Electrophoretic Characteristics of Bovine Myosin

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Story in Brief

Bovine myosin was isolated and purified from pre-rigor longissimus dorsi muscle. Electrophoretic characteristics of the myosin preparation were investigated by means of disc electrophoresis on polyacrylamide gels. In addition to establishing the general disc-gel patterns for bovine myosin, the effects of various gel conditions on the elctrophoretic behavior of myosin were determined. The electrophoretic pattern obtained on an aqueous solution of myosin exhibited seven faint, but distinct bands, which could be grouped into three zones along the gel. Zone 1, 2 and 3 contained 2, 3 and 2 bands, respectively. Results indicated that the major solubility problems with myosin could be circumvented by using 8M urea as a solvent.

The electropherograms of myosin dissolved in urea showed greater band density and definition. Also, the electrophorograms were similar to those obtained with myosin dissolved in water in terms of the number of zones, bands within each zone and overall mobility. The addition of a reducing agent (2-mercaptoethanol) to either the electrophoresis buffer did not significantly alter the myosin patterns. When the molecular sieving effect of the separating gel was reduced by decreasing its acrylamide concentration from 7 percent to 3.5 percent, the myosin patterns showed only one band in each of the three zones.

Introduction

To withstand growing competition from "synthetic" protein foodstuffs, new methods for processing and fabricating beef carcasses, which would increase marketing efficiency and enhance the quality of retail beef products, must be developed and implemented. To accomplish this the need for more detailed information on the basic biochemical make-up of beef muscle and the post-mortem alterations occurring therein, has become apparent. From the standpoint of market quality in beef, an area of primary interest centers around the interactions of its major fibrillar protein, myosin, during the development of rigor and the subsequent post-mortem changes which occur during the "aging" process.

The purpose of this study was to isolate and purify bovine myosin and to determine its electrophoretic behavior, being an important first

step in evaluating the role of myosin in the various port-mortem quality changes occurring in beef muscle.

Materials and Methods

The experimental material consisted of bovine longissimus dorsi muscle excised pre-rigor. After the muscle was excised, it was taken immediately to the cold room and all subsequent operations were conducted at 0°C. The muscle was freed of surrounding fat and connective tissue, then minced in a stainless steel grinder through a 3 mm. plate. Myosin was isolated from the minced muscle by a salt extraction procedure modified from that of Szent-Gyorgi (1943). The final myosin precipitate was dissolved in 0.5M KCI. For electrophoresis studies, an aliquot of the dissolved myosin was dialyzed against 50 volumes of 0.01M Tris-HCI buffer (pH 7.1) for 12 hours, and lyophilized.

Aqueous and 8M urea solutions of the lyophilized myosin preparation were electrophoresed on polyacrylamide gels according to procedures outlined by Davis (1964). All electrophoretic tests were performed at 2°C. A 7 percent separating gel of 5 mm. in diameter was used. The electrode buffers contained 14.4 gm. glycine and 3.0 gm. Tris in 1 liter of distilled water. This system stacks at pH 8.9 and runs at pH 9.5. The current setting for protein separation was 5 ma. per tube, and the dye front was allowed to migrate (cathode to anode) 5.7 cm. in about 45 minutes. Gels were stained with 1 percent Amido Schwartz in 11 percent acetic acid: 5 percent methanol and destained electrophoretically.

The biuret method was used to determine protein concentration, with crystalline bovine serum albumin serving as the standard (Gornall, 1949).

Results and Discusison

Purity of the myosin preparation was assessed by ultracentrifuge sedimentation. The electrophoretic pattern obtained on an aqueous solution of the myosin preparation exhibited seven faint, but distinct bands, which could be grouped into three zones along the gel. Zone 1, 2 and 3 contained 2, 3 and 2 bands, respectively (Figure 1).

It is pointed out that high inorganic salt solutions can not be successfully electrophoresed on polyacrylamide gels. Hence, for the electrophoretic studies the myosin preparation could not be dissolved in its usual (0.5M KCI) solvent. Considerable difficulty was encountered in getting sufficient lyophilized myosin to dissolve in water. Consequently, the electrophoretic patterns obtained on myosin in aqueous solutions

were always rather faint (Figure 1).

