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ACRYLAMIDE GEL ELECTROPHORESIS OF AGGREGATION AND DEGRADATION PRODUCTS OF MYOSIN

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SUMMARY

The acrylamide gel electrophoresis of myosin, its degradation products and aggregation forms is described. On a 5 % gel a myosin dimer and three types of myosin polymers could be observed. After tryptic digestion five bands additional to the heavy meromyosin (HMM) and light meromyosin (LMM) were observed: two of these seem to be closely related to the LMM and HMM fragment while two other are low-molecular weight fragments (mol. weight between $2–7 \cdot 10^4$). In contrast to the separation of myosin aggregates, the separation of myosin breakdown products is better on a 7.5 % gel; the 5 % gel separation of the tryptic digest of myosin does not result in the separation of the double band of the low molecular weight fragments and the LMM fragment (presumably LMM-1) is also undistinguishable from the LMM band. At least five additional fragments appear after trypsin digestion on the 7.5 % gel besides the LMM and HMM fragments during the tryptic digestion of myosin.

INTRODUCTION

It is surprising that knowledge concerning the separation of an intensively studied protein such as myosin is rather incomplete. Though it is possible to trace several attempts to separate myosin and its degradation and aggregation forms by column chromatography within the past ten years[1,2], no method allowing the attachment of molecular entities to respective individual fractions was available until last year[3]. Light meromyosin (LMM) and heavy meromyosin (HMM) fragments could be clearly separated from the intact molecule and myosin polymers by the technique described; but the more detailed separation of higher aggregation products was not possible. The reason was presumably the use of DEAE-Sephadex A-50 as carrier material. As far as electrophoretic techniques are concerned, the separation of the breakdown products has been obtained in 6 *M* urea as has been described by SMALL *et al.*[4]. Some further results on the chromatographic separation of myosin and its degradation

products using Sephadex G-200, Bio-Gel P-300 and DEAE-cellulose have been published by HOTTA AND USAMI[5] and YAGI AND YAZAWA[6]. In addition, several results dealing with the separation of small molecular weight fragments of myosin using acrylamide gel electrophoresis have been reported[7,8].

As well as a single separation technique being considered inadequate in protein chemistry, the reason for looking for another separation technique is mainly the desirability of separating myosin polymers more precisely.

Furthermore, a separation technique which permits the stepwise degradation of myosin during the proteolytic degradation of this protein would be quite helpful in structural studies of this molecule.

The main problem in using acrylamide gel electrophoresis in myosin separations was the precipitation of the main part of the sample in the spacer gel zone. This fact is due to the high sensitivity of myosin towards ionic strength changes. In order to prevent this effect low density gels and buffers of relatively high ionic strength were used in the experimental part of this work.

MATERIAL AND METHODS

Samples of myosin were prepared according to SERAYDARIAN *et al.*[9] and precipitated twice before being used for electrophoretic studies. The preparations were essentially free of nucleotides.

Trypsin treatment

For every 10 ml of myosin solution in 0.5 *M* KCl + 0.05 *M* phosphate buffer pH 6.2 (protein concentration approx. 1.5 %) 1 ml of 0.05 % trypsin solution was added with stirring at room temperature. The action of the trypsin was stopped by adding 0.1 ml of 0.1 % soybean trypsin inhibitor.

The LMM and HMM were prepared according to the technique of LOWEY AND COHEN[10]. Aggregated forms of myosin were obtained by keeping the myosin solutions under sterile conditions at room temperature and at 4° for a sufficiently long period of time (2–13 days). Preparations of myosin enriched in myosin dimer were obtained from the so-called "post rigor" muscles[11]. In all experiments rabbit white skeletal muscle myosin was used. However, no essential differences in the electrophoretic behaviour were observed in preliminary tests with white chicken muscle, rabbit heart, chicken heart and beef heart muscle myosins.

The acrylamide gel (7.5 %-spacer gel) was prepared following, in general, the procedure published by REISFELD *et al.*[12]. The following solutions were used for the preparation of the 5 % gel.

