ACTIN-LINKED REGULATION OF THE HUMAN PLATELET CONTRACTILE SYSTEM

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1. Introduction

Regulation of muscle contraction involves two basic mechanisms. In vertebrate muscles Ca^{2+} controls contraction by binding to troponin located on the tropomyosin–actin containing thin filament [1, 2]. In molluscan muscles troponin is absent and the calcium regulation is effected through binding to myosin light chains [3].

We have previously demonstrated that in human platelets, similarly to muscle systems, a saturating level of 10^{-6} M Ca^{2+} is required to switch on the natural actomyosin(thrombosthenin) Mg-ATPase activity [4]. Furthermore, we have isolated from human platelets a tropomyosin-like protein, possibly involved in the contractile regulatory system [4].

In order to elucidate the mechanism of platelet contractile regulation we applied a principle recently put forward by Lehman et al. [5]. In the absence of Ca^{2+}, the regulatory proteins, bound to the actin thin filament in vertebrates and arthropods and to myosin in mollusca and lower organisms, prevent interaction between actin and myosin, and as a result the Mg-ATPase activity is depressed. In the case of vertebrate and arthropod muscle, pure actin (tropomyosin- and troponin-free) added to an actomyosin system in the absence of Ca^{2+} turns on the Mg-ATPase activity; thus the actin interacts directly with the myosin. On the other hand, in the case of molluscan muscle where the regulatory system is bound to myosin [3], pure actin added to actomyosin in the absence of Ca^{2+} cannot interact with myosin. The application of this principle to the human platelet contractile system and the presently observed regulation conferred on rabbit myosin ATPase activity by platelet thin filaments are highly indicative that the regulation of platelet contraction is of the actin-linked type.

2. Materials and methods

Platelet natural actomyosin(thrombosthenin) was prepared according to Bettex-Galland and Lüscher [6] with some modifications. Isolated human platelets, devoid of red and white blood cells, were washed at 20°C in dithiothreitol (DTT)- containing isotonic solution [7]. All further operations were carried out at 4°C. Twenty g of washed platelet pellets were suspended in 100 ml of 40 mM KCl, 5 mM DDT, 6.7 mM sodium phosphate buffer pH 7.0, and homogenized in a Sorvall Omnimixer. The homogenate was centrifuged in a Spinco model L ultracentrifuge at 100 000 *g* for 1 hr. The sediment was suspended in 50 ml of Weber–Edsall solution (0.6 M KCl, 0.04 M $NaHCO_3$, 0.01 M Na_2CO_3) containing 10 mM DTT. The suspension was stirred overnight, centrifuged for 1 hr at 25 000 *g* and sediment was discarded. The supernatant, containing actomyosin, was slowly added to 3 vol of 2 mM $MgSO_4$, acidified with dilute acetic acid to pH 6.4, and the flocculate was centrifuged immediately for 15 min at 25 000 *g*. The immediate centrifugation is crucial for obtaining platelet natural actomyosin with Ca-sensitivity. The sedimented gel was dissolved in 10 ml of 0.6 M KCl, 5 mM DTT, 0.01 M histidine buffer pH 7.0, and the solution was slowly added to 10 vol of 2 mM $MgSO_4$. The flocculate was immediately centrifuged for 15 min at 25 000 *g*, the sedimented gel was dissolved in 10 ml of 0.6 M KCl, 5 mM DDT, 0.01 M histidine buffer pH 7.0, and dialyzed overnight against 100 vol of the same buffer.

Human platelet G-actin was prepared from acetone dried actomyosin according to Probst and Lüscher [8] using 2 mM DTT at all stages of preparation. Both the crude extract (non-gel filtrated) and the gel filtrated pure actin were used in a polymerized F form.

Rabbit skeletal muscle proteins: myosin was prepared

according to Holtzer and Lowey [9], actin according to Kendrick-Jones et al. [3], and actomyosin according to Sent-Györgyi [10].

Sodium dodecyl sulfate (SDS) gel electrophoresis was performed according to Weber and Osborn [11] with a modification in the processing of the sample which was left in boiling water for 3 min in the presence of SDS and β-mercaptoethanol, both at 1% concentration.

