Disc Electrophoresis of Weber-Edsall Extract and Actomyosin from Skeletal Muscle

J. H. Rampton,[1] A. M. Pearson,* J. E. Walker,[1] and J. G. Kapsalis[1]

Inconsistency and heterogeneity in electrophoretic patterns of Weber-Edsall extract from both rabbit and beef muscle could be largely overcome by avoiding heavy metal contamination and/or prolonged exposure to atmospheric oxygen. Adding an equal weight of sucrose also aided in stabilizing Weber-Edsall extract. Unreduced Weber-Edsall extract separated into "stationary" and "slow" myosin, two tropomyosin bands, a diffuse actin band, troponin, and two minor bands. Dithiothreitol (DTT) treatment effectively sharpened the actin band and eliminated one tropomyosin and one minor band from Weber-Edsall extract, apparently by inhibiting protein interactions. Disc electrophoresis of unreduced actomyosin resulted in a similar pattern to unreduced Weber-Edsall extract, except that one minor band occurred for rabbit and two for beef preparations. DTT reduction of actomyosin was inconsistent, resulting in altered and less repeatable electrophoretic patterns. The significance of these findings is discussed.

Earlier studies from our laboratories have reported on disc electrophoresis of actin, tropomyosin, troponin, α- and β-actinin and extra protein (Rampton *et al.*, 1970a) and upon myosin aggregation (Rampton *et al.*, 1970b) from skeletal muscle preparations. Dialysis of myofibrillar extracts against 8 *M* urea containing small amounts of DTT (dithiothreitol) improved the separation on disc gels and aided in identification of the various purified proteins (Rampton *et al.*, 1970a,b). Thus, such techniques should be applicable for more completely characterizing Weber-Edsall extract and actomyosin, which have not previously been well characterized by disc electrophoresis. Information of this nature should prove helpful in elucidating the role of the various myofibrillar proteins upon the physical properties of meat and meat products.

The present study describes the separation of Weber-Edsall extract and actomyosin by acrylamide gel electrophoresis in 7 *M* urea, both before and after reduction of SH-groups with DTT or reaction with sulfite. The results are also discussed in regard to possible quantitative applications.

METHODS

Preparation of Weber-Edsall Extract. Muscle was homogenized with 12 volumes of a solution composed of 0.25 *M* sucrose, 1 mM EDTA (ethylenediaminetetraacetic acid), and 0.05 *M* Tris buffer at pH 7.6. After allowing the mixture to stand for 10 min, the slurry was centrifuged at 20,000 $\times$ *g* for 15 min. The supernatant, which contained the sarcoplasmic proteins, was disregarded. The entire procedure was repeated a second time. The remaining washed muscle residue and washed myofibrils were prepared according to the procedure of Perry and Zydowo (1959) and utilized for preparation of Weber-Edsall extract.

The washed muscle residue was homogenized with 60 ml of Weber-Edsall solution (0.6 *M* KCl, 0.04 *M* $NaHCO_3$, and 0.01 *M* Na_2CO_3) for every 10 g of original muscle, or else the prepared myofibrils were concentrated by centrifuging for 20 min at 15,000 $\times$ *g*, weighed and extracted with 45 ml of Weber-Edsall solution for every 10 g of myofibril preparation. Extraction was allowed to proceed for 20–24 hr, after which the viscous mass was diluted with 180 ml of Weber-Edsall solution per 10 g of original muscle or 135 ml per 10 g of myofibril preparation. The suspension was centrifuged at 25,000 $\times$ *g* for 1 hr. The supernatant, which contained the salt soluble proteins, was saved and subsequently utilized in either preparation of actomyosin or directly as the Weber-Edsall solution.

Preparation of Actomyosin. Weber-Edsall extract prepared as outlined above was utilized for isolating and purifying actomyosin by the method of Tonomura and Sekiya (1961). All subsequent analyses were carried out on the purified actomyosin.

Gel Electrophoresis. Samples for electrophoresis were prepared by dialyzing against 8 *M* urea and electrophoresced as described by Rampton *et al.* (1970a) using a modification of the method described by Davis (1964). Gels were prepared from three stock solutions as outlined by Jolley *et al.* (1967) with acrylamide being added as Cyanogum at levels of 5.0 and 3.0% for the running and spacer gels, respectively. All gels contained 7 *M* urea and electrophoresis was carried out at 2 mA per tube. Staining was according to the procedure of Rampton *et al.* (1970a).

