

Differential binding of rabbit fast muscle myosin light chain isoenzymes to regulated actin

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The direct binding of S1(A1) and S1(A2) to regulated actin has been investigated by centrifugation. Binding was measured in the presence of either $\text{Mg} \cdot \text{AdoPP}[\text{NH}]P$ or $\text{Mg} \cdot \text{ADP}$ at 24 °C at various ionic strengths. At low ionic strength, in either the presence or absence of Ca^{2+} , the binding of S1(A1) to regulated actin was always stronger than for S1(A2). As the ionic strength was increased the differential binding between S1(A1) and S1(A2) was still maintained in the presence of Ca^{2+} but not in its absence. These data are discussed in terms of a modifying role for the N-terminal region of the A1 light chain in regulation of the contractile process.

Actin binding Myosin subfragment 1 isoenzyme Regulated actin Cooperative binding

1. INTRODUCTION

Myosins purified from different muscles contain different alkali light chains [1]. Furthermore, the light chain content and hence the type of myosin light chain isoenzyme present changes during muscle development [2] and in response to nerve stimuli [3,4]. Recently, different myosin light chain isoenzymes have even been found to occur within a single myosin filament [5].

The presence of a particular alkali light chain associated with the myosin head has been shown to influence the affinity of the heads for actin [6–9]. Subfragment 1 (S1) containing only the A1-type of light chain (S1(A1)) showed a greater affinity for actin than S1, possessing only the A2 type of light

chain (S1(A2)). Different alkali light chains have also been shown to influence the turnover of ATP in the presence but not in the absence of actin [10]. These differences, however, were only apparent at low ionic strength and disappeared as the ionic strength was increased [9,11]. Consequently, their physiological significance has become controversial, especially since it has been found that the isolated heavy chains, free from light chains, can both hydrolyse ATP and bind to actin [12,13]. These isolated heavy chains, however, were unstable and readily aggregated. Recombination with alkali light chains prevented further denaturation and subsequent loss of ATPase activity [14].

The major difference between the A1- and A2-type of alkali light chains from rabbit fast muscle resides in the extra 41 residues at the N-terminal end of the A1 light chain [15]. ^1H -NMR data from our laboratory show that this Pro,Ala,Lys-rich N-terminal region forms an extended structure [16] which interacts directly with the C-terminal region of actin [17]. This interaction is maintained at physiological ionic strength and is substantiated by chemical cross-linking studies of Sutoh [18]. Preliminary ^1H -NMR studies [19] suggest that the inhibitory protein of the troponin complex (Tn-I)

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Abbreviations: S1, myosin subfragment 1; S1(A1), S1(A2), rabbit fast-twitch muscle myosin subfragment 1 containing either the alkali 1 (A1 or LC1) light chain or the alkali 2 (A2 or LC2) light chain; $\text{AdoPP}[\text{NH}]P$, adenosine-5'-[β , γ -imido]triphosphate; Ap_5A , P^1 , P^5 , P^1 , P^5 -di(adenosine-5')pentaphosphate; Tn-I, troponin I, the inhibitory component of troponin

can specifically weaken the interaction between the N-terminal residue of the A1 light chain and actin. We therefore considered it pertinent to reinvestigate the binding of S1 isoenzymes to actin at high ionic strength, both in the presence and absence of regulatory proteins.

2. MATERIALS AND METHODS

2.1. Protein preparations

Myosin, S1(A1), S1(A2) and actin were prepared as described in [20]. Troponin-tropomyosin complex was prepared according to [21]. G-Actin was polymerised by addition of KCl to 50 mM and MgCl₂ to 5 mM. After 1 h at 4°C the F-actin was dialysed against 25 mM triethanolamine (pH 7.5) containing 50 mM KCl, 5 mM MgCl₂, 0.5 mM dithiothreitol for 2 h on a rocking dialyser to remove excess ATP from the actin.