ATPase activity ($\mu = 0.06$) was measured at 25°C in a system containing 25 mM Tris (pH 7.6), 2.5 mM $MgCl_2$, 2.5 mM ATP, and, when used, 0.1 mM ethylene glycol-bis-(2-aminoethyl ether)N,N'-tetraacetic acid (EGTA). The reaction was stopped by addition of 1 ml of 15% trichloroacetic acid to the 2 ml reaction volume. The inorganic phosphate liberated was determined by the method of Fiske and Subbarow [12]. Results are in μmol P_i/mg protein/min.

Protein concentrations were measured by the method of Lowry et al. [13]; standardized by Kjeldahl nitrogen determination.

3. Results and discussion

Pure rabbit F-actin, devoid of tropomyosin and troponin, released the 70% inhibition caused by the absence of Ca^{2+} on the Mg-ATPase activity of platelet natural actomyosin, similarly to the effect of F-actin on rabbit natural actomyosin (table 1). It follows that rabbit actin is able to interact with human platelet myosin which has been dissociated from platelet actin in the absence of Ca^{2+}. This result furthermore suggests the presence of regulatory proteins bound to the platelet actin.

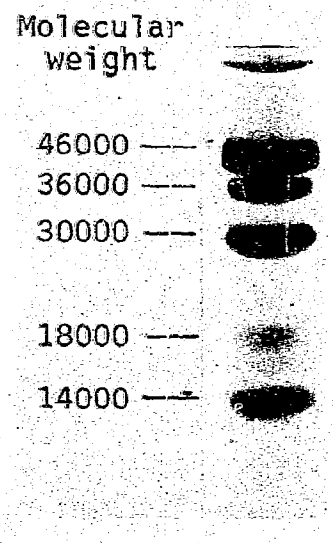

Fig. 1. SDS gel electrophoresis of human crude platelet actin ("thin filament").

Table 1

Actin-activation of natural actomyosin Mg-ATPase in the absence of Ca^{2+}.

	Mg	Mg + EGTA	Mg + EGTA + rabbit actin*
	ATPase activity, μmol P_i/mg protein/min		
Human platelet actomyosin	0.058	0.018	0.055
Rabbit muscle actomyosin	0.30	0.07	0.40

* Weight ratio of actomyosin:rabbit actin was 2:1.

It was further observed that in a rabbit muscle myosin–human platelet F-actin system in the absence of Ca^{2+} the crude actin preparation inhibited the Mg-ATPase activity by 50%, whereas the pure actin preparation was without any effect (table 2). Moreover, crude platelet F-actin, contaminated with tropomyosin and probably with troponin (fig. 1), behaved in SDS gel electrophoresis as natural thin filaments [5]. This electrophoretic pattern is similar to that of a non-

Table 2

Mg-ATPase activity of rabbit muscle myosin-human platelet F-actin system in the absence of Ca^{2+}.

Incubation mixture	μmol P_i/mg protein/min
Myosin	0.01
Myosin + crude platelet F-actin*	0.35
Myosin + crude platelet F-actin* + EGTA	0.18
Myosin + pure platelet F-actin*	0.30
Myosin + pure platelet F-actin* + EGTA	0.32

* Weight ratio of rabbit myosin:platelet actin was 2:1.

polymerizable fraction obtained by Probst and Lüscher [8]. The 30 000 subunit molecular weight band is the tropomyosin-like protein [4], the other bands (36 000; 18 000; 14 000) may correspond to troponin subunits [14, 15]. The presence of regulatory proteins bound to actin has already been suspected by Probst and Lüscher [8].

According to Lehman et al. [5] organisms in the early stages of evolution have a myosin-linked regulation of muscle contraction, whereas the actin-like regulation is of recent evolutionary acquisition. The present observations indicate that the contractile regulation in human platelets is of the "actin-linked" type and is mediated by tropomyosin and troponin. The actin-linked regulation of the human platelet contractile system might be brought to bear on the place of platelets in phylogenesis, they having been considered to be phylogenetic relics [16].

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