Experimentation revealed the myosin preparation to be readily soluble in 8M urea. This observation led to the comparison of the electrophoretic patterns of myosin dissolved in water and in 8M urea. The results presented in Figure 2 show that the myosin-urea electrophorograms are similar in the number of zones, bands within each zone and in overall mobility.

Recent studies with polyacrylamide gel electrophoresis have indicated that the ammonium persulfate catalyst, an oxidizing agent used in polymerizing the separating gel, can leave this gel in an oxygen-rich state which could result in increased electrophoretic heterogeneity of the separated compounds (Brewer, 1967). Since myosin has a high sulfhydryl content, it was considered possible that this phenomenon might have an

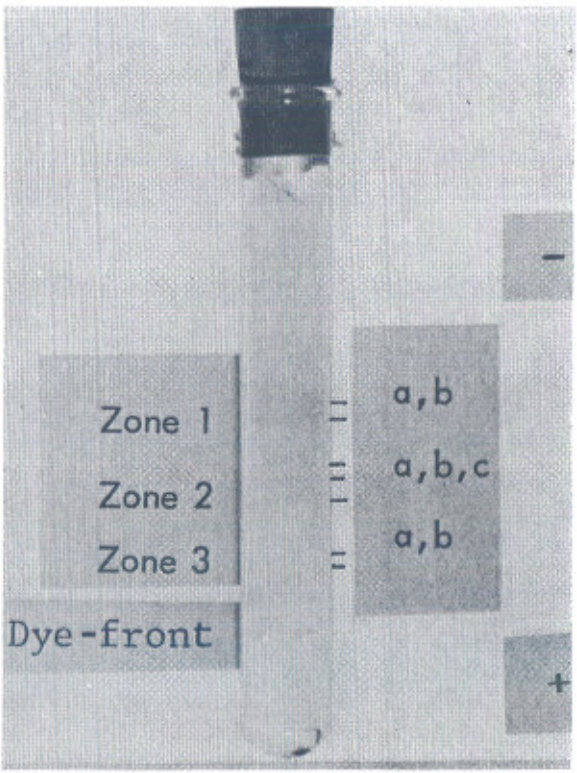

Figure 1. Electrophoretic separation of bovine myosin in aqueous solution.

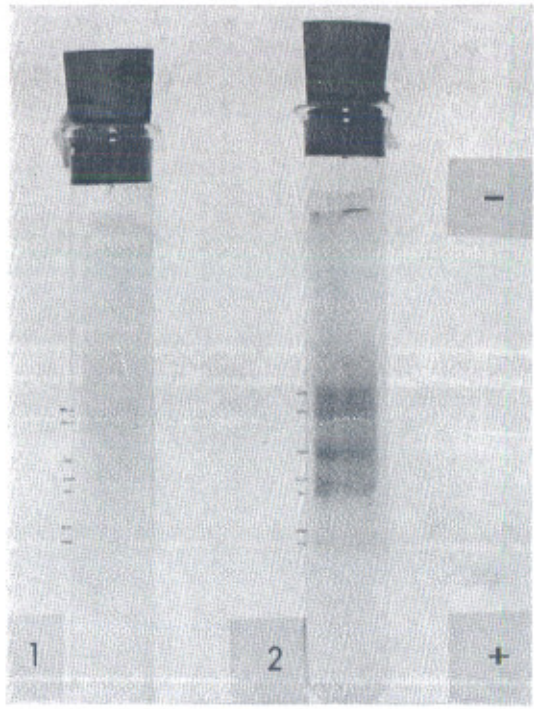

Figure 2. Electrophoretic separation of bovine myosin. 1) aqueous solution 2) 8M urea

adverse effect on the electrophoretic behavior of myosin. To test this condition, 2-mercaptoethanol (an anionic reducing agent) was added to the elctrophoresing buffer (to a final concentration of 1.4×10^{-3}M).

