Biophysical Chemistry 193–194 (2014) 20–26

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Effect of urea and trimethylamine *N*-oxide on the binding between actin molecules

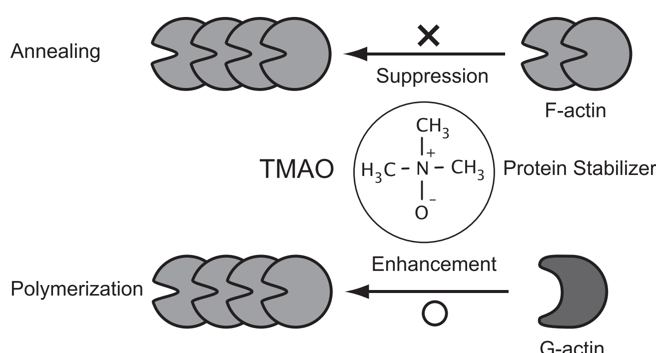
Kuniyuki Hatori *, Takuya Iwasaki, Reito Wada

Department of Bio-Systems Engineering, Graduate School of Science and Engineering, Yamagata University, Yonezawa 992-8510, Japan

HIGHLIGHTS

- Urea and TMAO decreased the end-to-end annealing rate constant of actin filaments.
- Only TMAO enhanced the thermal stability and polymerization rate of actin monomers.
- The deleterious effect of urea on polymerization was completely offset by TMAO.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 June 2014
Received in revised form 14 July 2014
Accepted 14 July 2014
Available online 23 July 2014

Keywords:
Denaturant
Protein stabilizer
Muscular protein
Polymerization
End-to-end annealing
Protein–protein interaction

ABSTRACT

Urea and trimethylamine *N*-oxide (TMAO) are known to denature and stabilize proteins, respectively. We examined two actin-binding processes, namely, end-to-end annealing of actin filaments (F-form) and the polymerization of actin monomers (G-form) into filaments, in the presence of urea, TMAO, and both solutes. Fluorescence microscopy for direct observation of actin filaments bound by fluorescent phalloidin revealed that the annealing rate constant decreased as the concentrations of urea or TMAO increased. Fluorescence spectroscopy with pyrene-labeled actin monomers showed that urea decreased the polymerization rate, whereas TMAO enhanced the rate. The decrease in the polymerization rate constant and thermal stability induced by 0.6 M urea was almost completely ameliorated by the addition of 0.3 M TMAO. These results suggest that TMAO-dependent stabilization of actin structure facilitates the binding of G-form actin to the ends of F-form actin filaments. Conversely, the binding between ends of mature filaments was impaired by TMAO.
© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Actin polymerization processes have been intensely studied because the morphology and dynamics of actin filaments are crucial for cell motility [1–3]. In *in vitro* experiments, actin monomers (G-form actin) spontaneously polymerize into filaments (F-form actin) when salt and magnesium ions are added to the actin solution. When G-form actin monomers associate with the ends of F-form actin filaments, they undergo conformational changes that are marked by a rotation between sub-domains [4–6]. It is likely that this structural change strengthens intermolecular interactions of actin constituents within filaments and enhances the stability of the filament structure [5]. These observations raise the issue of how stabilization of the actin structure contributes to the association between actin molecules.

Abbreviations: TMAO, trimethylamine N-oxide; ATP, adenosine-5′-triphosphate; TMR, tetramethyl-rhodamine; HMM, heavy meromyosin; NEM, *N*-ethylmaleimide; CD, circular dichroism; DTT, dithiothreitol.

* Corresponding author. Tel./fax: +81 238 26 3727.
E-mail address: khatori@yz.yamagata-u.ac.jp (K. Hatori).

http://dx.doi.org/10.1016/j.bpc.2014.07.001
0301-4622/© 2014 Elsevier B.V. All rights reserved.

To evaluate protein structure and function, investigators generally study protein unfolding and de-stabilization in the presence of denaturants or under various physical conditions [7–14]. In particular, urea and guanidine chloride are widely employed as protein denaturants. High concentrations of urea likely induce protein unfolding *via* the exposure of hydrophobic regions to an aqueous environment through solvation of the peptide backbone and side chains [7–9]. In contrast, trimethylamine-*N*-oxide (TMAO), a natural osmolyte found in water-stressed organisms [15], can act as a protein stabilizer and chemical chaperone [16–28]. The exclusion of TMAO solutes from protein surfaces through unfavorable interactions with the protein backbone tends to thermodynamically favor a folded (rather than extended) protein structure, which is suggested to underlie the stabilizing effects of TMAO [12,16–18]. However, it was proposed that TMAO can also interact with proteins [19] and modulate hydrogen bonds between water and proteins [20,21], and a consensus for TMAO-dependent protein stabilization has not yet been reached.

Although many studies on the effect of urea and TMAO on proteins have focused on the structural stability and folding/unfolding processes of proteins, little information is available regarding their effect on protein–protein interactions [29–31]. Our previous work revealed that TMAO suppresses the motility of actin filaments that interact with heavy meromyosin (HMM) molecules, even though the HMM structure is stabilized [31]. In addition, the deleterious effect of 0.6 M urea on motility was offset by the presence of TMAO at 0.3 M, a concentration that is within the physiological upper limits (386 mmol/kg) of a deep sea-dwelling organism [32]. In addition to physiological significance of urea and TMAO, to understand the biophysical role of these osmolytes, we here extended our studies of actin–myosin interactions to demonstrate that TMAO has differential effects on the binding between molecules composed of the G- and F-forms of actin.

