ROLE OF Ca^{2+} IN THE ALLOSTERIC REGULATION OF PLATELET ACTOMYOSIN[1]

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Summary: The role of Ca^{2+} in regulation of platelet actomyosin ATPase activity has been investigated. The results suggest that Ca^{2+} has at least two roles in the reaction mechanism; (a) it forms a complex with ATP to form the substrate, CaATP and (b) it forms a complex with the protein to activate the enzyme. Both the substrate and free Ca^{2+} bind cooperatively to the protein. The binding of free Ca^{2+} stimulates the enzymic activity and causes a change in the apparent K_m value. The apparent K_m value for CaATP is 0.15mM in the absence of free Ca^{2+} and 0.07mM in the presence of 2.5mM Ca^{2+}. Thus Ca^{2+} appears to act as a positive allosteric effector.

We recently demonstrated the existence of Ca^{2+}-dependent cooperative substrate interactions in platelet actomyosin (1). This was highly significant because 1) although myosin is composed of subunits and has multiple ATP binding sites, allosteric interactions had never been observed in other systems and 2) while Ca^{2+} has been shown to activate platelet actomyosin ATPase, there has been no direct evidence that this regulation occurs by means of a troponin-tropomyosin type interaction. Thus, the observation of an allosteric regulation may be physiologically significant for this system. From our previous studies (1), it was not clear whether there was a separate allosteric site for Ca^{2+} or whether the observed effect of

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Ca^{2+} was due entirely to its existence as a CaATP complex. This report describes the further characterization of the involvement of Ca^{2+} in cooperative interactions of platelet actomyosin. The data indicate a separate allosteric site(s) for Ca^{2+}.

Materials and Methods

Bovine platelets were prepared as previously described (1) by differential centrifugation of fresh blood using EDTA as anticoagulant. Actomyosin was extracted as described (2,3). The final product was further purified by filtration through a column (2.5 x 40 cm) of sepharose 4B. There was no significant difference in the enzymic activity whether the protein was passed through sepharose 4B or not. ATPase activity was determined in a medium of 0.6M KCl, containing 50mM Tris-HCl, pH 7.2, with Ca^{2+} and ATP added as indicated. The ATPase reactions were linear for at least 15 min. under all conditions. After 5 or 10 min. incubation at 37° the reaction was stopped with cold 10% TCA, the tubes were centrifuged and the inorganic phosphate in the supernatant was determined by the method of Marsh (4). Protein concentration was determined by the modified Folin method of Lowry *et al*. (5). Protein concentration ranged from 0.05 to 0.1 mg/ml.

Results and Discussion

The association constant of Ca^{2+} and ATP is very high (6) so that equimolar amounts of $CaCl_2$ and ATP in solution exist predominantly as a CaATP complex, with essentially no free Ca^{2+} or ATP. The dependence of ATPase activity on concentration of CaATP can thus be determined in either the presence or absence of free Ca^{2+}. The ATPase activity of platelet actomyosin

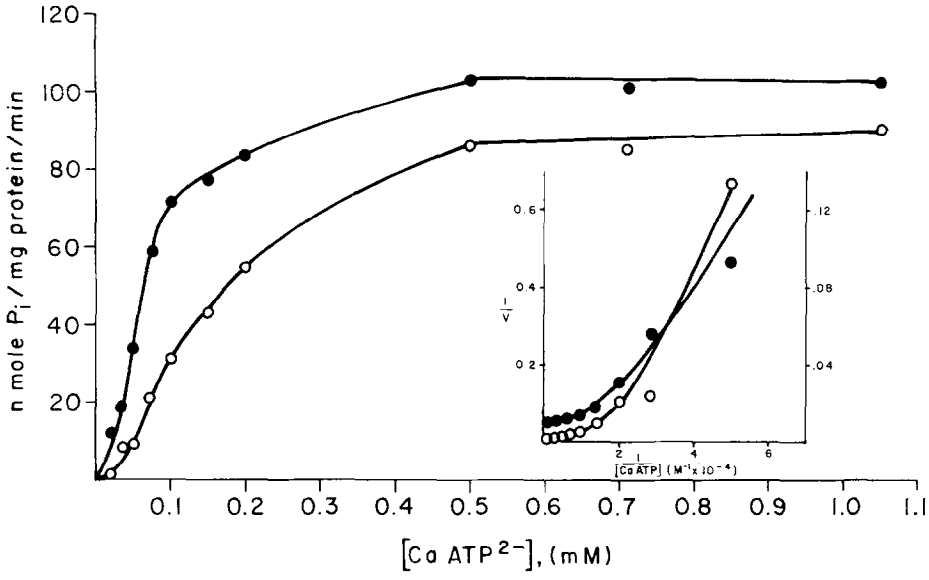

Fig 1. Plots of initial velocity as a function of CaATP concentration in the presence and absence of free Ca^{2+}. The assay conditions were as described in the text, ○—○ , Ca and ATP in equimolar concentrations; ●—● , in the presence of free Ca^{2+} (2.5mM).

Table I

Kinetic parameters for platelet myosin ATPase in the presence or absence of excess Ca^{2+}.

	K_m (mM)	V_{max} n mole Pi/ mg protein/min	Hill coefficient (n)
No free Ca^{2+}	0.15	88	2.0
Excess Ca^{2+} (2.5mM)	0.07	104	1.6

in 0.6M KCl, where actomyosin is known to be dissociated into its components actin and myosin (7,8), is plotted as a function of CaATP concentration in Fig. 1. The sigmoid curves indicate homotropic cooperative effects (9), and are similar to data published for several other enzyme systems (10-13). Addition of excess Ca^{2+} (upper curve), lowers the apparent

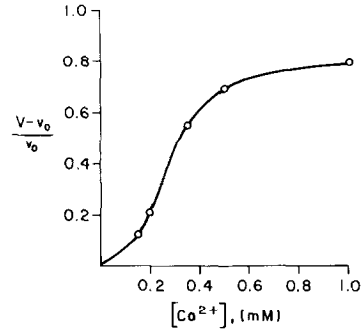

Fig 2. Effect of free Ca^{2+} on the initial velocity of platelet myosin ATPase activity. Enzyme activity is expressed as $\frac{V-v_o}{v_o}$ where V is the velocity at each free Ca^{2+} concentration and v_o is the velocity in the absence of any free Ca^{2+}. CaATP was constant at 0.1mM. Other assay conditions were as described in the text.

K_m, effectively increasing the rate 2 to 3 fold at low substrate concentrations. Both curves, when plotted in the double reciprocal form (Fig. 1, inset) show a non-linear relationship. Kinetic parameters, including Hill coefficients, for these two curves are shown in Table I. To further analyze the effect of Ca^{2+}, ATPase activity was plotted as a function of free Ca^{2+} concentration at low substrate levels (Fig. 2). The resulting curve is sigmoidal, indicating that free Ca^{2+} also exhibits cooperative effects.

Excess Ca^{2+} has three effects on platelet myosin ATPase; it lowers K_m, decreases the Hill coefficient and slightly increases V_{max}. Thus, it appears that Ca^{2+} is a positive allosteric effector. The data indicate that this enzyme has at least two interacting CaATP binding sites and at least two interacting sites for free Ca^{2+} and that binding of free Ca^{2+} enhances binding of ATP. These cooperative interactions may be characteristic of all myosins; we have observed similar interactions with smooth muscle myosin but with skeletal muscle

myosin no such effects can be seen because of the very low K_m. The Ca^{2+} effect described here may be important in regulation of contractility in platelets as well as in other non-muscle and smooth muscle systems.

Acknowledgement

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