MUSCLE FIBRILS: SOLUBILIZATION AND GEL ELECTROPHORESIS

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1. Introduction

The fibrous nature of the muscle proteins of the contractile apparatus, and in particular the insolubility of myosin at the low ionic strengths required for electrophoresis, has in the past mainly limited study to the individual purified proteins. This communication describes the use of the detergent sodium dodecyl sulphate (SDS) to dissolve myofibrils, which are the basic functional components of muscle. This method permits assessment for the first time of the total protein complement of the myofibril, with minimization of losses occurring during extraction procedures, and should further our understanding of the composition and biosynthesis of muscle. The identification and quantitation of the proteins will be described, and the existence is demonstrated within the myofibril of minor protein components which are distinct from the other well known muscle proteins (i.e. myosin, actin, α-actinin and the tropomyosin-troponin complex).

2. Materials and methods

The animals used were male albino rats of the Sprague-Dawley strain weighing 280–320 g. Muscles were removed under light ether anaesthesia, and subsequent manipulations were performed at 4°. Myofibrils prepared from cardiac or skeletal muscle [1] were washed five times by centrifugation and resuspension, and then further purified from contaminating sarcoplasmic proteins by centrifugation through zones of 40% and 50% sucrose [2]. The myofibrils from 1 g of muscle were dissolved in 5–10 ml of a solution containing 1% SDS, 6 M urea, 1% β-mercaptoethanol and 50 mM sodium phosphate (pH 7.1) with heating at 50° for 30 min (reference proteins were similarly treated). Microscopic examination of the myofibrils showed complete disruption of structural features and loss of cross striations after addition of SDS. Only 1–2% of the total protein content of the myofibril was not brought into solution by SDS treatment (the residue was dissolved in 2 M NaOH for protein estimation). Equivalent solubilization could be achieved in the absence of urea, but urea was included for reasons discussed below. The myofibril solution was used directly for electrophoresis on acrylamide gels containing SDS, in which the migration of a reduced SDS treated protein is a direct function of its molecular weight [3–5]. Identification of myofibril protein bands in the gels was attempted by comparison with purified proteins prepared from rabbit skeletal muscle by established methods documented below; estimates of the molecular weights and relative amounts of each component in the gel were also made (see figs. 1 and 2).

3. Results and discussion

Ultracentrifugal studies in the presence of dissociating agents [7–9], as well as SDS acrylamide gel electrophoresis [10, 11], indicate that myosin of molecular weight 470,000 is composed of two 'heavy' chains, each of molecular weight 200,000 and at least two 'light' chains averaging 20–30,000 molecular weight. The myosin used in the present study, donated by members of the MRC Muscle Biophysics Unit, King's College London (as were the actin and tropomyosin), was prepared by the method of Perry [12],

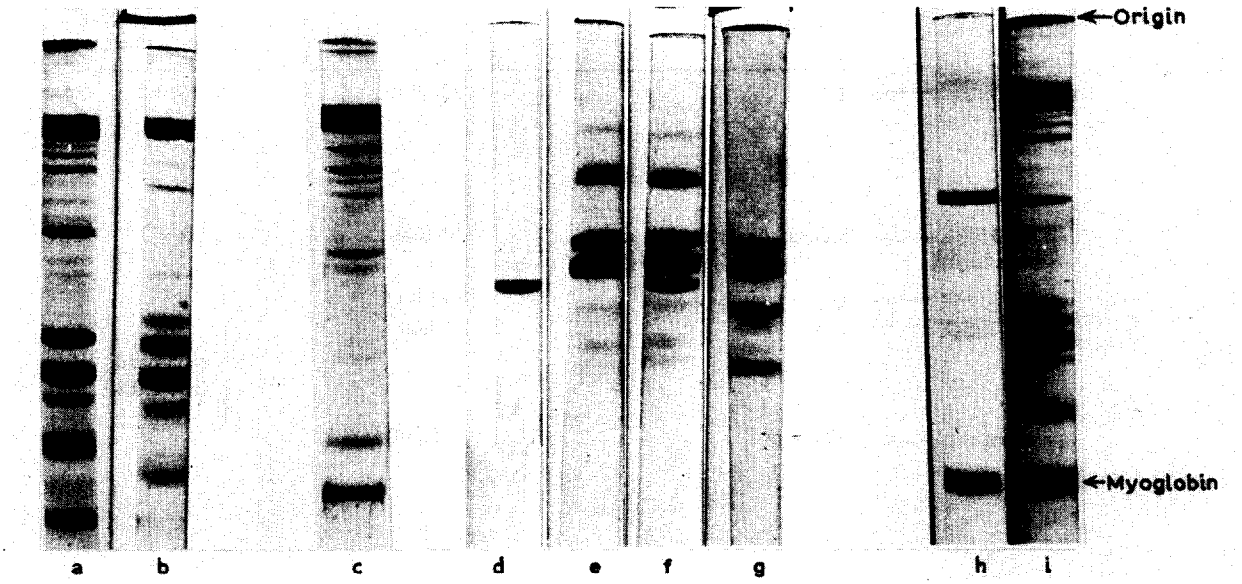
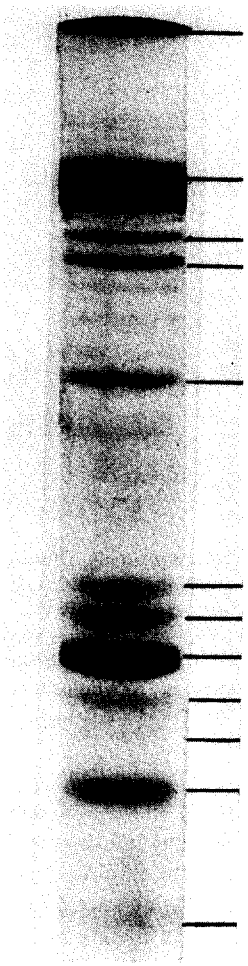


