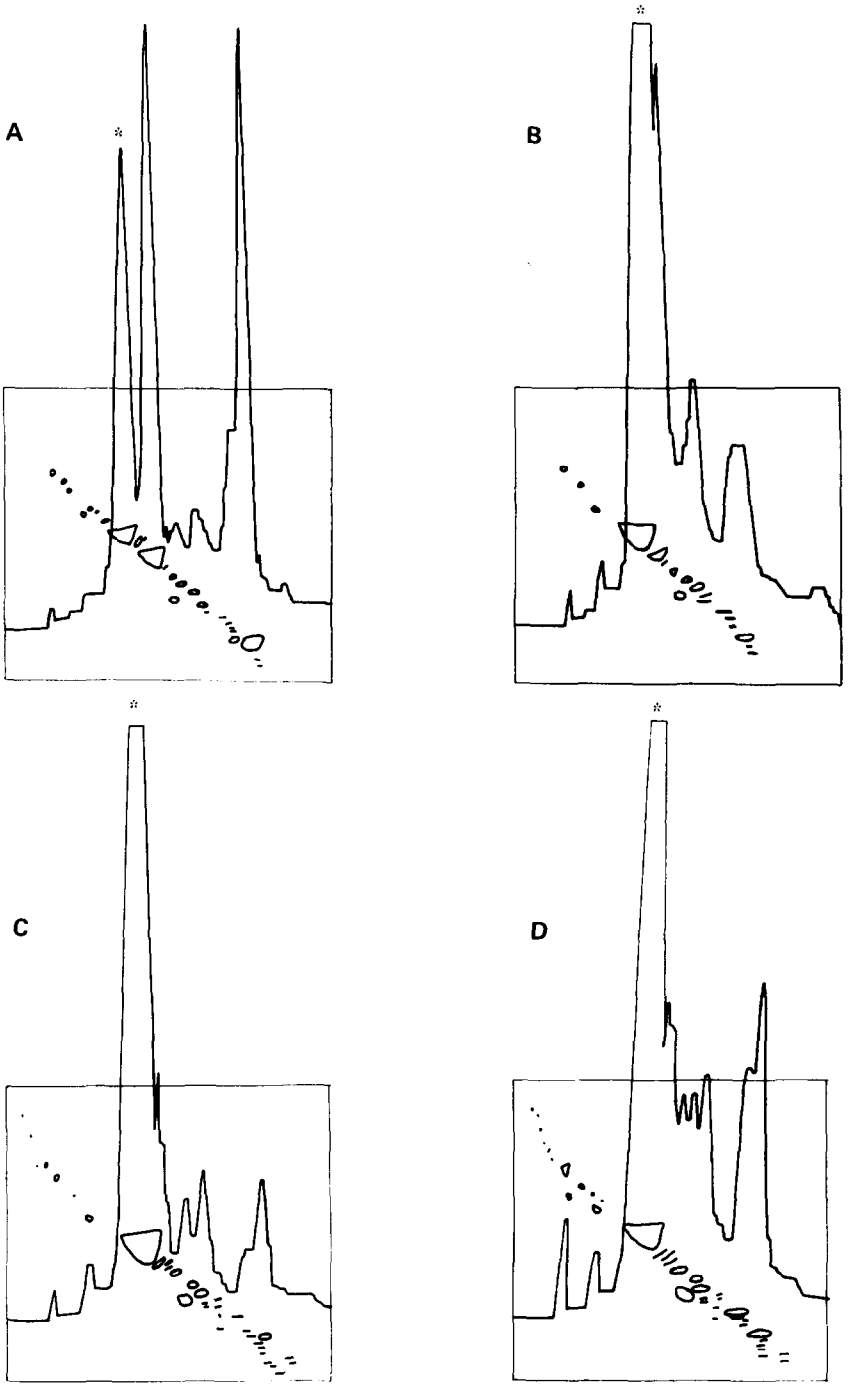


Fig.1. Two-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis of membrane proteins in the four muscle microsomal fractions. A-D refer to fractions 1–4 respectively. The figures consist of superimpositions of the densitometer tracings of the first dimension separations on drawings of the second dimension separations. Electrophoresis in the first dimension was from left to right in 5.2% gels and in the second dimension from top to bottom in 6–27% gradient gels. Further experimental details are given in Materials and methods. * Indicates the 100 000 dalton protein component.

