MYOSIN-LINKED CALCIUM REGULATION IN VASCULAR SMOOTH MUSCLE

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1. Introduction

As in all muscle, the contractile activity of vertebrate smooth muscle and the ATPase activity of its actomyosin is regulated by calcium ions in the micromolar range [1,2]. In contrast to skeletal and heart muscle however, the nature of the calcium sensing mechanism is still unknown and troponin, which is so important in skeletal muscle regulation could not be detected [3,4]. In many invertebrate muscles which also lack troponin, the regulation is mediated through 'light' polypeptide chains which are part of the myosin subfragment 1. In vertebrate smooth muscle, the possibility of myosin linked Ca^{2+}-regulation has recently been suggested mainly for two reasons: 1) In 'competition experiments' [5] added skeletal myosin, but not actin depresses the calcium sensitivity of smooth muscle crude actomyosin.2) Gizzard actomyosin (devoid of troponin) still exhibits calcium sensitivity after being purified from tropomyosin and other contaminating proteins [3]. The results presented here indicate that a simple reconstituted system of actin and smooth muscle myosin (or its heavy meromyosin subfragment 1) exhibits a calcium regulated actin-activated ATPase, and that this regulation is lost after removal of the light chains.

2. Methods

Vascular actomyosin was extracted from pig carotid arteries with a solution of pH 7.2 containing 80 mM KCl, 20 mM imidazole, 500 μM dithioerythritole, and 5 mM ATP [2,6]. The calcium sensitivity of the preparation was lost after desensitation [7], which removed tropomyosin and a 130 000 dalton component [8]. Vascular heavy meromyosin subfragment 1 [9] was prepared by papain digestion [10] of either calcium sensitive or desensitized actomyosin and purified by affinity chromatography as described elsewhere [11], using the immobilized ATP column described by Lamed [12,13]. The abbreviation HMM-S1 is used here for heavy meromyosin subfragment 1 from all preparations. It should be noted, however, that HMM-S1 from desensitized actomyosin is devoid of at least one light chain (see Results). Myosin [15] and actin [16] which was free of troponin and tropomyosin (fig.1) were prepared from rabbit muscle. ATPase activity was determined using the NADH-linked optical test methods as modified by Trentham et al. [18]. The incubation was carried out at 25°C in solutions of pH 7 containing 50 mM KCl, 10 mM imidazole, 5 mM $MgCl_2$, and either EGTA (ethylenglycol-bis(2-aminoethylether)*N,N'*-tetraacetic acid, relaxing conditions) or 100 μM $CaCl_2$ (activation conditions) or 5 mM Ca-EGTA buffer of the desired pH [20]. ATP (2 mM) was kept constant by the ATP regenerating system containing phosphoenolpyruvate and pyruvatekinase [18]. Ca^{2+}-sensitivity is defined as:

$$\frac{\text{ATPase activity at } 100\ \mu\text{M } Ca^{2+}}{\text{ATPase activity at } 10\ \text{nM } Ca^{2+}}.$$

3. Results and discussion

As shown in fig.2 the ATPase activity of arterial HMM-S1 was activated by adding increasing amounts of unregulated actin from skeletal muscle. The actin activated activity in the presence of 100 μM Ca^{2+} is nearly twice as high as the activity in the presence of EGTA (10 nM Ca^{2+}) (table 1). The ratio of these activi-

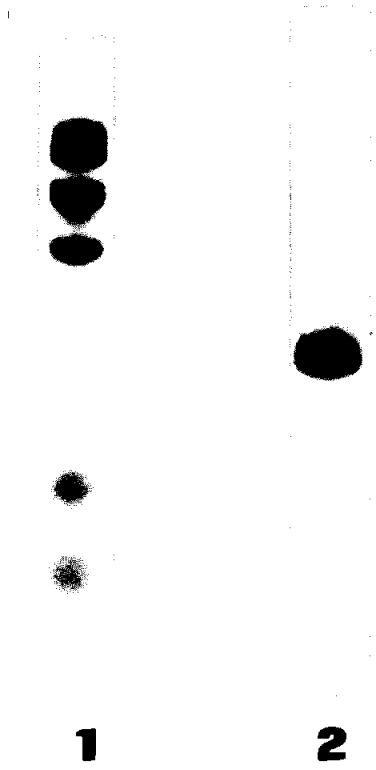

Fig.1. Electrophoresis on 8% polyacrylamide gels containing 0.1% SDS [17]. (1) Heavy meromyosin subfragment 1 from vascular muscle (The HMM-S1 obtained from Ca^{2+}-sensitive actomyosin shows 3 slow moving bands as in the case of skeletal muscle [14] and 2 fast moving bands, the 17 000 and the 20 000 dalton light chain.) (2) Unregulated actin from rabbit skeletal muscle.

Table 1
Dependence of the ATPase activity of acto-HMM-S1 on Ca^{2+}-concentration

$[Ca^{2+}]$	ATPase rate (s^{-1})
10^{-8} M (5 mM EGTA)	12.9×10^{-3}
10^{-5} M (5 mM Ca-EGTA)	24.1×10^{-3}
10^{-4} M (100 μM $CaCl_2$)	28.9×10^{-3}

The conditions were: 440 nM vascular HMM-S1, 6.6 μM skeletal muscle actin, 50 mM KCl, 10 mM imidazole, 5 mM $MgCl_2$, 100 μM dithioerythritole; T = 20°C, pH 7.

