

## Irrigation of Rabbit “Ghost” Single Fibers with Myosin from Normal and PSE Porcine Muscle

Muguruma, Michio

Laboratory of Chemistry and Technology of Animal Products, Faculty of Agriculture, Kyushu University

Izumi, Kenjiro

Laboratory of Chemistry and Technology of Animal Products, Faculty of Agriculture, Kyushu University

Ito, Tatsumi

Laboratory of Chemistry and Technology of Animal Products, Faculty of Agriculture, Kyushu University

Fukazawa, Toshiyuki

Laboratory of Chemistry and Technology of Animal Products, Faculty of Agriculture, Kyushu University

<https://doi.org/10.5109/23715>

---

出版情報：九州大学大学院農学研究院紀要. 25 (1), pp.9-13, 1980-08. Kyushu University  
バージョン：  
権利関係：



## **Irrigation of Rabbit "Ghost" Single Fibers with Myosin from Normal and PSE Porcine Muscle**

**Michio Muguruma, Kenjiro Izumi, Tatsumi Ito and  
Toshiyuki Fukazawa**

Laboratory of Chemistry and Technology of Animal Products,  
Faculty of Agriculture, Kyushu University 46-06, Fukuoka 812

*(Received January 12, 1980)*

The organization and quantity of myosin irrigation into myosin-free "ghost" fibers were compared between normal and PSE myosin. Considerable amounts of myosin were incorporated into the "ghost" fibers for both normal and PSE myosin. Electron microscopic study showed that the myosin filaments were formed all over the thin filaments after the irrigation. However, there was almost no difference in the quantity and filamentogenesis of the incorporated myosin into the "ghost" fibers between normal and PSE myosin.

### **INTRODUCTION**

It is generally accepted that the development of tension in muscle is due to the interaction of myosin with actin coupled with the hydrolysis of ATP. Muscle fiber loses its tension after the removal of myosin and the resulting myosin-free "ghost" fiber recovers its tension following irrigation with myosin. Therefore, the tension of myosin-irrigated "ghost" fiber has been used for elucidating the physiological properties of isolated myosin, although the extent of the tension developed by an irrigated fiber is much less than that of an intact muscle fiber (Oplatka *et al.*, 1974a, b; Hayashi and Maruyama, 1975; Tawada *et al.*, 1976).

It is known that PSE porcine muscle is in a partially denatured state and has a lower specific ATPase activity than does normal muscle (Sung *et al.*, 1976). In a previous paper, we found that upon addition of a contracting solution the tension of a "ghost" fiber is slightly increased by the irrigation with normal myosin, while no tension is developed in the case of a fiber irrigated with PSE myosin (Izumi *et al.*, 1977). However, the ultrastructure of rabbit "ghost" fibers irrigated with normal and PSE porcine myosin has not been published. The present study deals with a comparison of the organization and quantity of myosin from normal and PSE muscle following irrigation into "ghost" fibers prepared from rabbit muscle.

### **MATERIALS AND METHODS**

#### **Materials**

Normal and PSE porcine longissimus thoracis muscles were selected from

the carcasses at 24 hr after slaughter by testing the contractility of myofibrils as described in a previous paper (Sung *et al.*, 1976). Normal muscle (pH 5.54) myofibrils exhibited 100 % contractility upon addition of  $Mg^{2+}$ -ATP solution, while those from PSE muscle (pH 5.09) exhibited 0 % contractility. Myosin was prepared from these muscles according to the method of Tonomura *et al.* (1961) with slight modifications (Ito *et al.*, 1978): a modified Guba-Straub solution (0.3 M KCl, 0.15 M phosphate, 2 mM ethyleneglycol-bis-(2-aminoethylether)-N, N, N', N'-tetraacetic acid (EGTA), 5 mM  $MgCl_2$  and 5 mM ATP, pH 6.5) was used for extracting myosin from postmortem muscle, and the time for the extraction was 10 min.

