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Bioelectrochemistry 70 (2007) 53–57
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Mechanism of actin polymerization by myosin subfragment-1 probed by dynamic light scattering

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Received 4 June 2005
Available online 15 April 2006

Abstract

Monomeric actin (G-actin) polymerizes spontaneously into helical filaments in the presence of inorganic salts. The slowest, rate-limiting step of the polymerization process is formation of actin trimers, the smallest oligomers that serve as nuclei for fast filament growth (filament elongation) by monomer addition at the filament ends. In low ionic-strength solutions, actin can be polymerized by myosin subfragment-1 (S1). In early works it has been suggested that G-actin-S1 1:1 complexes (GS) assemble into filaments according to the nucleation-filament elongation scheme. Subsequent studies indicated that one S1 molecule can bind two actin monomers, and that oligomerization of the initial complexes is a fast reaction. This has led to suggest an alternative mechanism, with a ternary G_2S complex and its oligomers being predominant intermediates of S1-induced assembly of G-actin into filaments. We used dynamic light scattering to analyze the initial steps of S1-induced polymerization of actin. Our results suggest formation of GS complexes and their oligomers in the presence of S1 equimolar to or in excess over actin. We confirm formation of G_2S complexes as intermediates of S1-induced polymerization in the presence of actin in excess over S1.

Keywords: Actin; Myosin subfragment-1; Actin polymerization

1. Introduction

Actin is one of the most abundant proteins in eukaryotic cells. Its biological function is to participate in a variety of motile processes. Polymerization of its monomeric form (G-actin) into helical F-actin filaments, associated with hydrolysis of G-actin-bound adenosine-5′-triphosphate (ATP), provides forces generating changes in cell shape and driving cell locomotion as well as some forms of intracellular transport. In the filamentous form, it interacts with myosin and accelerates its ATPase activity, which powers contraction of muscle and contractile processes in non-muscle cells.

G-actin polymerizes spontaneously in the presence of inorganic salts that neutralize its net negative charge and induce a change in the monomer conformation. This fast initial step (activation) is followed by slow association of activated monomers into trimers that serve as nuclei for a fast growth (elongation) of the polymer by monomer addition at its ends (for a review, see [1]). In living cells, where ionic conditions are optimal for actin polymerization, the filament assembly/disassembly is spatially and temporally regulated by a number of actin-binding proteins (reviewed in [2,3]).

In low ionic-strength solutions, actin can be assembled into filaments by its electrostatic interaction with positively charged residues of myosin from vertebrate skeletal muscle or its subfragment-1 [4–6] encompassing the N-terminal part of the myosin heavy chain and one of the two "essential" light chains of the parent molecule, A1 or A2 [7,8]. Although S1 of skeletal muscle myosin is structurally and functionally similar to the class I of non-muscle myosins [9], there is no indication that these myosins directly participate in actin polymerization in the cell. Nevertheless, myosin S1-induced polymerization of actin has been extensively studied in an attempt to define the differences between the interaction of myosin with G-actin and F-actin, as only F-actin is able to activate the myosin ATPase activity [5,10–12]. Another aim is to get an insight into the mechanisms of the regulation of actin polymerization by other actin-binding proteins.

Formation by S1 of a 1:1 complex with G-actin [13–15], saturation of S1-induced polymerization of actin at this ratio of the two proteins [16], and an evidence for S1-induced changes in G-

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doi:10.1016/j.bioelechem.2006.03.035

actin structure [16,17] have led to conclusion that 1:1 G-actin-S1 complex (GS) forms "S1-decorated" filaments according to the activation–nucleation–elongation mechanism of salt-induced polymerization. Entirely different mechanism has been suggested based on fluorescence titrations of pyrene-labeled G-actin with S1, indicating that at G-actin/S1 ratios equal to or greater than 1, in the absence of free ATP in solution, one S1 molecule binds two actin monomers to yield a ternary G_2S complex [18]. Fluorescence and light-scattering measurements also revealed rapid association of the initial complexes into oligomers [19] contrasting with the slow nucleation step in salt induced polymerization of actin. It has been concluded that G_2S complex is the major intermediate in S1-induced polymerization of actin, its rapid self-association leads to massively formed oligomers in which the actin/S1 ratio is 2, and these yield S1-decorated filaments by a slow end-to-end annealing with concomitant further binding of S1 up to actin/S1 ratio of 1:1 [19,20].