Results obtained with the aqueous myosin solution indicated the protein still migrated as three primary zones and although the same total number of bands were apparent, a more definite primary band occurred within each zone (Figure 3). Also, these results suggest that the reducing agent facilitated the migration of more protein into the separating gel, since the same sample concentrations were used on both gels. In another test the reducing agent (0.7 u M 2-mercaptoethanol in 50 percent sucrose) was layered in front of the sample gel, since it was believed that the addition of the reducer to the electrophoresing buffer might result in the reduction of the disulfide bonds of myosin. Again the solubility problem with myosin was encountered and the gel could not be detected photographically. However, from the gel itself, it could be seen that the electrophoretic patterns was not significantly altered.

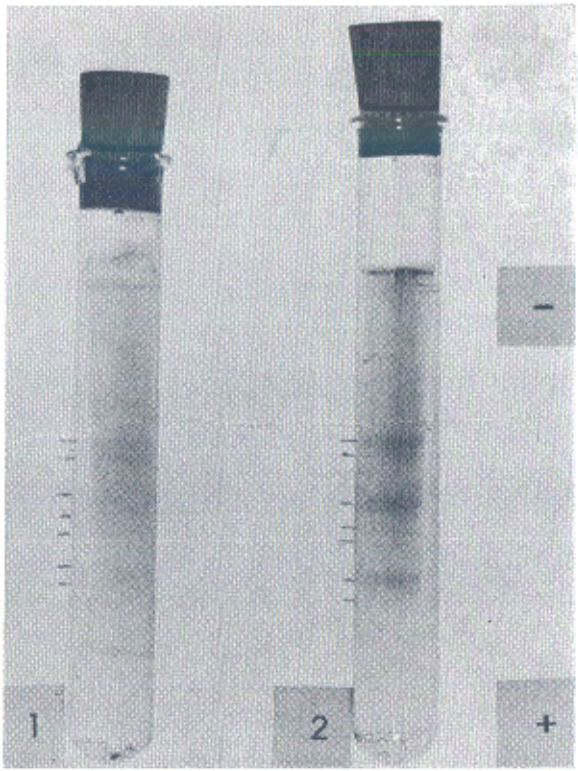

Figure 3. Electrophoretic separation of bovine myosin in aqueous solution. 1) no reducer 2) reducer added to electrophoresing buffer

When the molecular sieving effect of the separating gel was reduced by decreasing its acrylamide concentration from 7 to 3.5 percent, the myosin patterns obtained showed only one band in each of the three zones (Figure 4). As expected, greater migration of the protein occurred in the 3.5 percent gel. The three primary bands observed on the 3.5 percent separating gel are of major interest since this finding agrees with the hypothesis of Kielley and Harrington (1960) that myosin is composed of three major subunits.

Literature Cited

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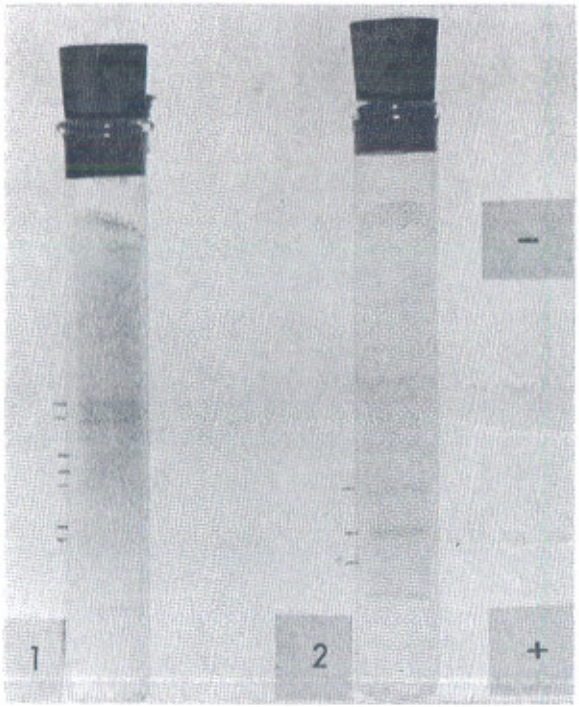

Figure 4. Electrophoretic separation of bovine myosin in aqueous solution. 1) 7% separating gel 2) 3.5% separating gel

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