Fig. 1. The identification of myofibril proteins in SDS-acrylamide gels by comparison with reference proteins. The sources of these reference proteins are indicated in the text. Electrophoretic conditions are as described in [3], with the following variations: gels are 4% 'Cyanogum' 41 (British Drug Houses) and contain 5 M urea in addition to 0.1% SDS. Gels were stained with Coomassie brilliant blue and destained electrolytically [6]. Some gels myoglobin (whale skeletal muscle) which was added as a marker for the measurement of relative electrophoretic mobility. Gels which are grouped together (a and b; d, e, f, and g; h and i) were electrophoresed at the same time. (a) Solubilized myofibrils from rat heart. (b) Mixture of myosin, actin and tropomyosin-troponin complex. (c) Myosin. (d) Actin. (e) Tropomyosin. (f) Tropomyosin + actin. (g) Tropomyosin-troponin complex. (h) α-actinin + myoglobin. (i) Solubilized myofibrils from rat heart + myoglobin.

with the addition of a batch treatment with DEAE-cellulose [13]. Electrophoresis of this myosin preparation shows (fig. 1c) in addition to the heavy and light chains a number of previously undetected components, whose possible significance has been investigated by Starr and Offer [14]. The molecular weights obtained for the light chains agree with other estimates on purified myosin (for example 17,000 and 21,000 from amino acid composition [15], and 17,000 and 25,000 from SDS gel electrophoresis [10]). A third light chain could not be identified in either cardiac or skeletal muscle, possibly because it had not been satisfactorily fixed or stained. The figure of 207,000 for the molecular weight of the heavy chain compares well with values obtained by other techniques (e.g. 200,000 by ultracentrifugation [9]).

Actin was extracted from acetone powders prepared by the Straub method as modified by Katz and Hall [16], using 0.5 mM ATP, 0.2 mM $CaCl_2$ and 0.5 mM dithiothreitol (pH 7.5), and purified by a single polymerization step. Electrophoresis showed a single band (fig. 1d). The value of 44,000 for the molecular weight of the isolated protein and the myofibril band is in good agreement with the finding of a molecular weight of 46–48,000 for skeletal actin [17].

Tropomyosin prepared by the method of Bailey [18] behaved differently on SDS electrophoresis depending on whether it had been dissolved in SDS containing 6 M urea or lacking urea. In the absence of urea a wide band, possibly a doublet, was found of molecular weight 36,000, which is the accepted value [19, 20]. In the presence of urea two bands of molecular weights 51,700 and 54,700 were seen (fig. 1e). This behaviour was constant and reproducible. The same effect was observed with the band pattern of whole myofibril and with tropomyosin-troponin complex. None of the other protein molecular weights were affected by the presence of urea. As tropomyosin was removed by urea treatment from the region in the gel where troponin and myosin light chains also migrate, this phenomenon is useful in reducing the number of gel bands in the molecular weight region



Protein	Molecular weight	Percentage of total protein
U 1	⩾ 200,000	16 ± 2
Myosin, heavy chain	207,000	33 ± 3
U 2	175,000	each band 0.5–2.0%
U 3	150,000	
α-actinin	102,000	
Tropomyosin (1) in urea	54,700	6.9 ± 0.9
Tropomyosin (2) in urea	51,700	
Actin	44,000	16 ± 2
Troponin (1)	38,000	5.9 ± 1.7
Troponin (2)	31,000	
Myosin, light chain (1)	24,000	7.4 ± 1.6
Myosin, light chain (2)	18,000	

Fig. 2.

