Shimane J. Med. Sci., Vol.17 pp.37-41, 1999

ADP-RIBOSYLATION OF FILAMENTOUS ACTIN INDUCES ITS DEPOLYMERIZATION - THE ROLE OF ADP-RIBOSYLATION IN CYTOSKELETAL REORGANIZATION

Masaharu TERASHIMA, Makoto SHIMOYAMA and Mikako TSUCHIYA

Department of Biochemistry, Shimane Medical University, Izumo 693-8501, Japan

(Accepted September 2, 1999)

Actin, a major constituent in all eukaryotic cells, is one of the most important components of cytoskeletal architecture, and is involved in a numerous cellular processes including phagocytosis, cell locomotion, and maintenance of cell shape. These functions depend on the capacity of actin to polymerize and depolymerize. We previously reported that chicken arginine-specific ADP-ribosyltransferase ADP-ribosylated globular (G-) and filamentous (F-) actins, and that the modification of G-actin inhibited its capacity to polymerize *in vitro* and *in situ*, suggesting the involvement of ADP-ribosylation in regulation of the cytoskeletal organization *in vivo*. In the present study, we demonstrated that ADP-ribosylation of F-actin resulted in the depolymerization of the actin. Thus, ADP-ribosylation may have a role to shift G-F actin equilibrium toward the G-actin through the modification of both G- and F-actins, and participate in the regulation of cytoskeletal reorganization.

Keywords: ADP-ribosylation / filamentous actin / depolymerization / arginine-specific ADP-ribosyltransferase

INTRODUCTION

Arginine-specific ADP-ribosylation is a post-translational modification, in which ADP-ribose moiety of NAD is transferred to the arginine residues in the target proteins. In eukaryotes, although arginine-specific ADP-ribosyltransferases (ADPRTs) have been detected in many species and tissues (1), the functions of the transferases through the modification of the target proteins remain obscure.

Correspondence: Dr. Mikako Tsuchiya, Department of Biochemistry I, Shimane Medical University, Izumo 693-8501, Japan. Tel:81-853-20-2121 Fax:81-853-20-2120 E-mail: mikat@shimane-med.ac.jp

We previously reported that the arginine-specific ADP-ribosyltransferase was present in the cytosol of chicken polymorphonuclear leukocytes (so-called heterophils) (2), and that the transferase could modify various isoforms of globular actin (G-actin) *in vitro*, thereby preventing the actin polymerization (3). ADP-ribosylation sites in skeletal muscle α-G-actin were determined to be Arg28 and Arg206, and the latter was proved to be crucial for actin polymerization and DNase I interaction (4). Moreover, an introduction of NAD into the heterophils inhibited the increase in filamentous actin contents induced by a chemotactic peptide formyl-methionyl-leucyl-phenylalanine (5). These results suggest that ADP-ribosylation of actin might regulate the cytoskeletal organization *in vivo.*

It is generally accepted that polymerization of actin and organization of the actin filaments are regulated by ATP hydrolysis and by numerous actin-binding proteins (6, 7). Inhibitory effect of ADP-ribosylation on actin polymerization *in vitro* and *in situ* (3-5) may implicate additional mechanism to regulate cytoskeletal organization. Although we reported that filamentous actin (F-actin) was also ADP-ribosylated (3), we have not elucidated how ADP-ribosylation of F-actin affects its polymerization state. Thus, we turned our attention to see whether ADP-ribosylation of F-actin affects the actin polymerization or equilibrium state between G- and F-actins. In this study, we examined the influence of ADP-ribosylation on polymerization state of F-actin, and show here evidence that ADP-ribosylation of polymerized F-actin induces its depolymerization and increases G-actin contents.