Solution A: 1 *N* HCl 48 ml; Tris 36.6 g; Tetramethylenediamine 0.23 ml; H_2O ad 100 ml (pH = 8.9).

Solution B: Acrylamide 28.0 g; N,N'-methylenebisacrylamide 0.735 g; H_2O ad 100 ml.

Solution C: Ammonium persulphate 140 mg/100 ml. The three solutions were mixed in the following proportions: 1 ml of A + 1.2 ml of B + 4 ml of C + 1.8 ml H_2O.

The electrode vessels were filled with a Tris buffer, pH 8.3 (Tris 6.0 g; glycine 28.8 g; double distilled water ad 1000 ml). This stock solution was diluted ten times

with distilled water before use. For staining, a 1 % solution of Amidoblack (Merck) in 7 % acetic acid was used. Staining was complete within one hour; destaining was done with 7 % acetic acid within three days in a gently stirred vessel. Tubes 9 × 50 mm were used; optimal amperage 5 mA per tube.

Individual zones in electropherograms were compared with electropherograms of individual peaks obtained by DEAE-Sephadex A-50 chromatography according to RICHARDS *et al.*[3] and Sephadex G-200 chromatography according to YAGI AND YAZAWA[6] in order to attribute corresponding molecular entities.

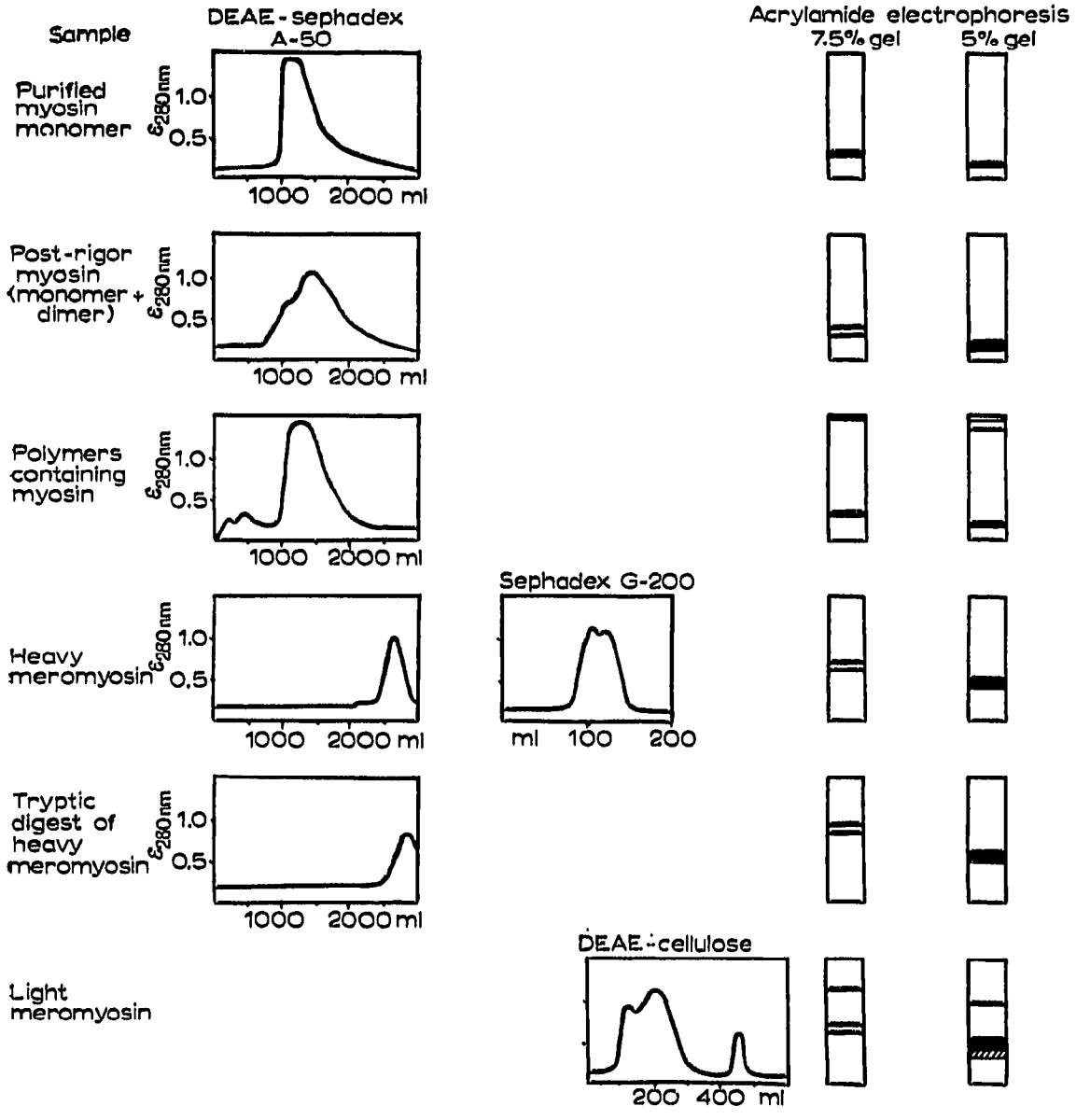

Fig. 1. Column chromatography of different myosin preparations and/or myosin degradation products and corresponding acrylamide gel electrophoretic patterns.

RESULTS

