Microscopic studies on severing properties of actin-binding protein: its potential use in therapeutic treatment of actin-rich inclusions

Han-ul Kim1, Anahita Vispi Bharda1, Jeong Chan Moon2, Dooil Jeoung1, Jeong Min Chung3* and Hyun Suk Jung1*

Abstract

Actin is an important unit of the cytoskeletal system, involved in many cellular processes including cell motility, signaling, and intracellular trafficking. Various studies have been undertaken to understand the regulatory mechanisms pertaining actin functions, especially the ones controlled by actin-binding proteins. However, not much has been explored about the molecular aspects of these proteins implicated in various diseases. In this study, we aimed to demonstrate the molecular properties of gelsolin, an actin-severing protein on the disassembly of the aggregation of actin-rich intracellular inclusions, Hirano body. We observed a decreasing tendency of actin aggregation by co-sedimentation assay and transmission electron microscopy in the presence of gelsolin. Therefore, we provide suggestive evidence for the use of actin-severing protein in novel therapeutic strategies for neurodegenerative conditions.

Keywords: Transmission electron microscopy, Actin, Gelsolin, Actin-binding protein, Actin-severing protein, Hirano body

Introduction

Actin is a fundamental cytoskeletal protein abundantly found in nearly all eukaryotic cells. It is highly conserved protein mediating many processes such as cell growth, motility, structural organization, as well as intracellular trafficking (Griffin et al. 2014). Being a critical cytoskeletal factor, tight regulation of the rapidly occurring assembly and disassembly of actin filaments is required for proper cellular operation (Remedios et al. 2003). Throughout the cell, actin is found in two standard forms: globular (G-actin) and filamentous (F-actin). The distribution and organization of these filaments into functionally relevant unit is regulated by several actin-binding proteins (ABPs) that modulate the underlying polymerization and depolymerization activities (Griffin et al. 2014; Remedios et al. 2003). These actin-binding proteins actively control the overall actin dynamics to perform different functions by modifying its structure both in vitro and in vivo (Fechheimer and Zigmond 1993; Pollard 2016). One of the representative classes of an actin-binding protein includes the gelsolin family, composed of six similar domains namely G1–G6 each having a Ca2+ binding site (Kinosian et al. 1998). This actin depolymerizing factor is found to bind tightly to the growing side of the actin filament thus decreasing its size and increasing the amount of monomeric actin present in the actin pool (Tellam 1985). Functionally, gelsolin causes rapid disassembly of actin filaments through severing, a mechanism involving breaking of the inner non-covalent bonds to produce shortened, broken filaments (Sun et al. 1999). Besides, the regulation of gelsolin and its counterpart is mainly affected by pH, phosphoinositides, lysophosphatidic acid and...
high micromolar concentrations of \( \text{Ca}^{2+} \) under physiological conditions (Sun et al. 1999; Spinardi and Witke 2007). In certain kinds of eukaryotic cells, gelsolin has been implicated to participate in numerous cytoskeletal events such as cytokinesis, cell movement and apoptosis besides its generic role as an actin-binding protein (Lappalainen 2016). Recent studies have shown its critical role in several conditions including amyloidosis, inflammation, cardiovascular diseases and cancer (Spinardi and Witke 2007). Another class of actin-binding protein, ABP34 is a small, 34 kDa crosslinking protein organizing F-actin into condensed bundles in a calcium dependent manner (Lim et al. 1999). Several in vivo studies have reported a part of this protein involved in the formation of Hirano bodies (Maselli et al. 2003). Hirano bodies are large, eosinophilic, actin-rich intracytoplasmic structures that contain paracrystalline arrays of F-actin (Goldman 1983) and ABPs (Galloway et al. 1987). Such cytoplasmic actin-rich aggregations comprising actin and ABPs are associated with various neurodegenerative diseases and can assemble as either actin/cofilin rods or Hirano bodies (Bamburg and Bloom 2009; Hirano 1994). Hirano bodies have been found to be associated with various neurodegenerative diseases such as Alzheimer’s disease (Gibson and Tomlinson 1977; Mitake et al. 1997; Schmidt et al. 1989), Pick’s disease (Schochet et al. 1968; New castle and Ball 1968), Guam amyotrophic lateral sclerosis and parkinsonism-dementia complex (Hirano et al. 1968), chronic alcoholism (Laas and Hagel 1994) as well as general aging (Hirano 1994). Despite a large body of literature describing the structure, incidence, and antigens associated with Hirano bodies, the mechanism of formation remains largely unexplored. Here we demonstrated the potential application of actin-severing protein, gelsolin on actin-rich intracellular inclusions Hirano body by co-sedimentation assay and TEM. In this study, we used gelsolin to compete with enormous bundled actin filaments for resolving the phenomenon of actin aggregation.