MATERIALS AND METHODS

[*adenylate*-^{32}P] NAD (29.6 TBq/mmol) was purchased from New England Nuclear. ADPRT was

purified from the chicken heterophils as described previously (2). G-actin was prepared from chicken breast muscle by polymerization-depolymerization cycles and an anion exchange column chromatography according to Spudich and Watt (8) and further purified by the gel filtration on a Sephadex G-150 column (3). Purity of the actin was more than 95% determined by SDS/PAGE, and the preparation should not contain ADP-ribosylated G-actin because the modified actin can not polymerize (3, 4). G-actin was incubated with 50 mM KCl and 2 mM $MgCl_2$ at 30℃ for 2 hr, and centrifugated at 105,000xg for 1 hr. Resultant precipitate was resolved in the buffer containing 10 mM imidazole, pH 7.5, 0.2 mM $CaCl_2$, 0.75 mM 2-mercaptoethanol, 50 mM KCl and 2 mM $MgCl_2$ and used as F-actin fraction (4).

Purified actin (5 μg) was incubated with or without purified heterophil ADPRT (10 ng) in the reaction mixture containing 50 mM Tris/HCl, pH 9.0, 5 mM dithiothreitol and 0.1 mM [^{32}P]NAD (7.4 kBq/nmol) at 25℃ for 15 min, and analyzed by SDS/PAGE and autoradiography. In some cases, radioactivity in the acid-insoluble fraction of the reaction mixture was measured using scintillation counter.

Increase in the viscosity of actin solution was used to estimate the increase in F-actin contents of the solution. The viscosity of the solution was measured at 25℃ as a flow time required for the solution to pass through a glass capillary tube, Cannon-Fenske viscometer (9). Relative viscosity, ηr, was determined as the ratio of the flow time of actin solutions incubated with $MgCl_2$ to that without $MgCl_2$. Specific viscosity, ηs, was calculated by the following equation; ηs = ηr-1. Contents of G- and F-actins in the solution were also determined with ultracentrifugation at 105,000xg for 1 hr followed by measurement of protein amounts in the resultant supernatant and precipitate fractions, respectively (9).

RESULTS AND DISCUSSION

ADP-ribosylation of G- and F-actins

To examine the extent of ADP-ribosylation of F-actin, polymerized actin was incubated with [^{32}P]NAD and ADPRT. As shown in Fig. 1, both G- and F-actins appeared as 43 kDa single bands and were apparently radiolabeled. The degree of the ADP-ribosylation of F-actin was nearly a half of that of G-actin (lanes 1 and 2). ADP-ribose incorporation into the F- and G-actins during 15 min incubation with ADPRT were 0.29 mol/mol and 0.57 mol/mol, respectively. These results are consistent with the data in which G-actin was modified at Arg28 and Arg206 on the molecule, whereas F-actin was Arg28 only (4). When ADPRT was omitted from the reaction mixture, F-actin was hardly labeled (Fig. 1, lane 3).

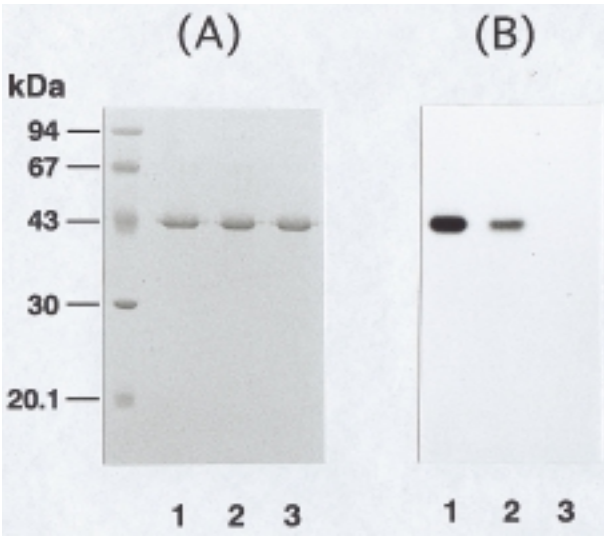

Fig. 1. ADP-ribosylation of G- and F-actins. Five μg of G- (lane 1) or F- (lanes 2 and 3) actin was incubated in the reaction mixture containing [^{32}P]NAD with (lanes 1 and 2) or without (lane 3) purified ADPRT at 25℃ for 15 min, and then subjected to SDS/PAGE followed by Coomassie brilliant blue staining (A) and autoradiography (B). Labels on the left indicate the sizes of molecular-mass markers.