Identification. Identification of the various myofibrillar proteins in the Weber-Edsall extract was achieved by comparison with purified preparations of the individually isolated fractions subjected to disc gel electrophoresis with and without DTT or sulfite reduction. The different myofibrillar proteins in the actomyosin preparation were also identified by comparing disc gel patterns for purified actomyosin with the individually isolated proteins.

Protection of Sulfhydryl Groups. In order to protect sulfhydryl groups of the various proteins against oxidation, DTT reduction and/or reaction with sulfite was/were utilized on a portion of the samples (Bailey, 1967). Thus, it was possible to compare bands from a given protein with or without protection of the SH-groups.

RESULTS AND DISCUSSION

Weber-Edsall Extracts. Weber-Edsall preparations tended to produce inconsistent and heterogeneous electrophoretic patterns typical of the one shown in Figure 1A. However, electrophoretic inconsistencies could be largely overcome by avoiding contamination with heavy metals or prolonged contact with atmospheric oxygen. Weber-Edsall extract could be stabilized for storage at 2° C by measuring the volume and adding an equal weight (w/v) of sucrose. Samples thus protected or stabilized yielded less heterogeneous and

Department of Food Science, Michigan State University, East Lansing, Mich. 48823

[1] Present address: U.S. Army Natick Laboratory, Natick, Mass. 01760

more reproducible electrophoretic results (Figures 1A and 1C).

Weber-Edsall extract from beef and rabbit myofibrils alike exhibited "stationary" myosin at the origin and "slowly migrating" myosin at $R_m = 0.00$. The bands at $R_m = 0.24$ and 0.43 have been reported previously in unreduced tropomyosin preparations (Rampton *et al.*, 1970a), while the diffuse, lightly-stained bands at $R_m = 0.27 - 0.33$ are typical of the unreduced actin preparations described by Rampton *et al.* (1970a). In addition, other minor bands were usually observed at $R_m = 0.50$, 0.53, and 0.88. Results suggest that Weber-Edsall preparations contained myosin, actin, tropomyosin and other minor myofibrillar proteins. However, there were no bands at $R_m = 0.02$ and 0.07, indicating α-actinin was not present in the Weber-Edsall preparation.

Weber-Edsall preparation could be washed at low ionic strength and stored in 8 *M* urea, in order to increase the amount of the "slowly migrating" myosin band, as previously described for isolated myosin (Rampton *et al.*, 1970b). Samples thus washed and stored did not usually produce the heterogeneous type of pattern shown in Figure 1A. However, storage often darkened the unreduced tropomyosin band at $R_m = 0.24$ and lightened the reduced tropomyosin band at $R_m = 0.43$.

Reduction and electrophoresis of Weber-Edsall preparation using DTT produced the typical pattern shown in Figure 1D. As can be seen, reduction by DTT caused the reduced tropomyosin band ($R_m = 0.43$) to darken. At the same time, the extremely sharp band at $R_m = 0.24$, which is characteristic of unreduced tropomyosin samples, disappeared. Furthermore, the diffuse, lightly-stained band for unreduced actin ($R_m = 0.27 - 0.33$) disappeared and a more well-defined band typical of reduced actin appeared at $R_m = 0.26$. In addition, a new band of $R_m = 0.11$ usually appeared. Although not apparent in Figure 1D, the "slow" myosin band ($R_m = 0.00$) decreased during reduction, as shown previously for isolated myosin (Rampton *et al.*, 1970b). The band at $R_m = 0.88$, when present, was darkened by DTT-reduction.

Electrophoretic results from Weber-Edsall preparation (Figures 1B, C, D) compared favorably with those obtained for individually isolated myofibrillar proteins. Results suggest that 7 *M* urea containing small amounts of DTT effectively inhibits the interactions that myofibrillar proteins exhibit toward each other.

Actomyosin. Actomysin preparations often produced heterogeneous electropherograms, unless the SH-groups were protected, as already described herein for Weber-Edsall preparations (Figure 1A). Actomyosin thus prepared from beef muscle produced the typical pattern shown in Figure 2A. It exhibited a diffuse, lightly-stained band at $R_m = 0.27 - 0.35$ and varying amounts of the bands at $R_m = 0.24$ and 0.43. In addition, faint bands at $R_m = 0.11$, 0.53, and 0.88 were often present. The above results indicate that the actomyosin complex consists of myosin (at origin $R_m = 0.00$), actin (unreduced $R_m = 0.27 - 0.33$), and tropomyosin (unreduced at $R_m = 0.24$ and SH-reduced at $R_m = 0.43$), as well as other minor myofibrillar proteins.