In this work, we used dynamic light scattering (DLS) to analyze the initial steps of S1-induced polymerization of actin. To determine the size of the initial G-actin-S1 complexes, the oligomerization step and formation of S1-decorated filaments were slowed down by using low protein concentrations and S1 (A2) isoform of S1. This isoform is less efficient than S1(A1) in assembling G-actin into filaments [13–15,18], and this difference is mostly due to lower equilibrium constant for the oligomer formation [19]. Our results confirm formation of G_2S complexes as intermediates of S1-induced polymerization of actin when actin is present in excess over S1. However, the measurements performed at a 1:1 S1/actin ratio suggest formation of GS complexes and their association into oligomers at saturating concentrations of S1.

2. Materials and methods

Rabbit skeletal muscle actin, isolated from acetone dried muscle powder, was purified according to Spudich and Watt [21] and, additionally, by gel filtration on Sephadex G-100, and was stored at 4 °C in a buffer containing 2 mM HEPES, pH 7.6, 0.2 mM ATP, 0.1 mM $CaCl_2$, 0.2 mM DTT and 0.02% NaN_3 (buffer G). Before measurements, ATP was removed from G-actin solutions by gel filtration on NAP™ 10 columns (Amersham Biosciences, Sweden).

Chymotryptic S1 was prepared from rabbit skeletal muscle myosin as described by Margossian and Lowey [22]. Isoforms S1(A1) and S1(A2) were separated by ion-exchange chromatography on SP-Trisacryl M [23], concentrated using Centriprep Centrifugal Filter Devices (Amicon, Millipore Co., Bedford, MA), and stored at 2 °C in 10 mM MOPS, pH 7.0, 80 mM NaCl and 0.4 mM DTT for up to 4 days. Before use, the preparations were dialyzed against ATP-free buffer G and clarified by 0.5 h centrifugation at 300 000 ×g.

The purity of protein preparations was controlled by SDS-PAGE [24].

Protein concentrations were determined spectrophotometrically using an absorbtion coefficient of 0.63 ml mg^{-1} cm^{-1} at 290 nm for G-actin [25] and 0.75 ml mg^{-1} cm^{-1} at 280 nm for S1 [22].

Static light scattering measurements were carried out in a Spex Fluorolog spectrofluorometer (Spex Industries, Edison, NJ). The measurements were taken at a 90° angle, at 450 nm.

Dynamic light scattering measurements were performed in a Nano ZS light-scattering apparatus (Malvern Instruments,