ADP-ribosylation of F-actin causes its depolymerization

Next, we examined effects of ADP-ribosylation of F-actin on its polymerization state by measuring viscosity of the actin solutions. G-actin solution including 2 mM NAD was incubated with 2 mM $MgCl_2$ for 60 min, and then, ADPRT was added to the solution. The flow time required for the solution to pass through a capillary tube was measured at each time indicated, and the specific viscosity was calculated. As shown in Fig. 2, viscosity of the solution was increased and reached to the maximum level, 0.20 at 60 min. After adding ADPRT, the viscosity was gradually decreased and the lowest viscosity 0.10 was obtained 2 hr after the addition. Prolonged incubation no longer decreased the viscosity (data not shown). These results suggest

that ADP-ribosylation decreases the F-actin content. This was confirmed by assessment of contents of G- and F-forms in the actin solution with ultracentrifugation. Incubation of polymerized actin with ADPRT in the presence of NAD for 2 hr reduced F-actin content from 96% to 45% and increased monomeric G-actin content from 4% to 55%. These results indicate that ADP-ribosylation of F-actin decreases its polymerized form. Taken together with the previous observation that ADP-ribosylation of G-actin inhibits its polymerization (3), ADP-ribosylation may shift the equilibrium state between G- and F-actins toward G-actin.

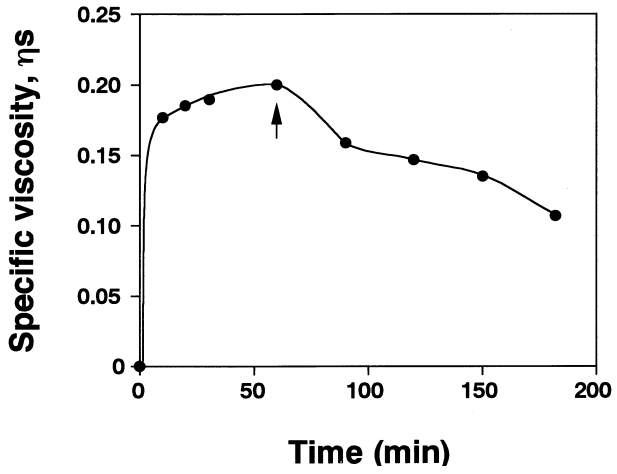

Fig. 2. Effect of ADP-ribosylation on the viscosity of F-actin. Purified actin (0.5 mg/ml) including 2 mM NAD was incubated with 2 mM $MgCl_2$ for 60 min, and then, ADPRT (10 μg) was added to the solution (indicated by an arrow). Incubation was continued for further 120 min. Specific viscosity of the actin solutions incubated for indicated times was determined as described in Materials and Methods.

ADP-ribosylation of actin affects its G-F equilibrium state

Actin, a highly conserved family of cytoplasmic proteins and major constituent in all eukaryotic cells, is one of the most important components of cytoskeletal architecture microfilament. Actin is involved in a wide variety of cellular processes, such as phagocytosis, secretion, cell locomotion, and the maintenance of the cell shape, besides muscle contractions. All these functions depend on the capacity of actin to polymerize and form filamentous actin, and to depolymerize to monomeric actin. In the resting state of the cell, monomeric G-actin and polymerized F-actin are in dynamic equilibrium state, which is regulated by various actin binding proteins (6, 7, 10). Actin filaments have two polar, non-equivalent ends for polymerization and depoly-merization; one is 'barbed end' and the other 'pointed end'. As a result of the difference in the assembly rates at the two ends, actin monomers can cycle through the filaments from the 'barbed end' to 'pointed end', thus keeping the equilibrium state (Fig. 3a).

