Biotechnology Advances 35 (2017) 867–888

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Covalent and non-covalent chemical engineering of actin for biotechnological applications

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ARTICLE INFO

Keywords:
Chemical engineering
Actin filament
Biomolecular motors
Nanofabrication
Self-assembly
Miniaturized sensing device

ABSTRACT

The cytoskeletal filaments are self-assembled protein polymers with 8–25 nm diameters and up to several tens of micrometres length. They have a range of pivotal roles in eukaryotic cells, including transportation of intracellular cargoes (primarily microtubules with dynein and kinesin motors) and cell motility (primarily actin and myosin) where muscle contraction is one example. For two decades, the cytoskeletal filaments and their associated motor systems have been explored for nanotechnological applications including miniaturized sensor systems and lab-on-a-chip devices. Several developments have also revolved around possible exploitation of the filaments alone without their motor partners. Efforts to use the cytoskeletal filaments for applications often require chemical or genetic engineering of the filaments such as specific conjugation with fluorophores, antibodies, oligonucleotides or various macromolecular complexes e.g. nanoparticles. Similar conjugation methods are also instrumental for a range of fundamental biophysical studies. Here we review methods for non-covalent and covalent chemical modifications of actin filaments with focus on critical advantages and challenges of different methods as well as critical steps in the conjugation procedures. We also review potential uses of the engineered actin filaments in nanotechnological applications and in some key fundamental studies of actin and myosin function. Finally, we consider possible future lines of investigation that may be addressed by applying chemical conjugation of actin in new ways.

1. Introduction

The protein-based cytoskeleton is central in the eukaryotic cell machinery. Its key filamentous structures are the actin filaments, intermediate filaments and microtubules. Out of these, the actin filaments have central roles in cellular shape changes and motility. The tasks are executed in interaction with molecular motors of the myosin superfamily and by virtue of polymerization, depolymerisation and branching, coordinated by cell signalling via a range of actin binding proteins (Blanchoin et al., 2014; Pollard, 2016). The actin-myosin interactions underlie aspects of intracellular transport and tension sensing but also cytokinesis and cell locomotion including muscle contraction. Thus, all these components, i.e. the cytoskeletal filaments together with the associated biomolecular motors and other accessory proteins, work effectively together as a biological nanomachine system. This complex system, with unique dynamic characteristics, has inspired hybrid devices or structures for diverse nanotechnological applications such as unique Lab-on-a-chip systems for capture and transportation of analytes to nanoscale sensors. In this regards the actomyosin system has been exploited mainly because of its higher transportation speed compared to the microtubule based systems also used for cargo transportation in nanotechnology (Korten et al., 2010).

In the developments towards actomyosin based nanodevices, several fields have been integrated such as nanotechnology, biotechnology, chemistry, material science, clinical medicine etc. Either myosin II motors together with actin filaments or actin filaments alone have been utilized in efforts towards a variety of applications. This includes the use of actin filaments as templates for the synthesis of nano-scale inorganic structures (Galland et al., 2013; Patolsky et al., 2004; Willner and Willner, 2010; Yao et al., 2009), capture and transportation of cargoes by motor propelled filaments (Iwabuchi et al., 2012; Kaur et al., 2011; Kumar et al., 2016; Kumar et al., 2013; Kumar et al., 2012; Mansson et al., 2004; Oyama and Hatori, 2016; Persson et al., 2013), integration of filaments and myosin motors into almost complete Lab-on-a-chip systems (Lard et al., 2013) and more recently, novel approaches for parallel biological computation (Nicolau et al., 2016).