### **Irrigation with myosin**

Myosin irrigation was done as described in a previous paper (Izumi *et al.*, 1977). After the preparation of single fibers from glycerinated rabbit psoas muscle, myosin was removed from the single fibers with Hasselbach-Schneider solution (0.47 M KCl, 1 mM  $MgCl_2$ , 10 mM sodium pyrophosphate and 0.1 M phosphate, pH 6.4) at 4°C for 4 hr. Then, the fibers were washed with a salt solution (0.1 M KCl, 1 mM  $MgCl_2$  and 6.7 mM phosphate, pH 7.0). The resulting "ghost" fibers were irrigated with normal (pH 5.54) and PSE (pH 5.09) myosin by incubating them with a large volume of myosin solution (5 mg/ml in 0.5 M KCl) at 4°C for 24 hr. After the irrigation, the fibers were rinsed with a large volume of the salt solution.

### **SDS polyacrylamide gel electrophoresis**

SDS polyacrylamide gel electrophoresis were carried out for the intact, "ghost" and myosin-irrigated "ghost" fibers by the previous procedure (Muguruma *et al.*, 1978), principally according to the method of Weber and Osborn (1969).

### **Electron microscope**

The fibers were fixed in 3 % glutaraldehyde buffered with 0.12 M sodium cacodylate at pH 7.2 for 2 hr, then washed briefly in the same buffer, and further fixed in 1 % osmium tetroxide buffered with sodium cacodylate at pH 7.2 for 2 hr. Most samples were stained *en bloc* with 1 % uranyl acetate in aqueous solution prior to dehydration. The fibers were dehydrated in an alcohol series and embedded into Epon 812. Thin sections for electron microscopy were cut with glass knives on a Porter-Blum MT-1 microtome, and stained with 1% lead acetate or doubly stained with 1 % lead acetate and uranyl acetate. The sections were examined with a Hitachi HS-9 electron microscope operating with an accelerating voltage of 75 kV.

### **pH determination**

Minced muscle (5 g) was homogenized in 10 ml of distilled water with a Waring blender for 3 min. The pH of the muscle homogenate was determined with a Hitachi-Horiba pH meter F-7 at 20°C.

### Protein concentration

All protein concentrations were determined by the biuret method (Gornall *et al.*, 1949). Bovine serum albumin was used as the standard.

### RESULTS

Figure 1 shows the electrophoretic patterns of intact muscle, "ghost" and myosin-irrigated "ghost" fibers. Myosin heavy chain almost disappeared after extracting myosin with Hasselbach-Schneider solution for 4 hr. but the thin

