ALLOSTERIC REGULATION OF PLATELET ACTOMYOSIN[1]

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Summary: The kinetic properties of platelet actomyosin have been examined to understand the mode of hydrolysis of its substrate ATP. In the presence of divalent cations, ATP hydrolysis deviated from Michaelis-Menten kinetics in such a way as to indicate cooperative effects, with a sigmoidal velocity vs. substrate curve and a Hill slope of 2.4. In the absence of added divalent cations, linear Michaelis-Menten kinetics were obtained and the Hill slope reduced to 1.0. These results indicate an allosteric regulatory site on platelet actomyosin.

Platelets are interesting mammalian non-muscle cells that contain an actomyosin-like protein with many of the characteristics of muscle actomyosin (1-3) but with some differences in enzymatic and physical properties (4,5). In the course of studies to develop a more general understanding of contractility, we are investigating the structure and mechanism of ATP hydrolysis by platelet actomyosin. We have obtained evidence for cooperative binding of ATP, an important characteristic not previously observed with other actomyosins.

Materials and Methods

Platelets from 1 liter of freshly drawn bovine blood collected into 0.1 volume of 120 mM NaCl and 54 mM EDTA as anticoagulant were prepared by differential centrifugation at 4° in a swinging bucket centrifuge. Red and white cells were

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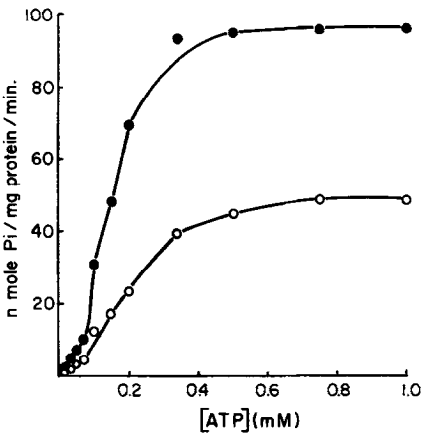

Fig. 1. Effect of ATP on the initial velocity of platelet actomyosin ATPase activity. The assay was as described in the text with 2.5 mM $CaCl_2$. O —— O, 0.1 M KCl; ● —— ●, 0.6 M KCl.

removed by centrifugation at 500 x g for 30 min and platelets were collected and washed twice in 150 mM NaCl, 2.5 mM EDTA, pH 7.0, by centrifuging at 1200 x g for 30 min. Approximately 2 ml of a platelet pellet was obtained. Actomyosin was extracted as described (4). ATPase activity was determined in a medium of 0.1 M KCl or 0.6 M KCl, containing 50 mM Tris-HCl, pH 7.2. 2.5 mM $MgCl_2$ or $CaCl_2$ were added as indicated. Protein concentration ranged from 0.15 to 0.5 mg/ml. After 10 or 15 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 (6).

Results and Discussion

The effect of substrate concentration on the initial velocity is shown in Fig. 1. Sigmoid curves were obtained with low salt (lower curve) and also with high salt (upper curve), where actomyosin in the presence of ATP is known to be dissociated into its components actin and myosin (7). The same data plotted in the double reciprocal form is non-

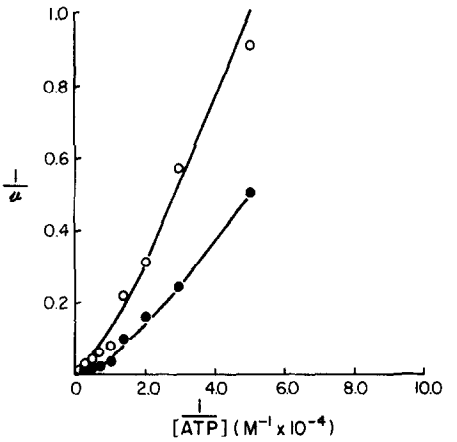

Fig. 2. The data of Fig. 1 plotted in the double reciprocal form. O —— O, 0.1 M KCl; ● —— ●, 0.6 M KCl.

linear (Fig. 2). Curves of this type (Fig. 1 and 2) are very similar to data published for several other allosteric enzyme systems (8-11), and indicate that ATP binds cooperatively to more than one site. For an enzyme possessing n number of mutually interacting substrate binding sites, it has been shown by Atkinson et al. (12) that the Michaelis equation may be put in the form

$$\log \frac{v}{V_{max}-v} = n \log [S] - \log K$$

This equation is also known as the Hill equation. As noted by a number of authors (12-14), the Hill plot is a useful tool for analyzing some allosteric effects and the value of the slopes, n, obtained are considered to be a measure of the number of interacting sites as well as the strength of their interactions. When the interaction is very strong, the value of the slope of the Hill plot approaches the number of binding sites for the ligand. The data in Fig. 1 are plotted in the form of the Hill equation for Fig. 3 and give straight lines of slope n = 2.3 and 2.4 for low and high KCl concentrations.