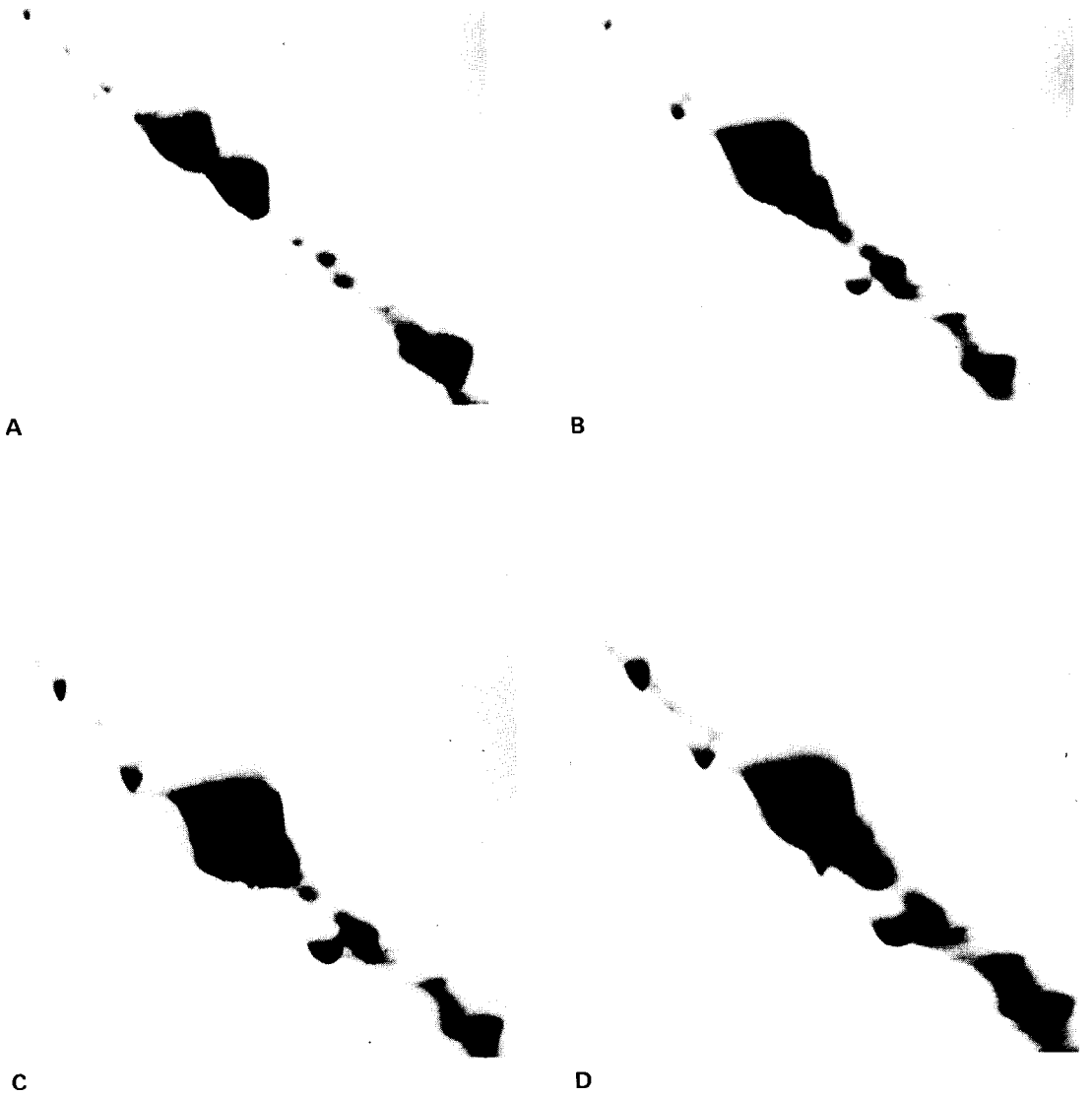


Fig.2. Photographs of gradient gel slabs after two-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis of membrane proteins from four muscle microsomal fractions. A-D refer to fractions 1--4 respectively. The electrophoretic procedure is described in the text.

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