53. Myosin I-like Protein in Gizzard Smooth Muscle

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In non-muscle cells, two classes of myosin have been described; myosin I composed of a single heavy chain (HC) of about 120 K dalton (kDa) and a single light chain, and conventional myosin composed of a pair of HC of 200 kDa and two pairs of light chains (1 for review). Myosin I was highly purified and well characterized in amoebal cells of *Acanthamoeba*^{2),3)} and *Dictyostelium*.^{4),5)} There is accumulating evidence to indicate that the 110 kDa-calmodulin complex in vertebrate is a myosin I-like protein.⁶⁾⁻⁹⁾ The complex is detectable in a few tissues including intestinal brush border and nervous tissues.^{10),11)} However, there is no report suggesting the presence of myosin I-like protein in smooth muscle.

We report here that myosin I-like protein, which has no calmodulin-binding activity, has been purified from chicken gizzard.

Materials and methods. The mince of chicken gizzard was homogenized with a Virtis homogenizer at 45 K rpm for 30 sec in 6 vol of 0.5 M NaCl, 0.1 M sucrose, 10 mM ATP, 10 mM imidazole (pH 7.5), 20 mM Tris-HCl (pH 7.5), 10 mM Na₄P₂O₇, 2 mM EGTA, 2 mM dithiothreitol (DTT), and protease inhibitors, i.e. 0.1 mM phenylmethylsulfonyl fluoride and 0.1 mM E64C.¹²⁾ The homogenate was centrifuged at 250 K×g for 60 min. The supernate, i.e. crude extract, was applied to a hydroxylapatite (HAP, Bio-Rad) column (1×15 cm) equilibrated with buffer containing 0.5 M NaCl, 20 mM Tris-HCl (pH 7.5), 1 mM NaN₃, 14 mM 2-mercaptoethanol (ME), 2 mM ATP and protease inhibitors. The column was washed by the same buffer, and then 200 ml of 0–1 M KH₂PO₄ (pH 7.0) gradient was applied to it. The fractions eluted from the column were subjected to the assay for K⁺-EDTA-ATPase activity and to SDS PAGE. Fractions containing the ATPase activity but without 200 kDa heavy chain of conventional myosin were pooled and dialyzed against the dialysis buffer containing 50 mM NaCl, 0.2 mM NaN₃, 20 mM Tris-HCl (pH 7.5), 14 mM ME and protease inhibitors. The dialyzate was mixed with 2 mM ATP of the final concentration and applied to a DEAE cellulose (DE-52 Whatman) column (1.5×20 cm) equilibrated with the buffer containing 50 mM NaCl, 20 mM Tris-HCl (pH 7.5), 2 mM ATP, 0.2 mM NaN₃ and protease inhibitors. After washing the column with the same buffer, 160 ml of 0.05–0.8 M NaCl gradient was applied to the column. Fractions eluted from the DE52 column were subjected to SDS PAGE, and those containing only 130 kDa band were pooled and concentrated by the dialysis against polyethylene glycol compound (PEG, Sigma, MW 15,000–20,000). The resulting concentrate was dialyzed against

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the dialysis buffer mentioned above and used as myosin I-like protein.

The fractions eluted from the HAP column and containing 200 kDa heavy chain of conventional myosin as judged from SDS PAGE were pooled and concentrated by the dialysis against PEG. The resulting concentrate was dialyzed against the dialysis buffer. The flocculent precipitate that was formed during the dialysis was removed by the centrifugation at $40 \times g$ for 30 min. The resultant supernatant was used as conventional myosin. Conventional myosin was also prepared from gizzard by the method of Ebashi.¹³⁾

Actin was prepared from chicken breast muscle as described previously.¹⁴⁾ Native actomyosin, or myosin B, was prepared from gizzard as described previously.¹⁵⁾ Calmodulin was prepared from bovine brain.¹⁶⁾

Protein concentrations were assayed colorimetrically assay using Bio-Rad protein assay using bovine serum albumin as a standard.

SDS PAGE was performed according to Laemmli,¹⁷⁾ and proteins were stained with Coomassie blue.

Gel overlay of ^{125}I -actin and ^{125}I -calmodulin was performed as described previously¹⁸⁾ by modifying the method of Snabes *et al.*¹⁹⁾

ATPase activity was measured by the colorimetric determination of inorganic phosphate released from ATP.²⁰⁾ The conditions for Ca^{2+} -ATPase activities: 0.6 M KCl, 20 mM Tris-HCl (pH 7.5), 3.8 mM CaCl_2 , and 2.5 mM ATP; those for K^{+} -EDTA-ATPase activities were 0.6 M KCl, 1 mM EDTA, 20 mM Tris-HCl (pH 7.5) and 2.5 mM ATP; those for Mg^{2+} -ATPase and actin-activated ATPase activities were 25 mM KCl, 3.8 mM MgCl_2 , 20 mM Tris-HCl (pH 7.5), 0.1 mM CaCl_2 and 2.5 mM ATP. The concentrations of F-actin and myosin I-like protein were 0.61 mg/ml and 0.13 mg/ml, respectively.

Results and discussion. Gel overlay experiment of crude extract of gizzard smooth muscle showed that not only 200 kDa HC of conventional myosin but also 130 kDa protein can bind actin (data not shown). The content of the 130 kDa protein in the extract was not negligible; the ratio of the 200 kDa band to the 130 kDa band in SDS PAGE was about 20–10 to 1 (not shown). The 130 kDa protein was purified by HAP column chromatography followed by DE 52 column chromatography. Table I shows its ATPase activities. The remarkable

Table I. ATPase activities of myosin I-like protein

Ca^{2+} -ATPase	4.0
K^{+} -EDTA ATPase	13.8
Mg^{2+} -ATPase	3.1
Actin-activated ATPase	9.5

n mol Pi/min. mg protein at 25°C.

feature is its high K^{+} -EDTA ATPase activity and low Ca^{2+} -ATPase activity. As suggested by gel overlay experiment, actin activated Mg^{2+} -ATPase activity. Thus the 130 kDa protein shows similar properties in its ATPase activities to those of myosin I of *Acanthamoeba* and *Dictyostelium*.^{4),12)} It must be noted that K^{+} -EDTA ATPase and actin-activated ATPase activity were lower than amoebal myosin I and bush-border 110 kDa-calmodulin complex. The effect of phosphorylation of this 130 kDa myosin I-like protein has remained to be examined.

Fig. 1 shows SDS PAGE of the 130 kDa myosin I-like protein. Unlike

Fig. 1. SDS PAGE of myosin I-like protein. a, myosin I-like protein; b, conventional myosin; c, mixture of myosin I-like protein and conventional myosin; Lc, light chains of conventional myosin.

amoebal myosin I, this protein appeared to have no light chain. Brush-border 110 kDa-calmodulin complex is composed of 110 kDa calmodulin-binding protein and calmodulin. The 130 kDa myosin I-like protein, however, has no sign of calmodulin binding according the gel overlay experiment with ^{125}I -calmodulin (data not shown).

The purification of the 130 kDa myosin I-like protein was carried out at 0–4°C in the presence of sufficient amount of protease inhibitors to minimize its proteolysis. However, we cannot rule out the possibility for the present time that it is a proteolytic product of conventional myosin. Further penetrating studies are needed.

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