For the purpose of these developments, actin filaments have been engineered in different ways, associated with attachment of certain

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http://dx.doi.org/10.1016/j.biotechadv.2017.08.002
Received 13 May 2017; Received in revised form 9 August 2017; Accepted 16 August 2017
Available online 19 August 2017
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biomolecules (Johnston et al., 2015; Kumar et al., 2016; Kumar et al., 2013; Kumar et al., 2012; Persson et al., 2013), metallic particles (Kaur et al., 2011; Patolsky et al., 2004; Willner and Willner, 2010), quantum dots (Mansson et al., 2004; Wang et al., 2013; Wu et al., 2003; Yao et al., 2009), organic fluorophores (Balaz and Mansson, 2005; Bombardier et al., 2015; Chazotte, 2010; Kumar et al., 2016; Kumar et al., 2012; Mansson et al., 2004; Pernier et al., 2016; Persson et al., 2013; Wulf et al., 1979), diamond nanoparticles (Bradac et al., 2016) etc. Moreover, engineering of actin filaments is important not only in nanobiotechnology but also in a range of fundamental cell biological and biophysical studies (e.g. (Barak et al., 1980; dos Remedios et al., 1987; Funatsu et al., 1995; Kaya and Higuchi, 2010; Knight and Offer, 1978; Kozuka et al., 2006; Kuhn and Pollard, 2005; Prochniewicz et al., 1993; Sheetz and Spudich, 1983a; Yanagida et al., 1984).

Both non-covalent and covalent conjugation methods have been used in recent efforts to engineer actin filaments by posttranslational chemical and biochemical methods. Here we will comprehensively review the methods used and the different types of cargoes or probes that have been attached to the actin filaments. Because of the relevance of actin biology and chemistry as well as actin-myosin interactions in this context we will also briefly review these issues. Furthermore, we consider relevant in vitro assays that have been important for developing applications in nanobiotechnology where engineered actin filaments are transported by myosin motors. Finally, we review key applications using engineered actin filaments alone or together with myosin motors.

2. Actin biology - a background to actin engineering

Actin is a globular protein (G-actin) with a molecular mass of about 42 kDa. It is composed of a single polypeptide chain of 375 amino acids (Collins and Elzinga, 1975). Actin is one of the most abundant eukaryotic proteins, it is found in all eukaryotic cells except for the nematode sperm and it is highly conserved between species (Galkin et al., 2010; Kabsch and Vandekerckhove, 1992; Pollard, 2016; Pollard, 1990). There are six isoforms of actin in mammals (Perrin and Ervasti, 2010) with only very small differences in amino acid sequence. Based on their isoelectric points, the isoforms are denoted α-actin (three different: skeletal, cardiac and smooth), β-actin and γ-actin (two different: smooth and cytosolic). The α-actins dominate in muscle whereas the β-actin and γ-actins dominate in non-muscular cells (Perrin and Ervasti, 2010). The striated muscle α-actins are the isoforms that, so far, have been utilized in nanotechnological applications (Gazit, 2007; Korten et al., 2010; Kumar et al., 2012; Lard et al., 2013; Mansson, 2012; Mansson et al., 2004; Patolsky et al., 2004; Persson et al., 2013; Yao et al., 2009). This fact is related to the vast previous experience from fundamental studies of α-actin and its expression at high levels in both skeletal muscle and heart muscle. The α-actin is also conveniently isolated and purified from striated muscle using standard protocols such as that in (Pardee and Spudich, 1982). At nearly physiological concentrations of KCl (> 50 mM), $MgCl_2$ (~1 mM) and ATP, G-actin monomers (with bound MgATP; MgATP-G-actin) self-assemble into filamentous actin (F-actin) (Fig. 1). Alternatively, $MgCl_2$ may be exchanged for $CaCl_2$ to obtain Ca-actin instead of Mg-actin filaments with different properties in certain regards e.g. related to the mechanism of nucleation of new filaments (Cooper et al., 1983) and the magnitude of the filament torsional rigidity (Yasuda et al., 1996).

The actin filaments (diameter ~ 8 nm; e.g.(Blanchoin et al., 2014)) have two protofilaments wound around each other in a right-handed double helix with cross-over every 36 nm (Fig. 1) (Hild et al., 2010; Kabsch et al., 1990; Schoenenberger et al., 2011). The two protofilaments contain 13 G-actin monomers per 36 nm (termed subunits in the following when incorporated into the filaments). The subunits with dimensions of 5.5 × 5.5 × 3.5 nm are connected to each other with the nearest neighbours along the filament and with two subunits in the other protofilament, an arrangement that may also be described as 6 left-handed turns per 36 nm. This geometry is relevant to bear in mind because it determines the geometrical arrangement of other molecules or particles conjugated to F-actin.

