# The Differential Effect of Ca - ATP and Mg - ATP on Platelet Actomyosin

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SUMMARY: SDS-gel electrophoresis of the myosin peak produced by Mg-ATP splitting of platelet actomyosin on a 5-20% sucrose gradient revealed large amounts of actin or actin-like protein present. Substitution of $Ca^{++}$ for $Mg^{++}$ in the sucrose gradient released a lighter protein from the myosin peak. SDS-gel electrophoresis showed little actin or actin-like protein in the myosin peak and only actin or actin-like protein in the light peak. The differential effect of Ca-ATP and Mg - ATP on platelet actomyosin is not observed with skeletal muscle actomyosin. Futhermore the importance of calcium in platelet physiology suggests some important role for this $Ca^{++}$-ATP sensitive actin or actin - like protein in platelet function.

The classical procedure for separating skeletal muscle actomyosin into its major components, actin and myosin, involves disssocation of the actomyosin complex with Mg-ATP and separation of the heavy f-actin polymer from the lighter myosin by ultracentrifugation (1). We have found this procedure to be unsatisfactory for separation of the actomyosin complex isolated from platelets; SDS - gel electrophoresis has always indicated a large actin contamination of myosin, an observation also reported by Adelstein, Pollard, and Kuehl (2). This suggested fundamental differences in the nature of the actomyosin complex isolated from skeletal muscle and platelets.

We have previously reported (3) enzymatic differences between skeletal muscle and platelet actomyosin that suggested that platelet actomyosin was more nearly like actomyosin obtained from smooth muscle. One striking result of these enzyme studies was that platelet actomyosin

hydrolyzes Ca-ATP about ten times faster than Mg-ATP, in contrast to skeletal muscle actomyosin, which hydrolyzes Mg-ATP as fast, if not faster, than Ca-ATP. Furthermore, the ratio $Ca^{++}$:$Mg^{++}$ in platelets is about 10 (4) whereas in muscle it is only about 0.2 (5), and large pools of stored $Ca^{++}$ are released by platelets under conditions where their contractile protein is presumed to function (6). We therefore tested the effect of Ca-ATP on the actomyosin complex, using ultracentrifugation on sucrose gradients to analyze the dissociation.

This report concerns the differential effects of Ca-ATP and Mg-ATP on conventionally prepared platelet actomyosin, and describes the existence of a possible third component in this system.

Actomyosin was prepared from fresh human platelets essentially as described by Bettex-Galland and Luscher (7) except that precipitation was at 0.1M KCl, pH 6.5, a condition that we have found to give optimum specific activity and yield (8). The three-times precipitated actomyosin was used within a day of preparation. Skeletal muscle actomyosin was prepared from chicken leg muscle by conventional means (9).

When platelet actomyosin is centrifuged on a 5 - 20% sucrose gradient without ATP, the protein is recovered in the sediment with essentially none observed on the gradient except for an occassional small amount of approximately 4.0 s protein (Fig. 1, dotted line). Skeletal muscle actomyosin under these conditions is also recovered as a sediment, and thus both platelet actomyosin and skeletal actomyosin behave as a large actomyosin complex in the

