ELECTROPHORETIC SEPARATION OF MYOSIN LARGE SUBUNITS*

R.G. HALE** and G.R. BEECHER**
Department of Biochemistry, Kansas State University, Manhattan, Kansas 66502, USA

Received 31 August 1971

1. Introduction

Considerable electrophoretic work has been performed on the myosin molecule. Small and coworkers observed several polydisperse bands in urea concentrations of 4–10 M [1]. In 12 M urea (45–50°) a single band, representing the myosin subunits, was observed. Studies have demonstrated an apparent heterogeneity of succinylated and acetylated myosin using disc gel electrophoresis [2]. Results of isoelectric focusing have similarly demonstrated a heterogeneity of unmodified myosin [3]. Recently, isoelectric focusing of alkylated myosin has resulted in two distinct bands representing the large subunits [4].

This communication presents evidence for two electrophoretically distinguishable myosin large subunits. A nomenclature is proposed for the subunits, designating them as myosin large subunit$_1$ ($MLS)_1$ and myosin large subunit$_2$ (MLS_2).

2. Materials and methods

Myosin was obtained from the adductor magnus of fasted New Zealand White male rabbits by the method of Harris and Suelter [5] and was stored in either 6 M urea at 4° or 0.6 M KCl-50% glycerol at −20° immediately after isolation.

Polyacrylamide disc gel electrophoresis was conducted [6] in 6–11 M urea using 2.0% (w/v) acrylamide gels in which comonomer was 5% by weight of monomer. The sample layers (containing approx. 100 μg of protein) and stacking layers were prepared without the use of a large pore gel. Electrophoresis was performed by applying a constant current of 2 mA per gel for 30 min followed by 4 mA per gel for the remaining time. All gels were fixed in 20% (w/v) sulfosalicylic acid for 20 min, stained in 0.25% (w/v) aqueous Coomassie brilliant blue [7] for 30 min, destained in deionized, distilled water, and stored in 7% (v/v) acetic acid. Photographs were obtained immediately after destaining.

Concentrations of cyanate in urea solutions were determined by a modification of Werner's procedure [8].

Molecular weight studies were performed in 3.0% (w/v) acrylamide gels by procedures described previously [9, 10]. Reference proteins used were 7 S gamma globulin (appr. 160,000 g·mole^{-1}), obtained from Schwarz-Mann (Orangeburg, N.Y., USA), and Component I (approx. 190,000 g·mole^{-1}), obtained from Dr. R.E. Clegg [11]. The myosin preparation and both reference proteins were separately equilibrated in 11 M urea (40°) for 48 hr, after which they were equilibrated with 1% (w/v) sodium dodecyl sulfate (SDS) for 5 hr. Finally they were equilibrated with 0.1% (w/v) SDS, 5% (w/v) sucrose, and Tris-glycine buffer (pH 8.3) which also was used as the tray and gel buffer during electrophoresis. Gels and tray buffer were also 0.1% (w/v) in SDS. A total volume of 100 μl of protein (approx. 3.5 mg per ml) was placed over each gel. Gels were fixed, stained, and destained as described earlier.

* Contribution No. 129, Department of Biochemistry, Kansas Agricultural Experiment Station, Manhattan, Kansas 66502, USA.
** Present address: Human Nutrition Research Division, Agricultural Research Service, USDA, Beltsville, Maryland 20705, USA.

3. Results and discussion

The purity of myosin was established by several criteria. Data indicating the removal of AMP deaminase and nucleic acids during the purification of myosin are presented in table 1. Disc electrophoresis of the final myosin preparation in 6 M urea and 2.0% (w/v) acrylamide gels resulted in several bands similar to those previously reported [1, 9, 12]. However, electrophoresis of myosin in 0.1% (w/v) SDS resulted in only one band.

Results of disc electrophoresis of myosin in 11 M urea and 2.0% (w/v) acrylamide gels are presented in fig. 1. Samples were dissolved in 11 M urea, electrophoresed immediately (0 hr) and after equilibration with the same solvent for 24, 48, 72, 96, and 168 hr. Electrophoresis immediately (0 hr) after dissolution in 11 M urea resulted in a series of bands, the lead band of which had migrated approx. one-third the length of the gel. A vaguely stained, very diffuse region was observed in the lower third of the gel. Exposure to 11 M urea for 24 hr resulted in a reduction in the intensity and an overall increase in the distance of migration of all bands, which was thought to be due to a slight variation in the pore size of the gels. After 48 hr of 11 M urea exposure, a subtle separation was observed to occur in the lead band, with almost complete disappearance of all trailing bands. In all samples exposed to urea for 72 hr or longer, the entire disappearance of all trailing bands was observed, with the