2.2. Binding studies

The binding of S1(A1)·AdoPP[NH]P, S1(A2)·AdoPP[NH]P, S1(A1)·ADP and S1(A2)·ADP to actin or regulated actin was determined in the presence of 0.25 mM diadenosine pentaphosphate to inhibit myokinase activity [22]. Solutions were prepared by adding increasing concentrations of either S1(A1) or S1(A2) to a fixed concentration of actin or regulated actin. Regulated actin consisted of actin and troponin-tropomyosin complex at a molar ratio of 7:2, respectively. Solutions were then incubated at 25°C for 30 min. Subsequently, 180 µl samples were centrifuged at 178000 × g for 40 min in a Beckman Airfuge. Prior to centrifugation a portion of the sample was assayed to obtain the total concentration of S1. Following centrifugation, triplicate samples from the supernatant were analysed to determine the free S1 concentration by measuring the NH₄⁺-EDTA ATPase activity [23]. The steady-state ATPase hydrolysis rates were measured by determination of inorganic phosphate released [24]. Assays were carried out at 25°C in a solution containing 5 mM ATP, 0.4 M NH₄Cl, 40 mM EDTA (triethanolamine salt), 50 mM triethanolamine, pH 7.5. The presence of actin and/or troponin-tropomyosin had no influence upon the rates obtained and had a protective effect in preventing loss of ATPase activity during the 30 min incubation before centrifugation.

2.3. Analysis of binding data

The concentration of bound S1 was obtained by subtracting the free S1 from the total concentration of S1. The number of moles S1 bound per actin monomer (θ) was determined by dividing the concentration of bound S1 by the total monomer concentration of actin. The association constant, K_a , is then given by:

$$K_a = \theta / ([S1]_{\text{free}} (1 - \theta))$$

This assumes that S1 binds to F-actin with a stoichiometry of one S1 per F-actin monomer [25].

3. RESULTS

These results are the combination of many experiments with several different protein preparations. In all cases the actin-activated MgATPase activity of S1(A1) and S1(A2) in the presence of troponin and tropomyosin was inhibited at least 90% in the absence of Ca²⁺.

3.1. Binding of S1(A1)·AdoPP[NH]P and S1(A2)·AdoPP[NH]P to regulated actin in the presence of Ca²⁺

The binding of S1(A1) and S1(A2) to regulated actin in the presence of AdoPP[NH]P and Ca²⁺ is shown in fig.1. This cooperative binding was originally described by Greene and Eisenberg [26] and has been extensively studied by these investigators [22,27]. Of particular significance, however, is the observation that the S1(A1)·AdoPP[NH]P complex undergoes the cooperative transition at a lower concentration of free S1 than does the corresponding S1(A2)·nucleotide complex. Furthermore, this difference is maintained over the whole binding range even in the presence of 50 mM KCl (total μ = 70 mM) (fig.1). Under these conditions, when 50% of the S1 binding sites on actin are filled, the relative binding affinities of S1(A1)·AdoPP[NH]P and S1(A2)·AdoPP[NH]P for regulated actin were: $K_a^{S1(A1)} = 3.2 \times 10^5 \text{ M}^{-1}$; $K_a^{S1(A2)} = 2.4 \times 10^5 \text{ M}^{-1}$.

In the absence of any added salt (fig.2, μ = 20 mM), the relative binding affinities for the two isoenzymes at θ = 0.5 were: $K_a^{S1(A1)} = 1.1 \times 10^6 \text{ M}^{-1}$; $K_a^{S1(A2)} = 4.9 \times 10^5 \text{ M}^{-1}$.