Our results are summarized in the form of representative electropherograms (see survey Fig. 1). Generally speaking in purified myosin preparations only one zone is observed. The so-called "post rigor" preparations enriched with a high molecular weight aggregate, presumably a myosin dimer, exhibit a double fast moving zone (Fig. 2) very close to the myosin monomer. Myosin aggregates of even higher molecular weight do not enter the 7.5 % gel at all and precipitate within the spacer gel area; they are quite clearly separated in the 5 % gel as one can see from Fig. 3, where a three- day-old non-purified rabbit white skeletal myosin preparation is compared with a heavily aggregated rabbit skeletal myosin. It thus seems reasonable to assume that on *in vitro* aging of the myosin preparations no more than two types of aggregates arise.

In trypsin treated myosin preparations three main zones can be distinguished (Fig. 4): the first zone being that of intact myosin followed by the HMM and LMM zone. From the separation point of view the quality of separation on a 5 % gel is not as good as that on a 7.5 % gel, but still adequate (Fig. 5). The over-all picture of

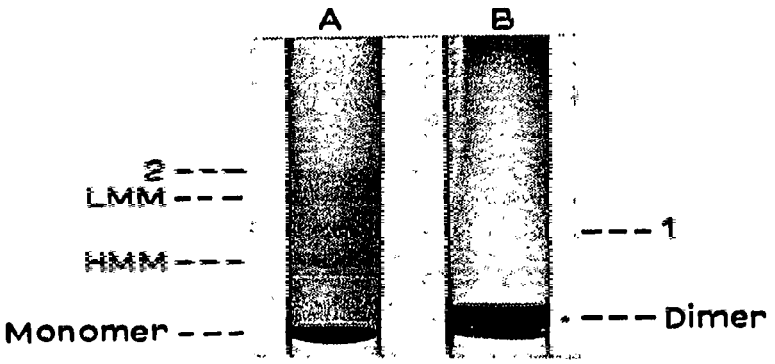

Fig. 2. 5 % acrylamide gel electrophoresis of trypsin treated myosin (A) and myosin isolated from post rigor muscle (B). Trypsin treatment was performed for 3 min. 1 = HMM derived; 2 = LMM derived + HMM derived. Traces of higher aggregates can be seen near the starting line.