Similar results were obtained for rabbit actomyosin, except that actomyosin from rabbit never exhibited the band at $R_m = 0.50$, which was present in actomyosin from beef. The band at $R_m = 0.50$ was occasionally observed in isolated beef tropomyosin. DTT-reduction of tropomyosin or of actomyosin preparations from beef removed the band.

The relative absence of the band at $R_m = 0.00$ in Figure 2A demonstrates the phenomenon of myosin aggregation, already

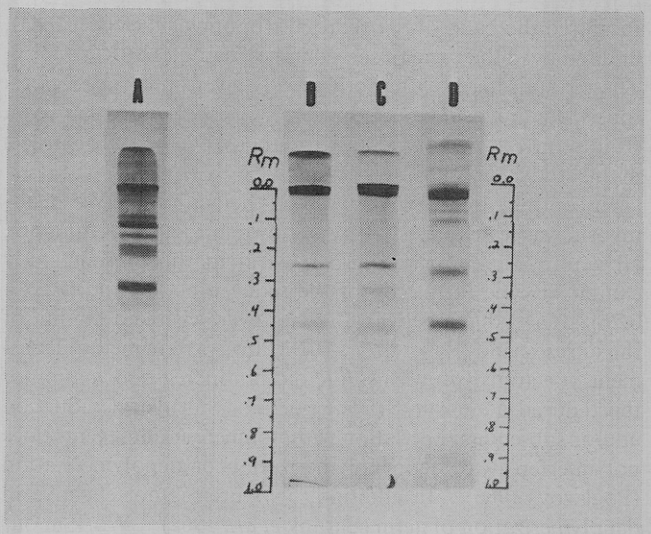

Figure 1. Disc electrophoresis of Weber-Edsall extract from beef myofibrils. A = 0.10 ml of a typical heterogeneous Weber-Edsall extract containing 0.10 mg protein/ml. B = 0.16 ml of Weber-Edsall extract containing 0.9 ml protein/ml. C = 0.14 ml of Weber-Edsall extract containing 0.12 mg protein/ml. Sample had been stored at 3° with an equal amount of sucrose (w/w). D = 0.20 ml of DTT reduced Weber-Edsall extract containing 0.8 mg protein/ml

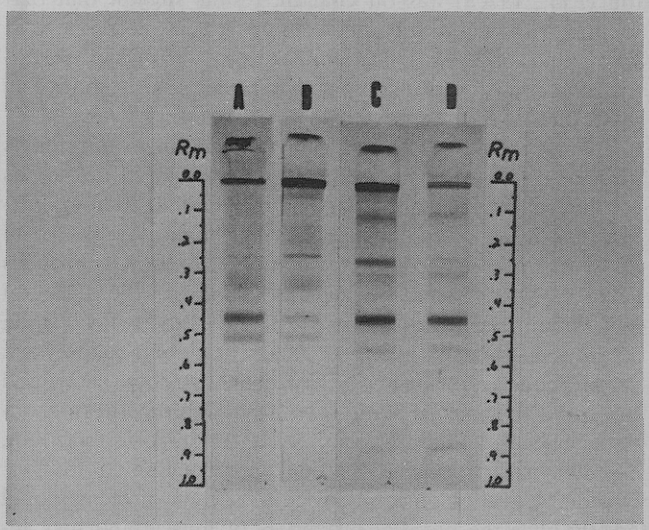

Figure 2. Disc electrophoretic pattern for actomyosin. A = 0.05 ml of beef actomyosin preparation containing 6.0 mg protein/ml. B = 0.05 ml of low ionic strength-treated actomyosin containing 6.0 mg protein/ml. C = 0.10 ml of DTT reduced sample from B (above) containing 5.5 mg protein/ml. D = 0.05 ml of DTT reduced actomyosin preparation containing 3.0 mg protein/ml

mentioned herein. Aggregation of myosin was a problem in many, although not all, actomyosin preparations. In contrast, actomyosin preparations, after washing in 0.04 *M* KCl (pH 7.0), exhibited an intense band at the top of the running gel ($R_m = 0.00$), as was already shown for isolated myosin by Rampton *et al.* (1970b). Typical results obtained on washing beef actomyosin at low ionic strength are shown in Figure 2B. Actomyosin thus washed also produced an intensified band at $R_m = 0.24$ (Figure 2B) which has been observed earlier in unreduced tropomyosin (Rampton *et al.*, 1970a).