The crystal structure of the G-actin monomer (Holmes et al., 1990; Kabsch et al., 1990; Otterbein et al., 2001) reveals two domains separated by an inter-domain cleft (Fig. 2A). Each of these domains, denoted small and large domain, are further subdivided into two smaller subdomains 1 to 4 where subdomains 1 and 2 together comprise the small domain and subdomains 3 and 4 the large domain. At high salt concentrations, actin monomers self-assemble into actin filaments (Fig. 2B). Moreover, actin polymerization is appreciably sped up by addition of ATP and divalent cations (Mg^{2+}/Ca^{2+}). The nucleotide binds in the cleft between the large and the small domain. All four subdomains are involved in the interaction with neighbouring subunits in the filament. The subdomains 1 and 3 are located at a part of a terminal subunit in F-actin to which new monomers are added most frequently upon filament elongation. This rapidly growing filament end is referred to as the “plus end” or the “barbed end”. The latter term is due to the filament appearance when it is labelled with myosin motor domains. The other end with lower polymerization rate is known as the minus end or the pointed end (Fig. 2). The critical concentration is the concentration of G-actin monomers at which the equilibrium length at a given end is maintained without net polymerization or depolymerization. The critical concentration is about 10-fold lower (Carlier, 1990) at the barbed end (~0.1 μM (Pollard, 1986b)) than at the pointed end (0.6 μM in (Pollard, 1986b)). This is related to the difference in intersubunit binding affinity along F-actin between ATP-actin and ADP-Pi actin subunits on the one hand and between ADP-actin-subunits, on the other (Carlier, 1990; Murakami et al., 2010; Pollard, 1986b). Because the ATP is hydrolysed to ADP and Pi (inorganic phosphate) followed by slow Pi-release after G-ATP subunits are added to the plus end, ATP-actin subunits predominate at the plus end whereas ADP-actin subunits dominate at the minus end. The critical concentration also varies with experimental conditions, e.g. $[Ca^{2+}]$, $[Mg^{2+}]$, ionic strength and pH. Clearly, the critical concentrations are important in governing actin filament length and dynamics under different conditions. Particularly, actin monomer concentrations below the critical concentration at the barbed end lead to filament disassembly over time. One unique feature of actin filaments is treadmilling occurring at steady-state G-actin concentrations between the critical concentrations at the plus end and minus end. In this process G-ATP-actin monomers are added at the filament plus end followed by hydrolysis, Pi-release and “transportation” of monomers within the filament towards the minus end where the “transported” monomers are disassembled as G-ADP-actin. (Brieher, 2013; Bugyi and Carlier, 2010; Guo et al., 2010; Kuhn and Pollard, 2005; Pollard, 1986a; Selve and Wegner, 1986; Wang, 1985).

The “critical concentrations” as well as other aspects of actin dynamics and mechanics (cf. (Cooper et al., 1983; Isambert et al., 1995; Jegou and Romet-Lemonne, 2016; Vavylonis et al., 2005; Weber, 1999) are relevant parameters to consider when engineering actin filaments for various purposes because the parameters may be modified by the engineering. Furthermore, the effect of the modification of a given amino acid residue may differ depending on the added molecule as illustrated (see below for details) by modifications at Cys-374 on actin (previously sometimes referred to as Cys-373). For this and other reasons, e.g. altered mechanical properties of F-actin upon certain modifications (Isambert et al., 1995; McCullough et al., 2008; Vikhorev et al., 2008), it is important with insights into relevant aspects of actin biology before extensive engineering efforts. In addition to the issues considered briefly above, further key details can be found in previous comprehensive reviews on the subject (Blanchoin et al., 2014; Carlier, 1990; Oosawa, 1975; Pollard, 2016; Reisler and Egelman, 2007).

3. Myosin II and the in vitro motility assay for studies of myosin propelled actin filaments

Biomolecular motors are bio-nanomachines that transduce chemical

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