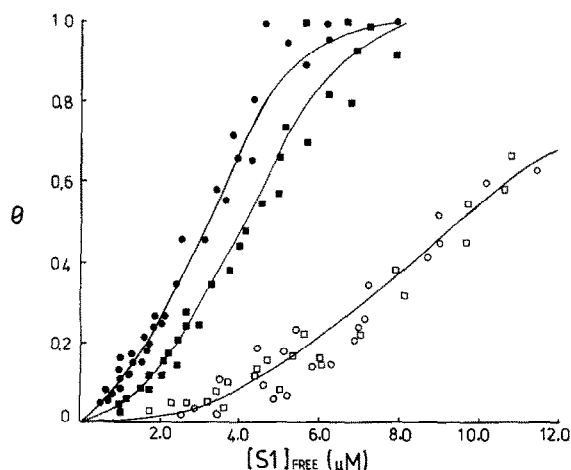


Fig. 1. The binding of S1(A1) and S1(A2) to actin in the presence of AdoPP[NH]P. Total ionic strength = 70 mM. Conditions: 50 mM KCl, 5 mM MgCl₂, 4 mM AdoPP[NH]P, 0.25 mM Ap₅A·P¹,P⁵, 0.5 mM dithiothreitol, 20 mM triethanolamine-HCl (pH 7.5), 25°C. The protein concentrations were 10 μM actin, 3 μM troponin-tropomyosin and 1–20 μM S1. Closed symbols denote the binding curves generated in the presence of 0.5 mM CaCl₂ while open symbols indicate the presence of 1 mM EGTA. (●, ○) S1(A1)·AdoPP[NH]P; (■, □) S1(A2)·AdoPP[NH]P.

3.2. Binding of S1(A1)·AdoPP[NH]P and S1(A2)·AdoPP[NH]P to regulated actin in the absence of Ca²⁺

In the absence of Ca²⁺ the cooperative transition occurs at a higher concentration of free S1 than in the presence of Ca²⁺ [26]. From fig. 2 it can be seen that at low ionic strength ($\mu = 20$ mM) the binding isotherms of S1(A1)·AdoPP[NH]P and S1(A2)·AdoPP[NH]P to regulated actin were different, and the binding affinities at $\theta = 0.5$ were: $K_a^{S1(A1)} = 2.8 \times 10^5$ M⁻¹; $K_a^{S1(A2)} = 1.7 \times 10^5$ M⁻¹.

In contrast, when the ionic strength is increased to 70 mM (fig. 1) the differences in binding between the two isoenzyme·nucleotide complexes disappeared and a single sigmoidal binding isotherm was generated with $K_a = 1 \times 10^5$ M⁻¹ at 50% saturation.

3.3. Binding of S1(A1)·ADP and S1(A2)·ADP to regulated actin in the presence and absence of Ca²⁺

S1·ADP binds more strongly to regulated actin

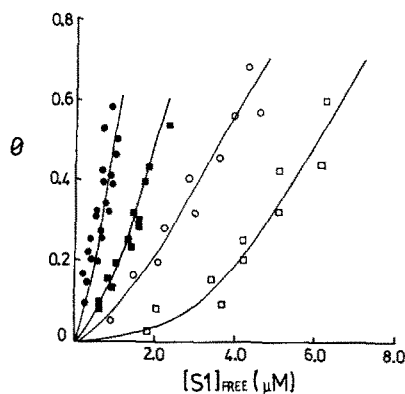


Fig. 2. The binding of S1(A1) and S1(A2) to actin in the presence of AdoPP[NH]P. Total ionic strength = 20 mM. Conditions are exactly as in fig. 1 except that no KCl was added. Closed symbols denote the binding curves generated in the presence of 0.5 mM CaCl₂. (●) S1(A1)·AdoPP[NH]P; (■) S1(A2)·AdoPP[NH]P. Protein concentrations were 8 μM actin, 2.4 μM troponin-tropomyosin and 1–7 μM S1. Open symbols indicate the presence of 1 mM EGTA; (○) S1(A1)·AdoPP[NH]P; (□) S1(A2)·AdoPP[NH]P. Protein concentrations were 8 μM actin, 2.4 μM troponin-tropomyosin and 1.5–12 μM S1.

than S1·AdoPP[NH]P [28] and so the binding of the S1 isoenzyme·ADP complexes to regulated actin was carried out at a higher ionic strength than above (total $\mu = 220$ mM). Even at this high ionic strength differences were still observed in the binding affinities of the two S1 isoenzyme·ADP complexes for regulated actin in the presence of Ca²⁺ and at $\theta = 0.5$; $K_a^{S1(A1)} = 4 \times 10^5$ M⁻¹; $K_a^{S1(A2)} = 2.5 \times 10^5$ M⁻¹. Under these ionic conditions in the absence of Ca²⁺ no differences in the binding of S1(A1)·ADP and S1(A2)·ADP to regulated actin are observed (at $\theta = 0.5$, $K_a = 1 \times 10^5$ M⁻¹).