The effect of DTT on beef actomyosin was also investigated. Typical patterns from DTT-reduced actomyosin are shown in Figures 2C and 2D. Reduction of actomyosin by DTT usually decreased the "slowly migrating" myosin band ($R_m = 0.00$), darkened the band at $R_m = 0.11$, removed the characteristically sharp unreduced tropomyosin band ($R_m = 0.24$),

increased the band of SH-reduced tropomyosin (R_m = 0.43), and removed the band observed in beef samples at R_m = 0.50. These results agree with those already given in earlier reports for isolated myosin (Rampton *et al.*, 1970b) and tropomyosin (Rampton *et al.*, 1970a) and for the Weber-Edsall extracts in this study.

In contrast, the effect of DTT on the actin moiety of actomyosin was less conclusive. As shown in Figures 2C and 2D, all actomyosin preparations analyzed in the present study exhibited some formation of the reduced actin band (R_m = 0.26) after reduction by DTT; however, visible amounts of the diffuse band (R_m = 0.27 – 0.33) always remained. Treatment of actomyosin with sulfite did not increase formation of the reduced actin band (R_m = 0.26). Sulfhydryl reduction and/or subsequent alkylation with nonmercurial reagents does not interfere with actin polymerization or depolymerization (Poglazov, 1966). In addition, 5 *M* urea is not effective in depolymerization of actin (Bárany *et al.*, 1962). Work in our laboratory (Rampton *et al.*, 1970a) has previously shown that reduction of purified actin with DTT usually gives only the reduced actin band (R_m = 0.26). Thus, results of the current study suggest that SH-groups of the actin moiety in actomyosin behave differently than SH-groups in isolated actin. Since reduction of the SH-groups in the earlier study (Rampton *et al.*, 1970a) was on G-actin, results suggest that SH-groups of the actin in actomyosin are not available to react with sulfite or DTT. This implies that F-actin in the actomyosin complex is not completely disassociated from myosin in 8 *M* urea with a subsequent polymerization.

The preceding studies indicate that actomyosin contains most of the proteins present in the Weber-Edsall extract, as can be seen by comparison of Figures 1 and 2. The heterogeneous nature of actomyosin was first implicated by Ebashi (1963). Discovery of several minor myofibrillar proteins (Ebashi, 1963; Szent-Györgyi and Kaminer, 1963; Ebashi and Ebashi, 1964; Maruyama, 1965) and of their effects upon actomyosin (Ebashi, 1966) suggested that some actomyosin preparations contained other proteins besides actin and myosin. The present study shows that actomyosin preparations probably contain myosin, actin, tropomyosin, α-actinin, β-actinin, and troponin, as well as other unknown proteins. Obviously, the presence or absence of these proteins could greatly alter the physical properties of muscle and may influence such factors as tenderness and water holding capacity.

Quantitative Applications. Presently, quantitative gel electrophoresis of actomyosin appears to be limited by two factors. As yet, there is no known method for consistently and quantitatively converting myosin into the electrophorescable species. A better understanding of the phenomenon of myosin aggregation appears to be necessary before this can be accomplished. The band obtained with reduced actin (R_m = 0.26) was very close to that produced by unreduced tropomyosin (R_m = 0.24), thus making it difficult to distinguish between the two. In the present paper, it was assumed that conditions producing complete reduction of isolated proteins would be equally effective in heterogeneous samples. This assumption was supported by the fact that the characteristically hypersharp band of unreduced tropomyosin at R_m = 0.24 was always removed by DTT-reduction, and the actin band at R_m = 0.26 in reduced samples never had this sharp appearance. Thus, a sharp band at R_m = 0.24 in unreduced samples was identified as tropomyosin, while a band at R_m = 0.26 in reduced samples was labeled as actin. However, since the actin moiety of actomyosin often resisted reduction, caution is necessary in interpreting these results.

Looking toward quantitative analysis of actomyosin preparations, it was found that actin and tropomyosin gave reducible densitometric responses between sample size of 10–200 μg and 1–30 μg, respectively. On the other hand, the densitometric response for myosin was not reproducible between different samples. Good agreement ($\pm 3\%$) on duplicate samples of actin and tropomyosin was obtained with certain precautions. The amount of sample applied to the gel was accurately measured. Consistent staining and destaining techniques were applied to all samples. Only clean, intact gels with a minimum of tailing were analyzed by densitometry.

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