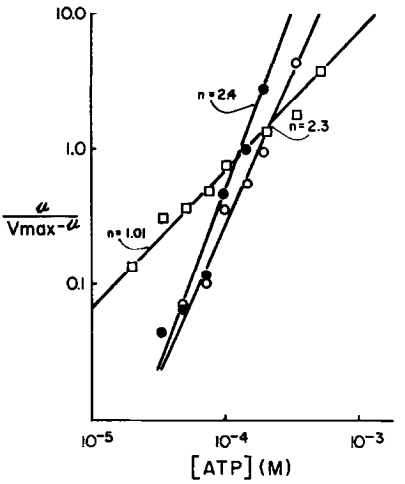

Fig. 3. Hill plot of data in Fig. 1. O —— O, .1 M KCl; O —— O, 0.6 M KCl; □——□, 0.1 M KCl without added divalent cation.

This demonstrates that there are at least two interacting ATP binding sites. Experiments were also done with Mg^{2+} as the divalent cation; the double reciprocal plot was non-linear and the Hill plot gave a slope of n = 1.5. This also suggests cooperativity of ATP binding.

In the absence of added divalent cations, the velocity vs. substrate concentration curve was hyperbolic, the double reciprocal plot was linear, and the Hill plot (Fig. 3) gave a slope n = 1.0. Thus, without divalent cations there are no interacting ATP binding sites.

The simplest and most likely explanation for the data obtained with the divalent cations Ca^{2+} and Mg^{2+} is that platelet actomyosin possesses at least two ATP binding sites which interact with one another in a cooperative manner. This type of homotropic interaction is characteristic of all allosteric proteins as described by Monod et al. (13,15). In contrast, without added divalent cation the predominant cation

is K^+ and no cooperative effects are observed. There are two possible explanations for these effects. 1) They may involve only changes in the nature of the substrate. Thus K-ATP either may not bind to the allosteric site or, if bound, may not induce the allosteric transition caused by binding of Ca-ATP or Mg-ATP. 2) They may be due to direct effects of different cations on the protein. That is, the conformation of platelet actomyosin may be dependent on specific cations, with only certain conformations exhibiting cooperative binding of the substrate. Such cation effects on enzyme conformation have been discussed in detail (16,17) and in addition to these allosteric effects, it is known that specific cations modify V_{max} of actomyosin ATPase. For example, the specific activity of platelet actomyosin in 0.1 M KCl is 7 $\pm$ 2.0 nmole Pi/mg protein/min; with addition of 2.5 mM $CaCl_2$ or $MgCl_2$ it is 100 $\pm$ 20 or 5 $\pm$ 2.

It is interesting to note that there is conflicting evidence whether there are one or two ATP binding sites on skeletal muscle myosin (18,19,20). Some authors have suggested that there are two interacting active sites (21,22) and it has also been proposed that there may be a regulatory site on skeletal muscle myosin (23,24). However, there has been no definitive experimental evidence to support these suggestions.

The significance of the apparent allosteric regulation of platelet actomyosin is not clear, nor is the exact role of the contractile system of platelets known, although it is presumably involved in some of the striking changes platelets undergo in response to certain external stimuli. Significantly, there are extensive Ca^{2+} fluxes associated with these changes (25) and it is interesting to speculate that Ca^{2+} might regulate

the contractile process in platelets by a mechanism different from that observed in skeletal muscle. There is no direct evidence for the presence of troponin (the calcium binding protein that regulates contraction of skeletal muscle) in non-muscle contractile systems and efforts to isolate a troponin-like protein from platelets have thus far been unsuccessful (E. McGowan and A. Stracher, personal communication). The allosteric effect of Ca^{2+} described here may thus be part of a physiological control mechanism.

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