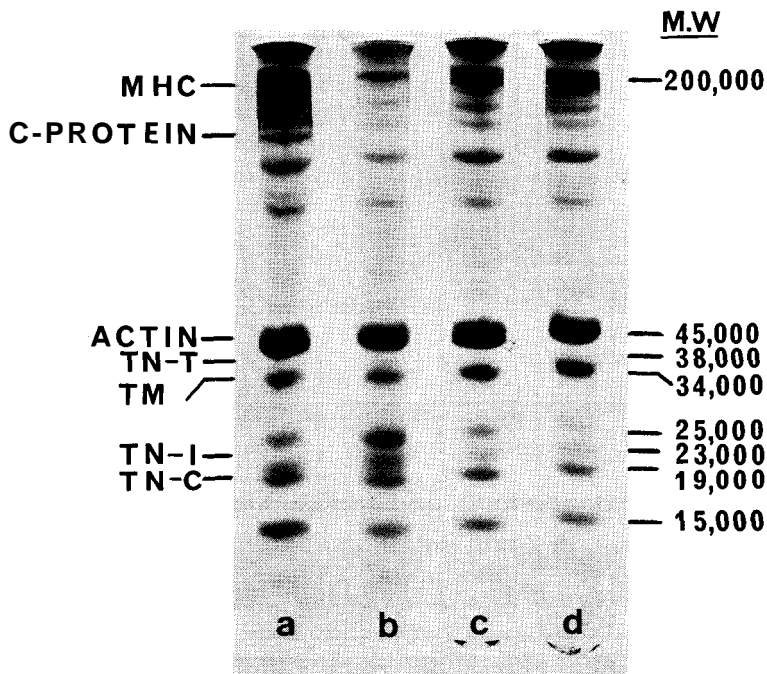


Fig. 1. SDS Polyacrylamide gel (7.5 %) electrophoretic pattern of intact, "ghost" and myosin-irrigated fibers. Each preparation was dissolved by heating at 100°C for 3 min in 10 mM sodium phosphate (pH 7.0) containing 1% SDS and 1%  $\beta$ -mercaptoethanol. The resulting solution was applied to the top of the gel and was electrophoresed at 8 mA/gel for 4 hr. Staining was with Commassie Blue.

- a) Intact rabbit single fibers (120  $\mu$ g protein loaded).
  - b) "Ghost" single fibers of rabbit psoas muscle after the extraction of myosin with Hasselbach-Schneider solution (60  $\mu$ g).
  - c) "Ghost" single fibers irrigated with normal myosin (85  $\mu$ g).
  - d) "Ghost" single fibers irrigated with PSE myosin (85  $\mu$ g).
- MHC; myosin heavy chain, TM; tropomyosin. TN-T; troponin-T, TN-I; troponin-I, TN-C; troponin-C.

filament proteins, i.e., actin, tropomyosin and troponin subunits, remained on the gel. After the irrigation with myosin, the intensity of myosin heavy chain band was increased (compare Figs. 1 c and d to b). In addition, there was almost no difference in the intensity of myosin heavy chain band between normal and PSE myosin from densitogram (data not shown).

Plates I and II show the electron micrographs of intact muscle, "ghost" and myosin-irrigated "ghost" fibers. Apparently, all of the thick filaments and M-lines disappeared after the extraction of myosin (Pl. I- Fig. 2), although complete removal of myosin from single fibers was difficult (Fig. 1 b). Almost intact I-Z-I structures and clear gaps between thin filaments are visible (Pl. I- Fig. 2). The irrigation filled up in part the gaps between the thin filaments with myosin filaments. However, the density of the myosin filaments in the gap regions was much less than that of intact muscle fibers. Rather, myosin filaments formed after the irrigation were equally distributed all over the thin filaments. However, the direction of the fibers was random (Pl. II- Fig. 3). In addition, quite similar structure of myosin filaments was found in the case of the irrigation with PSE myosin (Pl. II - Fig. 4) and also in the case of myosin prepared from porcine muscle having intermediate pH (pH 5.35; Data not shown).

## DISCUSSION

In the present study, we found that a considerable amount of porcine myosin was incorporated into rabbit "ghost" fibers by the irrigation. However, we could not irrigate myosin into the "ghost" fibers up to the original level of myosin in the intact muscle fibers (Fig. 1), although the myosins which we used in the present study contained minor components such as C-protein in addition to myosin heavy chain and light chains (Figs. 1 c and d). Thus, we can suppose the following three possibilities for the reason of the incomplete incorporation of myosin into the "ghost" fibers; (1) the insufficient population of irrigated myosin in the "ghost" fiber due to the incomplete diffusion of myosin into the thin filaments, (2) the incomplete filamentogenesis of myosin in the alignment of the thin filaments, and (3) the lack of M-lines substance.

In the present study, the length and shape of the filaments formed in the "ghost" fiber were apparently not uniform. This may be due to the insufficient formation of myosin filaments in the structure of the "ghost" fiber. In addition, the present results (Fig. 1 and Pl. II) show that there is no difference in the amount of the myosin incorporated into the "ghost" fibers or in the structure of the filaments formed regardless if irrigation was done with normal and PSE myosin. In addition, we have found that PSE myosin B also has an ability to form an arrowhead structure (Park *et al.*, 1975). These results suggest that in the "ghost" fibers there is no difference in the filamentogenesis between normal and PSE myosin, although it has found that PSE myosin loses in part its enzymatic activity, especially initial burst of phosphate liberation (Sung *et al.*, in preparation).

## ACKNOWLEDGEMENT

We wish to express our heartfelt appreciation to Professor Dr. Teru Aki Uchida, Kyushu University, for lending electron microscope.