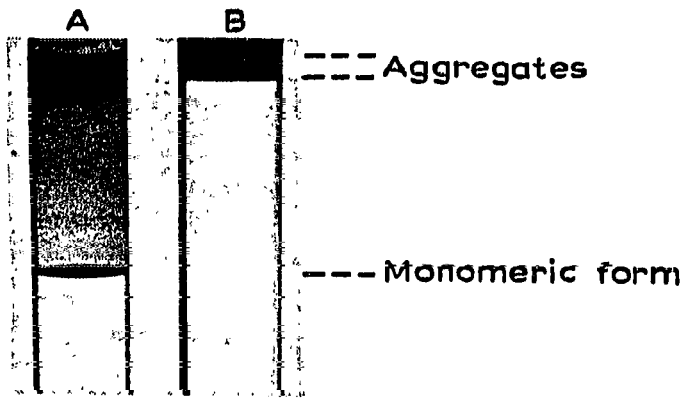

Fig. 3. 5 % acrylamide gel electrophoresis of myosin aged in solution. Aging was performed after sterilisation by ultrafiltration at 4°. Ancillary equipment was sterilized by U.V.-irradiation. A = 3 days aged myosin; B = 12 days aged myosin.

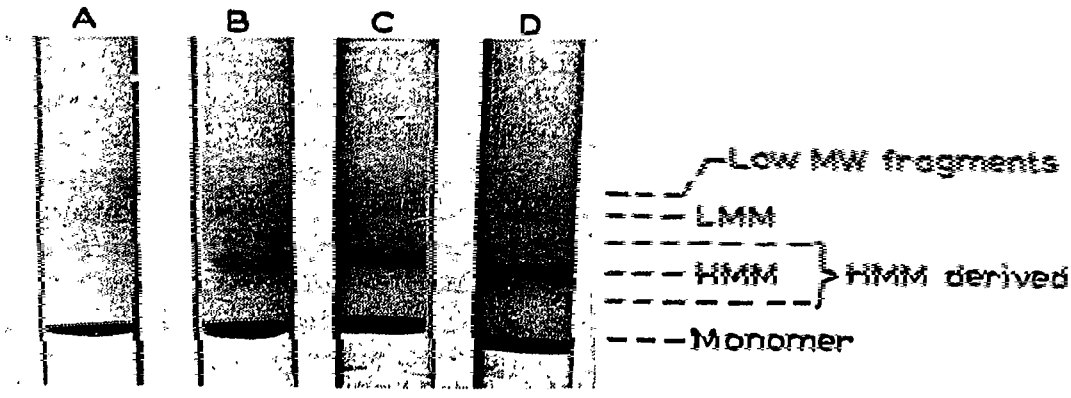

Fig. 4. 5% acrylamide gel electrophoresis of myosin and products of partial tryptic hydrolysis of myosin. A = Untreated myosin; B = 5 min trypsin treatment; C = 10 min trypsin treatment; D = 20 min trypsin treatment.

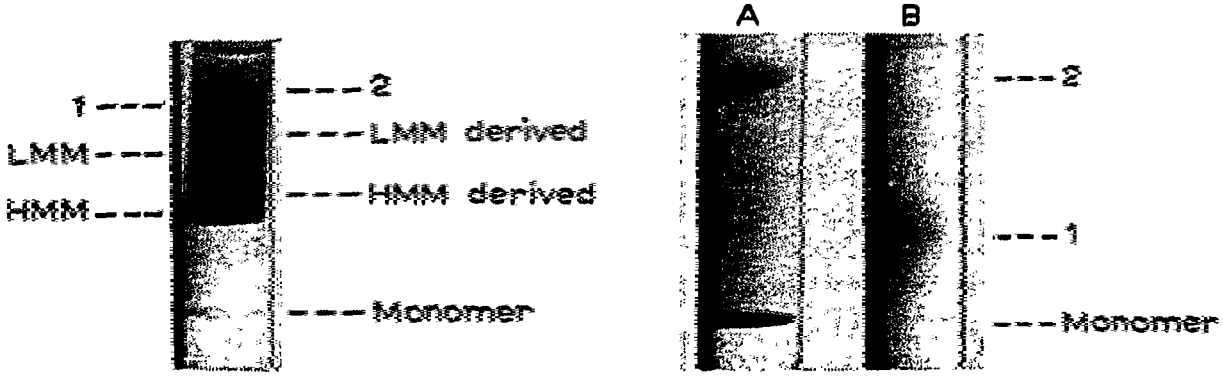

Fig. 5. 7.5% acrylamide gel electrophoresis of trypsin treated myosin. 1,2 = fractions derived from HMM and LMM; we did not succeed in resolving these fractions.