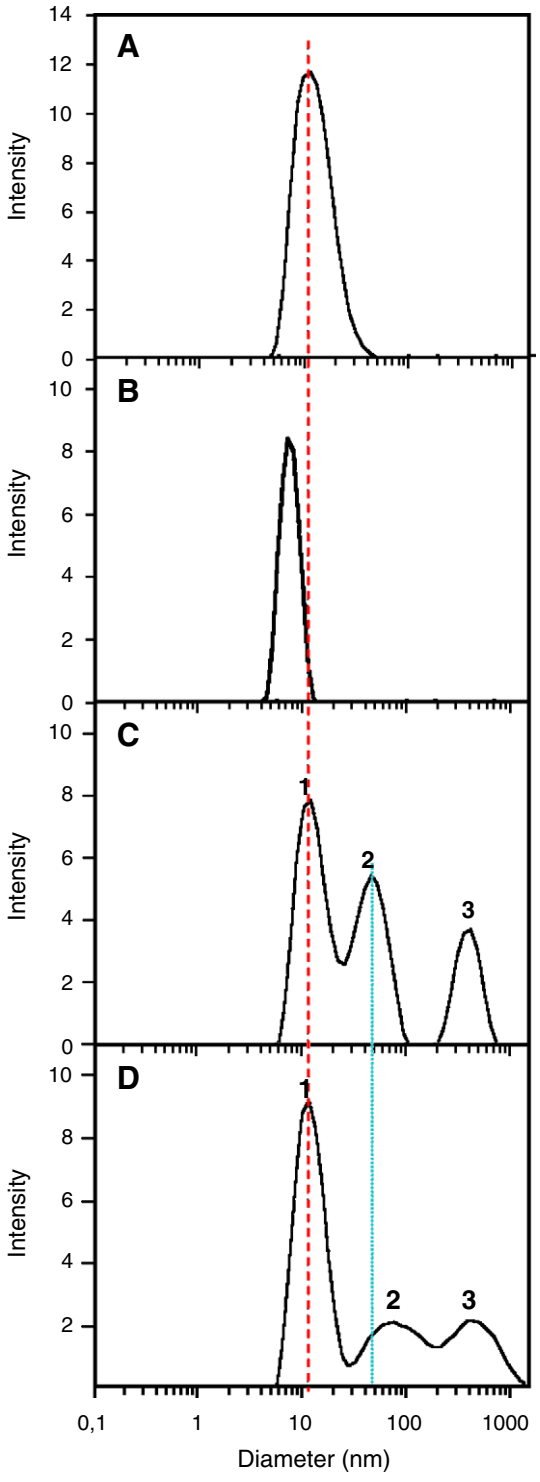

Fig. 1. Particle size distribution for S1(A2) alone (A), G-actin alone (B), and 1 μM G-actin mixed with either 0.5 μM (C) or 1 μM S1(A2) (D) in the absence of free ATP. DLS measurements were acquired as described in Materials and methods, at 25 °C, 10 min after mixing the proteins.