**Materials and methods**

**Protein sample preparation**

ABP34 protein was purified from *E. coli* BL21 (DE3) cells (Novagen, USA) (Chung et al. 2017), dialyzed against buffer containing 50 mM sodium acetate, 2 mM MgCl\(_2\), 1 mM EGTA, 20 mM MOPS, pH 7.0 and stored at -80 °C. Purification of gelsolin protein from bovine serum (Pel-Freez Biologicals, USA) was done as per (Kurokawa et al. 1990) which was subjected to dialysis in rigor solution (relaxing solution without mgATP), pH 6.5 and stored at -80 °C. Actin from rabbit skeletal muscle was a generous gift from M. Ikebe laboratory and stored at -80 °C.

**Acto-myosin preparation from scallop tissue**

The adductor muscle was dissected and cleaned from live scallops which were collected from a local market and placed in rigor solution (100 mM NaCl, 5 mM MgCl\(_2\), 5 mM PIPES, 5 mM Na\(_2\)HPO\(_4\), 1 mM EGTA, 1 mM Na\(_2\)PO\(_4\), pH 7.0) on ice. Muscles were then teased into thin strips and permeabilized with 0.5% saponin rigor solution for 4 h at 4°C with gentle agitation followed by
thorough rinsing in rigor solution (Jung and Craig 2008). The weighed muscle tissue (approx. 0.4 g) was washed thrice using wash buffer (40 mM NaCl, 5 mM phosphate buffer). It was efficiently chopped and homogenized using HG-15D homogenizer (Daihan Scientific, R.O.K) at 15,000 rpm for 1 min on ice. The resulting suspension was re-homogenized with a glass homogenizer (Wheaton, U.S) under “low ionic buffer” condition (20 mM PIPES, 50 mM KCl, 5 mM EGTA, 1 mM MgCl2, pH 7.0) on ice with 3–5 full passes (Jung et al. 2008a). The preparation was immediately centrifuged using Micro 17TR (Hanil Science, R.O.K) at 15,000 rpm for 10 min. The resulting pellet containing acto-myosin was resuspended up to 4 ml with relaxing solution, pH 6.5 and equally distributed into 4 tubes.

Gelsolin treatment in scallop muscle
In the first place, 1 mM mgATP was added to each tube containing the resuspended acto-myosin suspension. Next, each tube was treated for 30 min with different conditions involving 5.1 mM CaCl2 and 3 μM gelsolin either alone or in combination, except for the control. This was then centrifuged to confirm the migration of actin band by SDS-PAGE. The gels were stained with Coomassie Brilliant Blue R-250 (Bio-Rad) and analyzed using ImageJ software (Schneider et al. 2012) whereas the statistical procedures were performed using SigmaPlot (Systat Software, San Jose, CA, USA).

Actin co-sedimentation assay
Co-sedimentation assay was conducted to understand the binding activity of actin with ABP34 and gelsolin. Actin was first polymerized under low ionic buffer (20 mM PIPES, 50 mM KCl, 5 mM EGTA, 1 mM MgCl2, 1 mM mgATP, pH 7.0) for 1 h at room temperature forming F-actin. The F-actin was then mixed in equal proportion with ABP34 protein (3 μM) for 30 min at RT to form the actin bundles (Kim et al. 2015). Gelsolin (3 μM) was mixed with the former F-actin/ABP34 mixture for 30 min at RT followed by centrifugation in Micro 17TR
Kim et al. J Anal Sci Technol (2021) 12:49

The obtained supernatant and pellet fractions were collected and analyzed by gel electrophoresis. Low ionic buffer conditions were maintained throughout the experiment.

**Transmission electron microscopy**

An aliquot of 5 μl of the sample under investigation was applied to a carbon-coated grid formerly glow-discharged (Harrick plasma, USA) for 3 min in air. The grid was negatively stained with 1% uranyl acetate (Jung et al. 2008b). The prepared grids were examined by Technai 10 TEM system (FEI, USA) operating at 100 kV. Images were acquired with UltraScan 1000 CCD camera (Gatan, USA). Instrumentation was used in Kangwon Center for Systems Imaging, Chuncheon, Republic of Korea.