Fig. 6. 7.5% acrylamide gel electrophoresis of trypsin treated myosin. Effect of trypsin concentration. A = 0.1 ml of trypsin solution added (see description of trypsin treatment in text); B = 10 ml of trypsin solution added. 1 = Fraction corresponding to HMM; 2 = low-molecular fragment, mol. wt. 20,000.

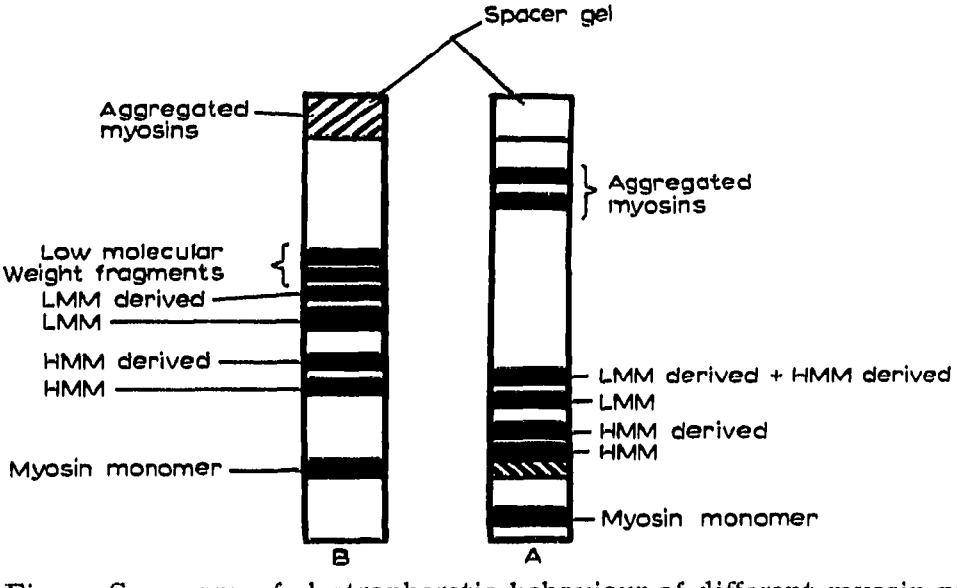

Fig. 7. Summary of electrophoretic behaviour of different myosin preparations and degradation products. A = 5% acrylamide gel; B = 7.5% acrylamide gel.

a trypsin treated myosin preparation, however, is far from being so simple. The distinct zone of myosin monomer sometimes doubled during trypsin digestion. Whether this doubling of the zone of monomeric myosin is due to myosin dimer formation or whether it is the result of a slightly modified myosin molecule deficient in some peripheric region we do not know. The HMM zone is always followed by a less intensive band and equally the main band of LMM is accompanied by a more slowly moving less intensive zone. This over-all pattern can be observed no matter which enzyme is acting and whatever protein ratio or digestion time are employed. Sometimes an additional very weak zone is observed on the 7.5 % gel in between the myosin monomer and the HMM band. This is only clearly separated on the 7.5 % gel; on the 5 % gel it forms a tailing area in front of the HMM band. The last area in trypsin digested samples is the zone of low-molecular weight substances which is rather close to the start but still has a much higher mobility compared to the myosin aggregates. On the 7.5 % gel these low molecular weight substances form a double zone. The molecular weight of this fragment, judged according to comparative Sephadex chromatogram, is between $2–7 \cdot 10^4$.

An over-all summary of the mobilities of the individual myosin fractions is presented in Fig. 7.

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