Fig. 2. The molecular weight and percentage composition of myofibril proteins from rat heart. Electrophoretic conditions are described in the legend to fig. 1. Tentative identification of known bands is indicated, and selected unknown bands are numbered sequentially from the origin (cathodal end), with the prefix U. The molecular weights of the proteins used to establish the molecular weight calibration curve were those quoted by Weber and Osborn [3]. Densitometry of stained gels was performed using a modified Hilger-Guilford scanner with a slit width of 0.2 mm. The recorded peaks from the chart paper were traced, cut out and weighed. Results are from measurements on cardiac muscle from five rats, and percentages are expressed as means + standard error of the mean.

below 44,000. A sample of tropomyosin-troponin complex [2] from skeletal muscle powder and further purified by two ammonium sulphate precipitation steps was donated by Dr. J. Spudich of the MRC Molecular Biology Research Laboratory, Cambridge. This gave four bands on gel electrophoresis (fig. 1g), two of which behaved identically to tropomyosin. The other two are considered to be troponin. Schaub and Perry [22] find two components on electrophoresis of troponin in acrylamide gels containing urea, but there are not yet reliable published figures for the molecular weights of the constituents of the troponin complex.

α-actinin, prepared by this method [23], was a gift

from Professor Darrel E. Goll of Iowa State University, Ames, USA. The purified protein had an electrophoretic mobility identical to that of a solubilized myofibril protein of molecular weight 102,000 (fig. 1h).

An attempt to duplicate the myofibril band pattern was made by mixing together myosin, actin and tropomyosin-troponin complex in approximately the proportions found in the myofibril. The mixture was heated for 30 min at 50° and electrophoresed. The result was a simple addition of the bands of the individual proteins, and the unidentified bands were those present in the myosin preparation (fig. 1b). Myofibrils of skeletal and cardiac muscle from normal rats, mice and rabbits were compared electrophoretically; apart from variations in myosin light chain mobilities there were no marked differences. This is not surprising as the separation in the presence of SDS is a crude one, on the basis of molecular size rather than amino acid charge difference.

Comparison with the purified reference proteins has thus allowed identification of myosin, actin, α-actinin, tropomyosin and troponin in SDS gels of solubilized myofibrils. It must be stressed that this identification is tentative and dependent on the purity of the standards used. All the present work was performed at a gel concentration of 4% acrylamide, which allows demonstration of the whole spectrum of proteins found in the myofibril. At this concentration low molecular weight proteins migrate rapidly and tend to diffuse. Variation in acrylamide concentration and cross linkage should yield more satisfactory results in the study of individual proteins.

Some of the unidentified proteins seen on gel electrophoresis of the solubilized myofibrils may be multimers of smaller proteins. Paterson and Strohman [11] found that sulphonation of myosin solutions by treatment with sodium sulphate prevents the formation in SDS acrylamide gels of high molecular weight bands identified by them as myosin heavy chain dimers and trimers. The band labelled U 1 in fig. 2 may represent such an aggregate. Contamination by non-myofibril proteins or by degradation of myofibril proteins cannot be excluded. Alternatively some unidentified bands could be 'minor' myofibril proteins which may have important structural or regulatory roles, and merit further investigation.

Quantitation of the proteins (fig. 2) assumes that the integrated optical density of stained gel bands is proportional to the mass of protein present; the affinity of the dye for the different proteins may however be variable. The method does not permit measurement of the minor protein bands with marked reliability. These constituted 0.5–2.0% each of the total protein. Apart from the large amount of the high molecular weight protein near the origin (fig. 2, U 1), and the contribution of the minor bands, the results are in fair agreement with previous estimates of the relative amounts of the major contractile proteins in muscle. Katz [24], for example, quotes the figures 35, 15, 5 and 2.5 for myosin, actin, tropomyosin and troponin respectively, expressing the amount of protein in g/kg wet weight of myocardium.

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