**Results and discussion**

**Activity of actin-binding proteins in the presence of calcium**

Negatively stained electron micrographs were analyzed to understand the activity of actin in the presence of Ca\(^{2+}\) with or without ABPs such as ABP34 and Gelsolin. In the presence of Ca\(^{2+}\), G-actins were polymerized to from a filamentous actin called F-actin (Fig. 1a, b). However, when the F-actins were treated with gelsolin, they were depolymerized causing the formation of relatively shorter and fewer F-actin molecules (Fig. 1c, d). Compared to that, ABP34 effectively caused the formation of F-actin bundles in the presence of Ca\(^{2+}\) (Fig. 1e, f). These implies that in the presence of Ca\(^{2+}\), both gelsolin and ABP34 effectively execute their functions over F-actin such severing and bundling activities, respectively.

**Poor tendency of actin aggregation in the presence of gelsolin**

To understand the effect of gelsolin on the actin-bundling activity of ABP34, co-sedimentation assay was conducted (Fig. 2). When actin was incubated with gelsolin, it was found in the supernatant, while the band were predominantly seen in the pallet when it was treated with ABP34 (Fig. 2a). And also, the actin band significantly shifted to the supernatant when the two APBs were mixed together along with actin (Molar ratio 1:1:1), indicating that the severing activity of gelsolin somehow affect the actin-bundling function of ABP34. To evaluate the mechanism of the inhibition effect of gelsolin on the actin-bundling activity of ABP34, actin was incubated with both APBs in different orders. In the experiment, the inhibition effect of gelsolin was more effective when it was incubated with actin before adding ABP34, suggesting that both ABP34 and gelsolin share same binding sties on F-actin (Fig. 2b, d, e). Notably, the actin bundles formed by ABP34 was eliminated by the doubled concentration of gelsolin, indicating that gelsolin has stronger binding affinity then ABP34 (Fig. 2c, f).

**Gelsolin activity on actin aggregation in muscle**

To understand the effect of gelsolin on the actin aggregation in the physiological environment, co-sedimentation assay was demonstrated using scallop muscle tissue. Based on the analysis of migration pattern of actin band, addition of Gelsolin in the presence of Ca\(^{2+}\) showed actin-band shift from pallet to supernatant significantly,

(Hanil, R.O.K) at 15,000 RPM for 10 min (Kinosian et al. 1998).
and this indicates that gelsolin enables its inhibition effect on the actin bundling formation in the physiological conditions (Fig. 3a, b).

In this study, a decreasing tendency of aggregated actin by gelsolin molecules was determined through co-sedimentation assay and EM analysis. Taken together, we proposed that addition of gelsolin can eliminate aggregated form of actin, and this can be used to purify actin more efficiently from muscle tissue (Fig. 4). Furthermore, this finding can also provide fundamental clue on further studies devising a novel therapeutic approach to treat various actin aggregation related diseases (Carnell and Insall 2011; Gourlay and Ayscough 2005). A previous study demonstrating the role of actin-severing proteins in a variety of neurodegenerative conditions showed increased vulnerability to excitotoxicity when gelsolin deficiency was present in hippocampal neurons, both in cell culture and in vivo (Furukawa et al. 1997). Moreover, a study investigating the effect of antibiotics on the actin aggregates concluded that the actin aggregates are significant therapeutic target site not only for the treatment of various neurovegetative diseases, but also neurodevelopmental disorders (Pathak et al. 2016).

Although, this is in very early stage of studying new therapeutic strategies for neurogenerative conditions associated with actin aggregation, it provided a fundamental knowledge of how actin-severing proteins could be used to solve problems involved in the neurovegetative diseases (Furukawa et al. 1997; Pathak et al. 2016; Moseley et al. 2006).

Acknowledgements
Authors gratefully acknowledge Prof. M Ikebe for his gift of actin from rabbit skeletal muscle.

Authors’ contributions
Conceptualization was done by HSJ. Data curation was done by KHU and JHS. Formal analysis was performed by H-UK and JHS. Methodology was done by H-UK, JMC, AVB, JCM, and HSJ. Software was provided by H-UK. Validation was carried out by H-UK, JMC, and HSJ. H-UK carried out the investigation. Writing—original draft was done by H-UK, JMC, and HSJ. Writing—review and editing was done by H-UK, AVB, DI, JMC, and HSJ. All authors read and approved the final manuscript.

Funding
This research was supported by a Grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (Grant Number: HI20C0344 to H-UK Kim), 2018 Research Grant (PoINT) from Kangwon National University, the research Grant of Kangwon National University in 2021, the National Research Foundation of Korea(NRF) Grant funded by the Korea government(MSIT) (2021R1A2C1009404 to HS Jung), (2017M3A9G7072417 to D Jeoung, 2021R1A2C1009404 to HS Jung).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
Competing interests
The authors declare that they have no competing interests.