2. Materials and methods

2.1. Chemicals and proteins

Urea and TMAO were purchased from Nacalai Tesque (Kyoto) and MP Biomedicals (California), respectively. Tetramethylrhodamine–phalloidin (TMR–phalloidin) was purchased from Sigma-Aldrich (St. Louis, MO). All chemicals were of special grade and were used without further purification. Actin monomers (G-actin) were purified from rabbit skeletal muscles according to the method of Spudich and Watt [33] and were stored at 4 mg/mL in G-buffer (2 mM Tris–HCl (pH 8.0), 0.2 mM ATP, 0.1 mM $CaCl_2$, 0.02% 2-mercaptoethanol, 0.05% NaN_3) at 4 °C. Purified actin monomers were used within 2 weeks.

2.2. Measurement of actin filament length for estimation of the annealing rate constant

The length of the actin filaments was measured by fluorescence microscopy. Initially, actin filaments (0.1 mg/mL) were fluorescently labeled and stabilized by TMR–phalloidin in a standard solution (25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 2 mM $MgCl_2$, 1 mM ATP, and 1 mM DTT). The sample was incubated for 2 days at 4 °C. Subsequently, the prepared sample (50 μL) was added to 450 μL of the standard solution containing urea and/or TMAO, and the mixture was immediately sonicated using an ultrasonic homogenizer to make short filaments (<1 μm). The sonicated sample was incubated at 25 °C, at which point annealing was initiated. The annealing was performed in the presence of 1 mM ATP. At 10 min intervals, 10 μL aliquots were taken from the specimen and added to 990 μL of the standard solution containing oxygen scavengers (3 mg/mL glucose, 0.02 mg/mL catalase, and 0.12 mg/mL glucose oxidase) because the annealing reaction was terminated by dilution. Immediately, 10 μL of diluted sample was dropped on a poly-lysine-coated thin slide glass (No. 1, 24 × 50 mm, Matsunami, Japan) and was observed under a fluorescence microscope (objective TIRF 100× H; Ti–U, Nikon, Japan). Fluorescence images were taken with an EM-CCD camera (DE-500, Hitachi Kokusai, Japan). The lengths of 100 individual filaments at each time point were measured using ImageJ software (Rasband, W.S., ImageJ; National Institutes of Health, Bethesda, Maryland, USA). The length was obtained in terms of actin units based on an estimated value of 389 actin subunits/μm. To determine the annealing rate constant, data from the time course were fitted to the equation derived by Andrianantoandro et al. [34]:

$$-dN/dt = k_a N^2/L, \quad (1)$$

where N is the concentration of filaments, dN/dt is the annealing rate, k_a is the annealing rate constant per subunit length, and L is the mean length of actin filaments in subunits. Using P as the initial actin concentration (0.24 μM), N = P / L was substituted into Eq. (1). Solving this differential equation, we obtained the relationship between length and time (t) as follows:

$$L^2 = 2k_a Pt + L_0{}^2, \quad (2)$$

where L_o is the initial filament length.

2.3. Fluorescent measurement of pyrene-labeled actin monomer polymerization

Actin monomers were labeled with N-(1-pyrenyl)iodoacetamide according to the method of Kouyama and Mihashi [35]. To start the polymerization, 100 μL of actin monomers containing 8% pyrene-labeled actin (1 mg/mL) were added to 400 μL of polymerization buffer (final concentrations in 500 μL: 25 mM KCl, 25 mM HEPES (pH 7.4), 1 mM ATP, 2 mM $MgCl_2$, and 0.5 mM DTT, with various concentrations of urea and TMAO). Immediately, 500 μL of the sample was infused into a cuvette (optical path for excitation, 0.2 cm; optical path for emission 1.0 cm), and then the fluorescent intensity was monitored using a fluorescence spectrometer (F-2500, Hitachi, Japan) at 25 °C. Excitation was set at 365 nm (band pass 2.5 nm), and emission was set at 407 nm (band pass 5.0 nm). Development of intensity with time (except for data obtained during the clearing phase) was fitted into the equation for a simple polymerization model, which only considers the association with, or dissociation from, single filaments [1] as follows:

$$-dC/dt = k_{on} mC - k_{off} m, \quad (3)$$

where C is the concentration of actin monomers, and −dC/dt is the decrease rate of C. k_{on}, k_{off}, and m denote the polymerization rate constant, the dissociation rate constant, and the concentration of the actin filament terminals, respectively. Because the concentration of polymerized actin molecules (P) is equal to the difference between the total concentration (C_o) and concentration of actin monomer (C), Eq. (3) is transformed into the following equation:

$$P = C_o - k_{off}/k_{on} + (k_{off}/k_{on} - C_o)\exp(-k_{on} m t). \quad (4)$$

In this study, the concentration of filament terminals (m) was not determined, and we assumed that emission intensity was proportional to the concentration of polymerized pyrene-labeled actin. In this study, C_0 was set at 4.8 μM.

2.4. Observation of polymerization of actin monomers into filaments

Polymerization of actin monomers was directly observed under a fluorescent microscope according to the method of Ishiwata et al. [36]

Download English Version:

https://daneshyari.com/en/article/5370985

Download Persian Version:

https://daneshyari.com/article/5370985

Daneshyari.com