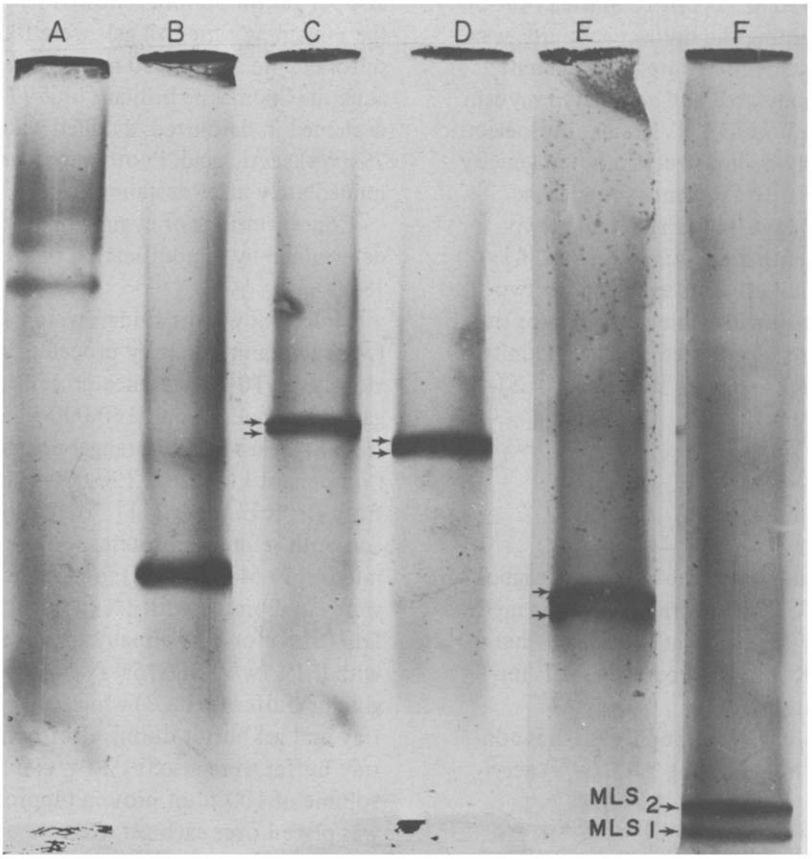

Fig. 1. Disc electrophoresis of myosin in 2.0% (w/v) acrylamide gels after equilibration in 11 M urea (40°) for various times. Gel A, 0 hr; Gel B, 24 hr; Gel C, 48 hr; Gel D, 72 hr; Gel E, 96 hr; Gel F, 168 hr. Gels A through D had a total electrophoresis time of 75 min, Gel E, 95 min, and Gel F, 105 min. Migration was from the top (–) to the bottom (+) of the gels.

Table 1
Myosin AMP deaminase activity and absorbance ratios.

Step in purification procedure	AMP deaminase specific activity (μmoles·min^{-1}·mg protein^{-1})	$A_{280\ nm}$:$A_{260\ nm}$
Before cellulose phosphate chromatography	0.035	1.16
After cellulose phosphate chromatography	< 0.001	1.25
After DEAE-cellulose chromatography	< 0.001	1.70

major molecular species existing as the two closely migrating bands. No other bands were observed to enter the gel, even after 3.5 hr of electrophoresis time. It is pertinent to note that these studies were conducted in 11 M urea on unmodified myosin using a 2.0% (w/v) acrylamide discontinuous electrophoretic system. Other investigators have electrophoresed either unmodified myosin in a 12 M urea – 3.75% (w/v) acrylamide continuous system [1] or both unmodified and modified myosin in a 9 M urea – 2.2% (w/v) acrylamide discontinuous system [2].

An 11 M urea solution was found to be 11.6 mM in cyanate after several days of incubation at 40° but less than 1 mM in cyanate if maintained at pH 2 (40°). Thus electrophoresis was performed on myosin which had been equilibrated for 48 hr in 11 M urea at pH 2 (40°). The two closely migrating bands were again visible as the major molecular species. Recent evidence has demonstrated that incomplete carbamylation of a protein in high concentrations of urea results in a time-dependent variety of molecular species [13]. One would therefore expect to observe a number of bands, rather than only two, if incomplete carbamylation were occurring. However, one would also expect that complete carbamylation, as none at all, would result in bands representative of the number of molecular species present. Hence the possibility of the bands representing polypeptide chains which were completely carbamylated must be considered. Experiments were also performed on myosin stored in 11 M urea for at least 48 hr under N_2 in the presence of a 1000-fold molar excess of dithiothreitol to investigate the possibility that partial oxidation could be occurring. Electrophoresis again resulted in two closely migrating, distinct bands. It therefore appeared that the two bands were not due to partial modification by carbamylation or oxidation.

Polyacrylamide gel electrophoresis of the myosin preparation in 0.1% (w/v) SDS established an approximate molecular weight of 200,000 g·mole^{-1} for the major molecular species (represented by MLS_1 and MLS_2 in fig. 1). Since myosin is known to dissociate completely to its major subunits in urea solution of at least 10 M [1], it is quite reasonable to assume that the major molecular species observed were the large subunits of myosin. From the results reported here using unmodified myosin equilibrated with 11 M urea for at least 48 hr and a dilute acrylamide discontinuous electrophoretic system, the myosin large subunits now appear to be distinguishable. The faster migrating subunit is designated myosin large subunit$_1$ (MLS_1) and the slower, myosin large subunit$_2$ (MLS_2).

Acknowledgements

This study has been supported by the Kansas Agricultural Experiment Station, National Institutes of Health Biomedical Sciences' Grant FR 07036 and National Institute of Arthritis and Metabolic Diseases' Grant AM 13764.

The authors wish to thank Dr. B.A. Cunningham for his advice and Dr. R.E. Clegg for his contribution of Component I.

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