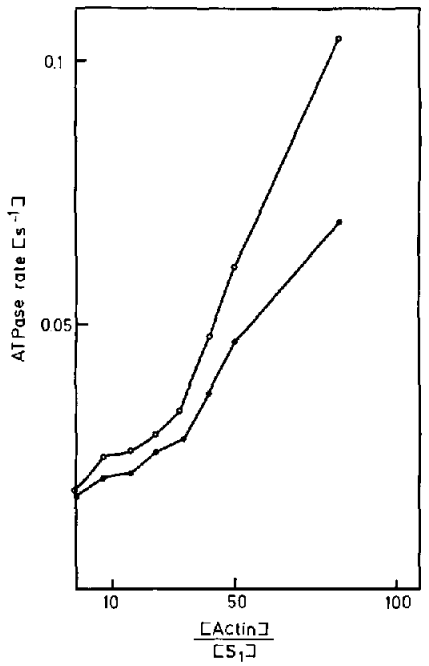

Fig.2. Dependence of ATPase activity of acto–HMM-S1 on actin concentration at 10 nM Ca^{2+} (lower curve) and at 100 μM Ca^{2+} (upper curve). The proteins shown in fig.1 were used. The assay conditions were: 670 nM HMM-S1, 50 mM KCl, 10 mM imidazole, 5 mM $MgCl_2$, 100 μM dithioerythritole; T = 20°C, pH 7. The molar concentrations of actin were varied.

Table 2
Ca^{2+}-sensitivity of various reconstituted actin-myosin systems

System	Ca^{2+}-sensitivity of the ATPase
Actin + HMM-S1 from native vascular actomyosin	1.89
Actin + HMM-S1 from desensitized vascular actomyosin	0.98
Actin + HMM-S1 from skeletal muscle myosin	1.01

The conditions were: 380 nM HMM-S1, 18 μM actin, 50 mM KCl, 10 mM imidazole, 5 mM $MgCl_2$, 2 mM ATP, 100 μM $CaCl_2$ or 5 mM EGTA resp., 100 μM dithioerythritole; T = 25°C, pH 7.

Table 3
'Competition test' on the localisation of Ca^{2+}-regulation in vascular actomyosin

Protein mixture	Ca^{2+}-sensitivity of the ATPase
Native vascular actomyosin	1.78
Native vascular actomyosin + pure actin from skeletal muscle	1.67
Native vascular actomyosin + HMM-S1 from skeletal muscle	1.0

2 mg/ml actomyosin, 2 mM ATP, 5 mM $MgCl_2$, 10 mM imidazole, 100 μM dithioerythritole, 100 μM $CaCl_2$ or 5 mM EGTA resp., T = 25°C; pH 7. Added excess actin: 50 μM, added excess HMM-S1: 7 μM.

ties – the Ca^{2+}-sensitivity – is independent of the actin concentration. In table 2, the Ca^{2+}-sensitivities of various reconstituted actin–myosin systems are given. The value is close to unity when actin is combined with skeletal muscle HMM-S1 (which is not Ca^{2+}-regulated), or with HMM-S1 prepared from desensitized arterial actomyosin. Native HMM-S1 contains 2 light chains of mol.wt 20 000 and 17 000 (fig.1) HMM-S1 prepared from desensitized actomyosin was found either to be devoid of 2 light chains (in 2 preparations) or only devoid of the heavier light (in 1 preparation). Though tentative, these results seem to suggest that one of the light chains, possibly the 20 000 light chain, confers Ca^{2+}-sensitivity to the contractile system of smooth muscle. The activity of fully Ca^{2+}-activated acto-HMM-S1 was similar to that of natural actomyosin [6,19] or desensitized actomyosin from arteries. However, for reasons which are not yet understood, the inhibition induced in natural actomyosin by removal of Ca^{2+} with EGTA was in the order of 70 to 80%, while it was less than 50% in the synthetic system of actin and HMM-S1. Preliminary experiments show (table 1) that higher concentrations of Ca^{2+} are required to activate the latter system than were needed to fully activate the natural actomyosin. There remains the puzzling discrepancy that unregulated (desensitized) HMM-S1 is devoid of light chains, whereas desensitized actomyosin contains both light chains somehow attached to the myosin. In extension of these studies we also examined whether the 'competition test' developed for demonstrating myosin-linked regulation in invertebrate muscle [5] was positive i.e. whether addition of pure unregulated skeletal HMM-S1 to actomyosin did decrease the calcium sensitivity of vascular actomyosin, while addition of unregulated skeletal actin did not. The results (table 3) confirm recent results obtained with chicken gizzard [3] suggesting, albeit rather indirectly, a myosin linked Ca^{2+}-regulation.

In summary, our experiments suggest that the calcium sensing system of vascular smooth muscle appears to be localized in the myosin head (HMM-S1) where it seems to be associated with one (or both) light chains. In contrast to the molluscan muscle, which is also known to posess myosin linked Ca^{2+}-regulation, the calcium sensitivity of vascular smooth muscle is retained in the HMM-S1 preparations.

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