When G-actin is ADP-ribosylated, ADP-ribose moiety covers the 'pointed end' of the actin at Arg206 to inhibit polymerization by a steric hindrance (3, 4, 11) (Fig. 3b). Mechanism of the inhibition seems different from that caused by clostridial ADP-ribosylating toxins, *C. botulinum* C2 and *C. perfringens* iota toxins, since both toxins ADP-ribosylate the 'barbed end' of actin at Arg177 (11, 12).

Though we demonstrated that ADP-ribosylation of actin in the 'pointed end' at Arg206 might cause the inhibition of actin polymerization (3, 4), the role of ADP-ribosylation of actin Arg28 which is located in the 'lateral surface' had been remained obscure. In this study, we demonstrated that ADP-ribosylation of F-actin induces its depolymerization. The result suggests that ADP-ribosylation of Arg28 in the F-actin causes the disruption of ordered conformation of the filament, and may stimulate depolymerization of F-actin. When F-actin is ADP-ribosylated, ADP-ribose moiety attaches to the 'lateral surface' of actin filaments at Arg28 and probably causes conformational changes in F-actin (4, 11, 13), leading to induce the depolymerization (Fig. 3c). In this state, additional modification may occur in the 'pointed end' on the depolymerizing G-actin which has been already modified at the 'lateral surface'. Thus, ADP-ribosylation would facilitate actin depolymerization, through both inhibition of the polymerization and induction of depolymerization. Taken together, ADP-ribosylation of G- and F-actins may have functional roles to inhibit actin polymerization and induce actin depolymerization, respectively, and by the sum of these effects, actin equilibrium would be shifted to the G- actin-dominant state.

Cellular concentration of G-actin is much higher than the critical concentration of G-actin for actin polymerization *in vitro*, and the phenomenon has been ascribed to numerous actin-binding proteins (6). It has been also shown that post-translational modifications

of actin including phosphorylation (14) may be involved in the regulation of actin polymerization. We previously reported the *in situ* ADP-ribosylation of actin in saponin-permeabilized polymorphonuclear leukocytes and the inhibitory effect of the ADP-ribosylation on actin polymerization (3, 4). We postulate here that ADP-ribosylation may have a role to the regulation by shifting G-F actin equilibrium toward G-actin through the modification of both G- and F-actins. Taken together with our recent study that ADP-ribosylation of tubulin, which is also a major component of cytoskeleton, was ADP-ribosylated and lost the capacity to form microtubule (15), ADP-ribosylation may participate in the regulation of cytoskeletal reorganization in the cells.