3.4. Binding of S1(A1)·AdoPP[NH]P and S1(A2)·AdoPP[NH]P to non-regulated actin

Greene and Eisenberg [25] showed that in the absence of regulatory proteins, S1 binding to actin was independent and resulted in a hyperbolic binding curve that was not influenced by Ca²⁺. Furthermore, this group [9] found that S1(A1)·AdoPP[NH]P bound more tightly than S1(A2)·AdoPP[NH]P at low ionic strength but that this difference disappeared as the ionic strength was raised. Our results are in general

agreement with these observations for at low ionic strength ($\mu = 20$ mM), $K_a^{S1(A1)} = 2 \times 10^5$ M⁻¹ and $K_a^{S1(A2)} = 1 \times 10^5$ M⁻¹ ($\theta = 0.5$), whereas when the ionic strength was raised to 70 mM, these differences were essentially eliminated ($K_a = 6 \times 10^4$ M⁻¹ at $\theta = 0.5$).

4. DISCUSSION

This study clearly shows a difference in the binding affinity of S1(A1) and S1(A2)·nucleotide complexes for actin. The binding of S1(A1) being stronger than S1(A2), irrespective of the nucleotide present. Of particular significance is the observation that when the regulatory proteins and calcium are present these differences are maintained at physiological levels of ionic strength. In contrast, when calcium is absent and the ionic strength is raised these differences are essentially eliminated.

The only significant structural difference between the two S1 isoenzymes is the additional 41 residues at the N-terminus of the A1 light chain. This segment of polypeptide forms an additional site of interaction with actin, specific to the acto-S1(A1) complex, binding close to the C-terminus of actin [17]. The binding data presented here show that it also influences the thermodynamic parameters of the regulated actin-myosin interaction.

The cooperative binding of S1 nucleotide to regulated actin is characterized by a weak binding state of actin at low free concentrations of S1 and a strong binding state at higher concentrations of S1. The rate and magnitude of the transition from weak to strong binding being influenced by factors such as Ca²⁺, nucleotide and ionic strength [22,25,27]. In light of our results it would appear that the N-terminal region of the A1 light chain can also exert its effect on this transition. The latest model, proposed by Hill et al. [29] to explain this cooperative equilibrium binding of S1 to regulated actin, envisages that the tropomyosin molecule can occupy a continuum of positions on the F-actin filament, where the exact position occupied depends upon the nature of the binding 'ligand' (e.g., S1, S1·ADP, S1·AdoPP[NH]P, etc.). Our data suggest that the N-terminal region of the A1 light chain can also influence the position of tropomyosin and hence the conformation of the actin regulatory unit.

Some insight into how this may occur in molecular terms has come from separate ¹H-NMR studies which have shown that the binding of Tn-I to actin specifically weakens the interaction between the N-terminal segment of the A1 light chain in S1(A1) and actin, without effecting the heavy chain interaction site(s) [19]. Since Tn-I does not appear to compete with this light chain for the same site on actin this must reflect the transmission of information through actin [19]. Conformational changes in actin induced by S1 have been noted by others (e.g., [30-32]).

The reciprocal binding of Tn-I and the A1 light chain to actin appears to correlate well with the binding data reported here. Thus, at low ionic strength, when the interaction between actin and S1 is maximised, A1 light chain binding is able to exert its effect on thin filaments even in the absence of Ca²⁺. When the ionic strength is raised, the differential binding is only seen with regulated actin in the presence of Ca²⁺, since, under these conditions, the interaction between actin-tropomyosin and troponin is at its weakest [33].

It must be emphasised that these proposals are highly speculative and more experiments are in progress to examine these possibilities. However, it is interesting to note that the distribution of A1-type light chains closely parallels that of the troponin regulatory system in vertebrates.

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