Author details
1 Division of Chemistry, Department of Biochemistry, College of Natural Sciences, Kangwon National University, Chuncheon, Gangwon 24341, Republic of Korea. 2 Division of Restoration Research, Research Center for Endangered Spices, National Institute of Ecology, Yeongyang 36531, Republic of Korea. 3 Department of Biotechnology, The Catholic University of Korea, Bucheon-si, Gyeonggi 14662, Republic of Korea.

Received: 28 July 2021 Accepted: 14 October 2021 Published online: 28 October 2021

Fig. 4 A schematic outline of actin-bundling and severing activity from this study. This schematic model elucidates a consolidated approach and correlation of the three key proteins involved in this study shown as actin-bundling (F-actin-ABP34) to actin-severing (F-actin-ABP34-Gelsolin) activities: t, time; Green circle, actin; yellow circle, ABP34; red circle, gelsolin.
Kim MK, Kim JH, Kim JS, Kang SO. Structure of the 34 kDa F-actin-bundling protein ABP34 from Dictyostelium discoideum. Acta Crystallogr D Biol Crystallogr. 2015;71(Pt 9):1833–49.

Kinosian BJ, Newman J, Lincoln B, Selden LA, Gershman LC, Estes JE. Ca2+-regulation of gelsolin activity: binding and severing of F-actin. Biophys J. 1998;76(6):3101–9.

Kurokawa H, Fujii W, Ohki K, Sakurai T, Nonomura Y. Simple and rapid purification of brevin. Biochem Biophys Res Commun. 1990;168(2):451–7.

Laas R, Hagel C. Hirano bodies and chronic alcoholism. Neuropathol Appl Neurobiol. 1994;20(12):1–21.

Lappalainen P. Actin-binding proteins: the long road to understanding the dynamic landscape of cellular actin networks. Mol Biol Cell. 2016;27(16):2519–22.

Lim RW, Furukawa R, Eagle S, Cartwright RC, Fechheimer M. Three distinct F-actin-binding sites in the Dictyostelium discoideum 34,000 dalton actin bundling protein. Biochemistry. 1999;38(2):800–12.

Maselli A, Furukawa R, Thomson SA, Davis RC, Fechheimer M. Formation of Hirano bodies induced by expression of an actin cross-linking protein with a gain-of-function mutation. Eur J Cell Biol. 2003;84(4):778–87.

Mitake S, Ojika K, Hirano A. Hirano bodies and Alzheimer's disease. Kaohsiung J Med Sci. 1997;13(1):10–8.

Moseley JB, Okada K, Balcer HJ, Kovar DR, Pollard TD, Goode BL. Twinfilin is an actin filament-severing protein and promotes rapid turnover of actin structures in vivo. J Cell Sci. 2006;119(Pt 8):1547–57.

Pathak S, Parker H, Tripathi S, Kale A. Ofloxacin as a disruptor of actin aggregates 'hirano bodies': a potential repurposed drug for the treatment of neurodegenerative diseases. Front Aging Neurosci. 2020;12:59179.

Pollard TD. Actin and actin-binding proteins. Cold Spring Harb Perspect Biol. 2016;8(8):a018226.

Newcastle NB, Ball MJ. Electron microscopic structure of the “inclusion bodies” in Pick's disease. Neurology. 1968;18(12):1205–13.

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9(7):671–5.

Schochet SS Jr, Lampert PW, Lindenberg R. Fine structure of the Pick and Hirano bodies in a case of Pick's disease. Acta Neuropathol. 1968;27(2):167–82.

Jung HS, Craig R. Ca2+-induced tropomyosin movement in scollop striated muscle thin filaments. J Mol Biol. 2008;383(3):512–9.

Jung HS, Komatsu S, Ikebe M, Craig R. Head-head and head-tail interaction: a general mechanism for switching off myosin II activity in cells. Mol Biol Cell. 2008a;19(8):3234–42.

Jung HS, Burgess SA, Billington N, Colegrave M, Patel H, Chalovich JM, et al. Conservation of the regulated structure of folded myosin 2 in species separated by at least 600 million years of independent evolution. Proc Natl Acad Sci USA. 2008b;105(16):6022–6.

Kim MK, Kim JH, Kim JS, Kang SO. Structure of the 34 kDa F-actin-bundling protein ABP34 from Dictyostelium discoideum. Acta Crystallogr D Biol Crystallogr. 2015;71(Pt 9):1833–49.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.