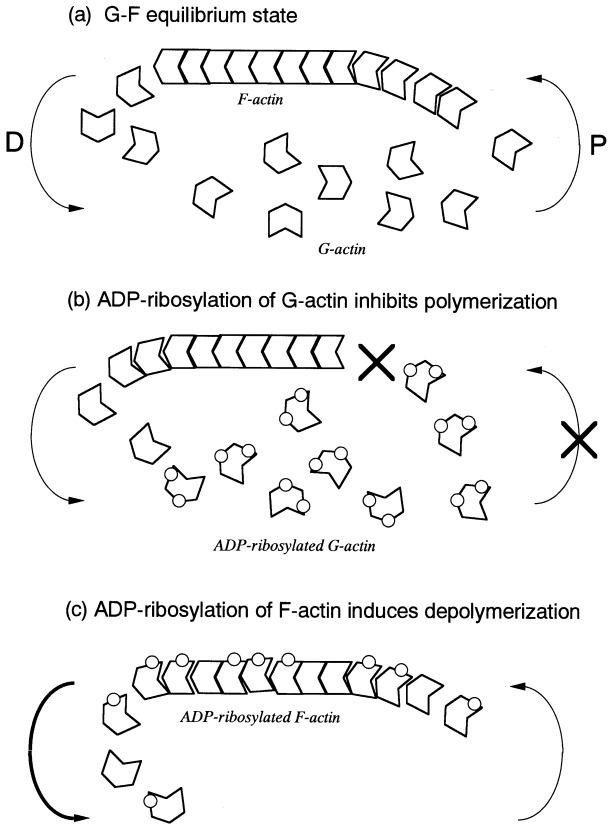

Fig. 3. Schematic representation for the possible effects of ADP-ribosylation of actins on the polymerization-depolymerization state. In the steady state, G- and F-actins are in dynamic equilibrium (a). When the ADP-ribosylation occurs on G-actin, modified G-actins are no longer integrated into the filamentous form (b). When the ADP-ribosylation occurs on F-actin, its ordered conformation are disrupted and F-actins are gradually depolymerized to the monomeric form (c). Actin is illustrated as a monomeric or filamentous form. ADP-ribose moieties are depicted open circles. Abbreviations of P and D mean polymerization and depolymerization, respectively.

ACKNOWLEDGMENTS

We thank H. Osago for the technical assistance. This work was supported by a Grant-in-Aid for Scientific Research 05858090, Ministry of Education, Science, Sports, and Culture, Japan.

REFERENCES

1) Zolkiewska A, Okazaki IJ and Moss J (1994) Vertebrate mono-ADP-ribosyltransferases. *Mol Cell Biochem* 138:107-112.

2) Mishima K, Terashima M, Obara S, Yamada K, Imai K and Shimoyama M (1991) Arginine-specific ADP-ribosyltransferase and its acceptor protein p33 in chicken polymorphonuclear cells: co-localization in the cell granules, partial characterization, and *in situ* mono (ADP-ribosyl) ation. *J Biochem* 110:388-394.

3) Terashima M, Mishima K, Yamada K, Tsuchiya M, Wakutani T and Shimoyama M (1992) ADP-ribosylation of actins by arginine-specific ADP-ribosyltransferase from chicken heterophils. *Eur J Biochem* 204:305-311.

4) Terashima M, Yamamori C and Shimoyama M (1995) ADP- ribosylation of Arg28 and Arg206 on the actin molecule by chicken arginine-specific ADP-ribosyltransferase. *Eur J Biochem* 231: 242-249.

5) Terashima M, Shimoyama M and Tsuchiya M (1999) Introduction of NAD decreases fMLP-induced actin polymerization in chicken polymorphonuclear leukocytes - The role of intracellular ADP-ribosylation of actin for cytoskeletal organization. *Biochem Mol Biol Int* 47:615-620.

6) Weeds A (1982) Actin-binding proteins - regulators of cell architecture and motility. *Nature* 296:811-815.

7) Korn ED, Carlier M-F and Pantaloni D (1987) Actin polymerization and ATP hydrolysis. *Science* 238:638-644.

8) Spudich JA and Watt S (1971) The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J Biol Chem* 246:4866-4871.

9) Cooper JA, and Pollard TD (1982) Methods to measure actin polymerization. *Methods in Enzymol* 85:182-210.
10) Sheterline P and Sparrow JC (1994) Actin. *Protein Profile* 1:1-121.
11) Kabsch W, Mannherz HG, Suck D, Pai EF and Holmes KC (1990) Atomic structure of the actin: DNase I complex. *Nature* 347:37-44.
12) Aktories K (1994) Clostridial ADP-ribosylating toxins: effects on ATP and GTP-binding proteins. *Mol Cell Biochem* 138:167-176.
13) Holmes KC, Popp D, Gebhard W and Kabsch W (1990) Atomic model of the actin filament. *Nature* 347:44-49.
14) Furuhashi K, Hatano S, Ando S, Nishizawa K and Inagaki M (1992) Phosphorylation by actin kinase of the pointed end domain on the actin molecule. *J Biol Chem* 267:9326-9330.
15) Terashima M, Yamamori C, Tsuchiya M and Shimoyama M (1999) ADP-ribosylation of tubulin by chicken NAD-arginine ADP-ribosyltransferase suppresses microtubule formation. *J Nutr Sci Vitaminol* 45:393-400.