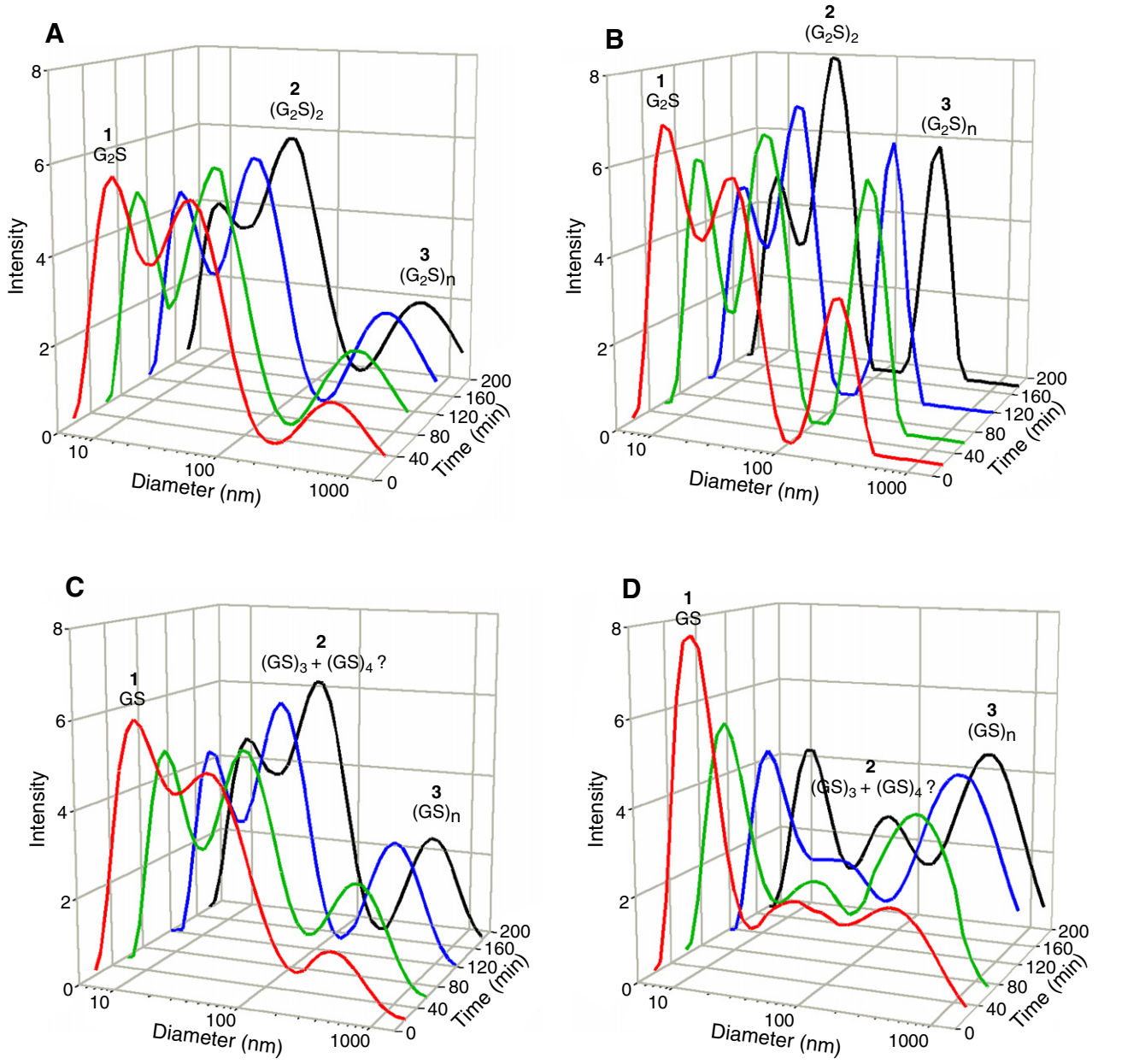

Fig. 2. Time dependent changes in particle size distribution during polymerization of 1 μM G-actin by S1(A2) at G-actin/ S1 molar ratio of 2:1 (A, B) or 1:1 (C, D). The measurements were performed in the absence of free ATP (B, D) or in the presence of 10 μM ATP (A, C). DLS measurements were acquired as described in Materials and methods, at 25 °C.

Worcestershire, U.K.). The time-dependent autocorrelation function of the photocurrent was acquired every 10 s, with 15 acquisitions for each run. The sample protein solution was illuminated by a 633 nm laser light, and the intensity of light scattered at an angle of 173° was measured by a solid-state avalanche photodiode. The z-average diameter and the polydispersity index of the sample were automatically provided by the instrument.

3. Results

In agreement with earlier observations, polymerization of 1 μM G-actin by myosin subfragment-1 isoform S1(A2), as monitored by measuring an increase in the intensity of static light scattering, was an extremely slow process. After 3-h incubation with either 0.5 or 1 μM S1 (A2) at 25 °C, the intensity of scattered light was only about 35% of steady-state levels reached with S1 (A1) within ~90 min. At both S1 concentrations, the filament assembly was preceded by a lag phase of 10–15 min (data not illustrated). The same conditions were used in DLS analysis of the initial G-actin complexes with S1(A2) and their oligomers.

Fig. 1 shows the particle size distributions for G-actin alone, S1(A2) alone, and mixtures of G-actin and S1, recorded 10 min after mixing. For G-actin alone and S1 alone, the size distributions were monomodal, with a hydrodynamic diameter of

Table 1
Particle size distributions in solutions of G-actin mixed with S1(A2) at 2:1 or 1:1 molar ratio

G-actin/S1 molar ratio	Presence of 10 μM ATP	Diameter (nm)
2:1	−	Peak 1: 13 Peak 2: 40 Peak 3: 350–400
2:1	+	Peak 1: 13 Peak 2: 45 Peak 3: 600–700
1:1	−	Peak 1: 13 Peak 2: 65 Peak 3: 450–500
1:1	+	Peak 1: 13 Peak 2: 60 Peak 3: 450–500

Data from Fig. 2.

about 7.5 and 13 nm, respectively. The particle size distributions in the mixtures of the two proteins indicated incorporation of all actin into G-actin-S1 complexes (disappearance of particles with the mean diameter of 7.5 nm) independent of whether G-actin was mixed with S1 in a 2:1 molar ratio or in equimolar ratio. This was not accompanied by a disappearance of particles with z-average diameter (the mean diameter based upon the intensity of scattered light) of 13 nm, the size of free S1 in solution. As one can see in Fig. 2, particles of this size persisted over the whole time course of the experiment (180 min) independent of the G-actin/S1 ratio and the initial presence or absence of free ATP in solution (that is rapidly converted into ADP by S1 ATPase). This indicates that the hydrodynamic diameter of the elongated S1 molecule (about 14 nm long in S1 crystals [26]) and of its complexes with one or two actin molecules (5.5×5.5×3.5 nm in actin crystals [27]) are not significantly different. Particles with z-average diameter of 40 nm and larger ones, which appear already in the early records, undoubtedly represent oligomers of the initial G-actin-S1 complexes. This is also indicated by the time dependent changes in size distribution as shown in Fig. 2. The number of particles with z-average diameter of 13 nm diminished with time concomitantly with accumulation of particles with a diameter in the range 40–65 nm.