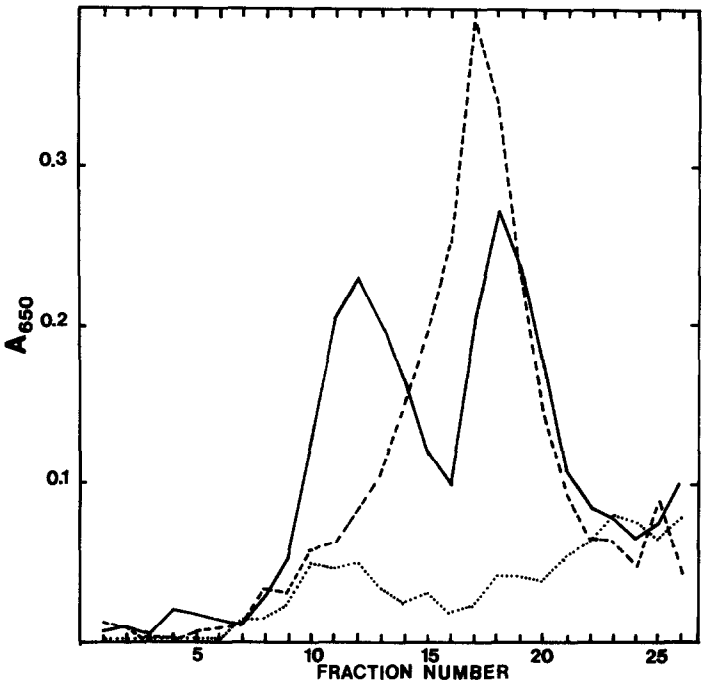


Fig. 1 5-20% (W/V) sucrose gradients with 0.5 M KCl, 10 mM Imidazole, pH 7.5, and either 10 mM Ca-ATP, 10 mM Mg-ATP, or no additions were made by layering 1.25 ml portions of 5,10 15 and 20% sucrose solutions in appropriate buffer in order of decreasing sucrose concentration, and allowing these gradients to age for 8 hrs. at 5°C (11). 0.4 ml of platelet actomyosin (1.15mg) dissolved in the same buffer as in the gradient was then layered on top and the gradients were centrifuged at 50,000 rpm for 16 hrs. at 3°C in a Beckman SW 50.1 rotor. Fractions were collected by time from the top of the gradient by pumping 40% sucrose at a constant rate up through a hole punched in the bottom of the 5 ml. polyallomer centrifuge tube. A 0.05 ml aliquot from each fraction was then analyzed for protein by the procedure of Lowry et al (12) using the appropriate controls. Sedimentation coefficients were calculated using the procedure of Martin and Ames (13) assuming a partial specific volume of 0.725 cc/g for all proteins. Dashed line- 10mM Mg-ATP, solid line-10mM Ca-ATP, dotted line - no additions.

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absence of ATP. When platelet actomyosin is centrifuged on a similar gradient with 10mM Mg-ATP, 70 - 80% of the protein is recovered as a broad asymmetrical peak of about 6.0 s (Fig. 1, dashed line). Analysis of this peak by SDS-gel electrophoresis (Fig. 2c) indicated that it is

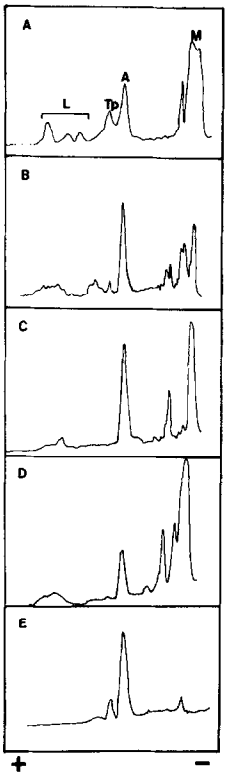


Fig. 2 SDS-gel electrophoresis was performed on 8.25% acrylamide gels (bis-acrylamide: acrylamide, 1:40) essentially as described by Weber and Osborn (14) except that the running buffer was 0.075 M Tris-acetate, 0.1% SDS, 0.1% B-mercaptoethanol, pH 7.9, and the gels were run at 3 mamp/ gel for 1.75 hrs. Between 5-20 ug of protein was applied to each gel. Densitometer tracings of the gels were made with a model K Canalco densitometer using a filter that had peak transmittance at 560 mu. In the protein range applied to our gels we have found good correlation between absorbance at 560 mu and protein concentration. A,-skeletal muscle actomyosin, B-platelet actomyosin, C-Mg-ATP 6.0 s material (fractions 16-19), D-Ca ATP 6.4 s material (fractions 17-20), E-Ca-ATP 4.0 s material (fractions 11-13). M=myosin, A=actin, Tp=tropomyosin, & L=Light chains or troponin.

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predominantly myosin with a substantial amount of actin or actin-like material. When skeletal muscle actomyosin is centrifuged under the same conditions, a symmetrical 6.0 s peak that contains little actin is observed, consistent with the dissociation of actomyosin by Mg-ATP into f-actin (sediment) and myosin (6.0 s). When platelet

actomyosin is centrifuged with 10mM Ca-ATP, (Fig. 1, solid line), 70 - 80% of the protein is recovered on the gradient as two peaks, one of about 6.4 s and one 4.0 s. SDS-gel electrophoresis indicated that the 6.4 s peak contains myosin with a small amount of actin (Fig. 2d) and the 4.0 s peak is almost entirely a protein with the molecular weight of actin (Fig. 2e). Centrifugation of skeletal muscle actomyosin with Ca-ATP gives the same result as with Mg-ATP.

The total protein in the two peaks produced by Ca-ATP equals that found in the Mg-ATP peak. Furthermore, the Mg-ATP peak contains both myosin and actin-like protein while in the Ca-ATP gradient the 6.4 s peak is predominantly myosin and the 4.0 s peak is predominantly actin-like protein. Thus it is obvious that the actin-like protein found in the 4.0 s peak came from protein found in the Mg-ATP peak.

Considering a molecular weight of platelet myosin of about 500,000 (10) and of actin-like protein of 40,000 there seems to be about ten moles of actin-like protein per myosin molecule. Conceivably this actin-like protein could polymerize, and the fact that it migrates with platelet actomyosin in the absence of Mg-ATP or Ca-ATP suggests two possible alternatives. One, it could exist as a complex with platelet myosin and be dissociated away by Ca-ATP but not by Mg-ATP. Secondly, it could be a large polymer whose degree of polymerization is differently affected by Mg-ATP and Ca-ATP, but which is not physically bound to platelet actomyosin. This would require that it also have the same solubility properties as platelet actomyosin, since our preparations are reprecipitated three times.

It is not known how platelet actomyosin causes

contraction in platelets or what other proteins participate in this process. If the actin-like protein reported here is isolated as a complex with myosin, it must have some important role in the function of platelet actomyosin. Even if it is isolated as an ATP-sensitive polymer not physically bound to the actomyosin, the unusual effects of Mg-ATP and Ca-ATP suggest that it has some essential platelet function.

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