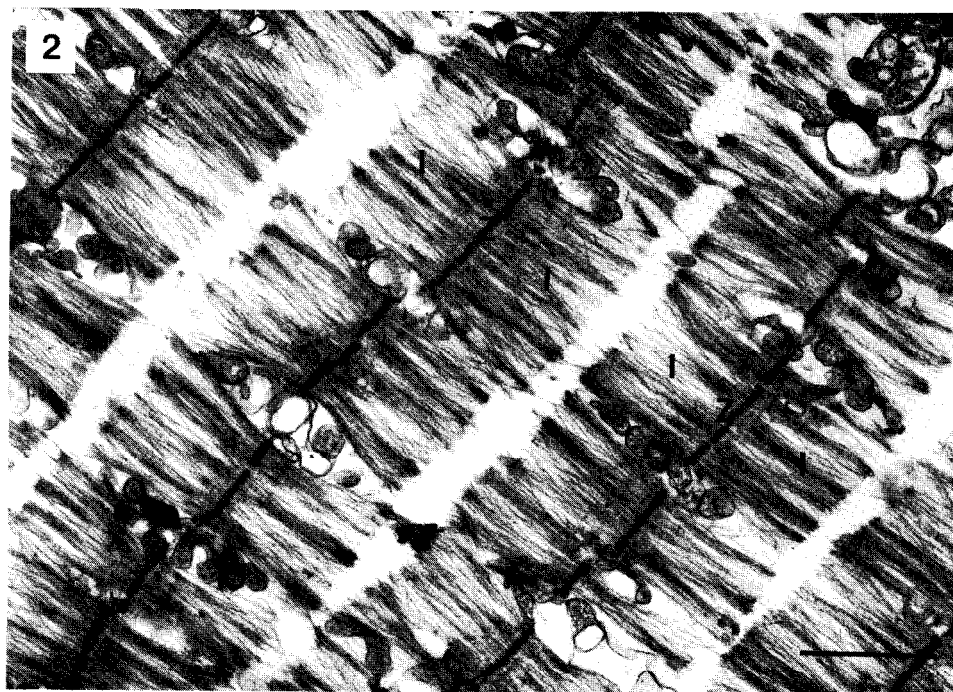
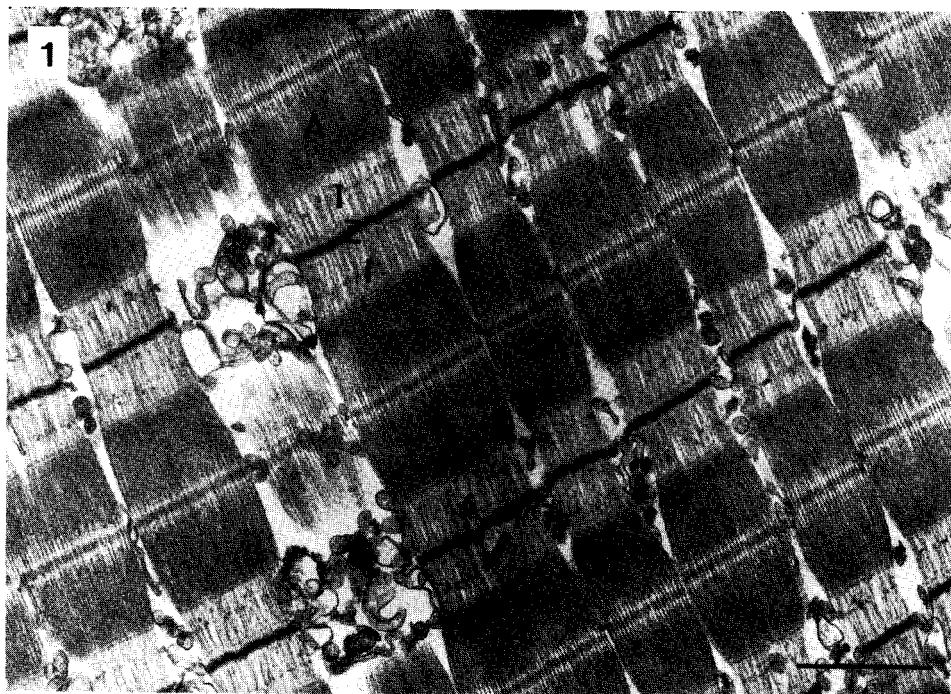
## REFERENCES