The fast consumption of free G-actin at 2:1 G-actin/S1 molar ratio in the mixtures of the two proteins confirms formation of G_2S complexes in the presence of G-actin in excess over S1. On the other hand, the apparent similarity of the hydrodynamic diameters of free S1 and its complexes with one or two G-actin molecules, consistent with predictions from the geometry of S1 binding to F-actin [28,29], precludes direct evaluation of actin/S1 ratio in the complexes formed at equimolar concentrations of the two proteins. An indirect evidence indicating formation of a different type of complex under these latter conditions comes from comparison of z-average diameters of the oligomeric species formed at 2:1 and 1:1 G-actin/S1 ratios (Table 1). In view of the helical structure of acto-S1 oligomers as predicted from the structure of F-actin [30,31] and F-actin decorated with S1 [28,32], the oligomers with the diameter of 40–45 nm, formed in the presence of G-actin in a twofold molar excess over S1, are likely to represent dimers of G_2S complexes. The larger size of the oligomers formed at equimolar concentrations of G-actin and S1 (z-average diameter of 60–65 nm) indicates higher content of the elongated S1 molecules in these oligomers, suggesting initial formation of 1:1 complexes of the two proteins (GS). Since the "longitudinal" actin dimer formation is an intermediate step in formation of the helical actin trimers and higher order oligomers (reviewed in [1]), the oligomerization of GS complexes seems to proceed through their dimerization and subsequent association of $(GS)_2$ with GS into $(GS)_3$ or self-association of $(GS)_2$ into $(GS)_4$. Thus, peak 2 in Fig. 2C and D may represent either $(GS)_3$ or $(GS)_4$, or a mixture of these two oligomeric species.

It has been suggested [19] that the reported 1:1 actin/S1 ratio in G-actin-S1 complexes [13,15] was due to carrying out the measurements in the presence of free ATP which inhibits the interaction between G-actin and S1. From experiments in which the fluorescence of pyrene-labeled actin was used to monitor G-actin interaction with S1 it has been concluded that MgATP, in the micromolar range of its concentrations, is much more efficient in dissociating G_2S than GS complexes [33]. As one can see in Table 1, the presence of 10 μM ATP (in our experiments complexed with Ca^{2+}) did not result in diminishing the size of the small oligomers arising from G_2S complexes formed in the presence of G-actin in excess over S1. Also the time dependent changes in the particle size distribution in the presence or absence of free ATP in solution (Fig. 2) were essentially similar except that removal of ATP accelerated both the oligomer formation and short filament assembly (peak 3).

4. Discussion

The DLS measurements strongly suggest that preferential formation of G_2S complexes as intermediates in S1-induced polymerization of actin is limited to conditions when G-actin is present in excess over S1. Since actin alone does not form stable dimers even when the net negative charge of the monomers is reduced by the charge-screening effect of inorganic salts ([34] and references therein), the G_2S complexes seem to be formed by sequential binding of two actin monomers to one S1 molecule as originally suggested by Carlier and coworkers [18–20]. An alternative possibility is that neutralization of the acidic residues in subdomain 1 of actin by their charge interaction with S1 [4–6] is sufficient to enable the association of the S1-bound monomer with the second actin monomer and, consistent with predictions from the atomic model of S1-decorated F-actin [28,29], the actin–actin contact is then stabilized by S1 interaction with the two monomers. In both mechanisms, formation of the 1:1 complex between G-actin and S1 seems to be the first event in G-actin interaction with S1. Formation of GS complexes at S1 concentrations equal to or higher than the concentration of G-actin is consistent with this view.

It has been argued [19] that only G_2S complexes can self-associate because their fast oligomerization shifts the equilibria and prevents formation of GS when [S]>[G]. If this were true, one could expect that the oligomers formed with S1 equimolar to G-actin, and with G-actin in excess over S1 would be of the

same or similar size. Our results show that this is not the case. The larger z-average diameter of the oligomers formed at equimolar concentrations of G-actin and S1 suggests the higher content of S1 molecules in these oligomers than in those formed when G-actin is in excess over S1, indicating association of GS complexes into dimers and trimers and/or self-association of $(GS)_2$ that are formed first. Further studies are needed to differentiate between these two possibilities.

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