- Gornall, A. G., C. T. Bardawill and M. M. David 1949 Determination of serum protein by means of the biuret reaction. *J. Biol. Chem.*, 177: 751-766
- Hayashi, T. and K. Maruyama 1975 Myosin aggregates as a requirement for contraction and a proposal to the mechanism of contraction of actomyosin system. *J. Biochem.*, 78: 1031-1038
- Ito, T., S. K. Sung and T. Fukazawa 1978 Change of acto-heavy meromyosin ATPase of rabbit skeletal muscle during postmortem storage. *J. Agric. Food Chem.*, 26: 324-326
- Izumi, K., T. Ito and T. Fukazawa 1977 Isometric tension development of glycerinated fibers prepared from normal and PSE porcine muscles and the effect of myosin irrigation on the tension development of "ghost" fibers. *J. Food Sci.*, 42: 113-116
- Muguruma, M., M. Yamada and T. Fukazawa 1978 Effect of calcium on extraction of Z-band proteins from I-Z-I brushes of rabbit striated muscle. *Biochim. Biophys. Acta*, 532: 71-80
- Oplatka, A., J. Borejdo and H. Gadashi 1974a Tension development in stretched glycerinated muscle fibers and contraction of "ghost" myofibrils induced by irrigation with heavy meromyosin. *FEBS Lett.*, 45: 55-58
- Oplatka, A., H. Gadashi and J. Borejdo 1974b The contraction of "ghost" myofibrils and glycerinated muscle fibers irrigated with heavy meromyosin subfragment-1. *Biochem. Biophys. Res. Commun.*, 58: 905-912
- Park, H. K., M. Muguruma, T. Fukazawa and T. Ito 1975 Relationship between superprecipitating activity and constituents of myosin B prepared from normal and PSE porcine muscle. *Agr. Biol. Chem.*, 39: 1363-1370
- Sung, S. K., T. Ito and T. Fukazawa 1976 Relationship between contractility and some biochemical properties of myofibrils prepared from normal and PSE porcine muscle. *J. Food Sci.*, 41: 102-107
- Tawada, K., A. Yoshida and K. Morita 1976 Myosin-free ghosts of single fibers and an attempt to reform myosin filaments in the ghost fibers. *J. Biochem.*, 80: 121-127
- Tonomura, Y., S. Tokura, K. Sekiya and K. Imamura 1961 Influence of solvent composition on the molecular shape and the enzymic activity of myosin A. *Arch. Biochem. Biophys.*, 95: 229-236
- Weber, K. and M. Osborn 1969 The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.*, 244: 4406-4412

#### Explanation of Plate I

**Fig. 1.** Electron micrograph of intact rabbit single fibers. A; A-band, I; I-band, Z; Z-band. The calibration bar represents  $0.5\ \mu\text{m}$ . Magnification  $\times 18,000$ .

**Fig. 2.** Electron micrograph of rabbit "ghost" single fibers after the extraction of myosin with Hasselbach-Schneider solution. I; I-band, Z ; Z-band. The calibration bar represents  $0.5\ \mu\text{m}$ . Magnification  $\times 18,000$ .





#### Explanation of Plate II

Fig. 3. Electron micrograph of “ghost” single fibers irrigated with normal myosin. I; I-band, Z; Z-band. The calibration bar represents  $0.5\ \mu\text{m}$ . Magnification  $\times 18,000$ .

**Fig. 4.** Electron micrograph of “ghost” single fibers irrigated with PSE myosin. I; I-band, Z; Z-band. The calibration bar represents  $0.5\ \mu\text{m}$ . Magnification  $\times 18,000$ .

