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Non diaphanous formin delphilin acts as a barbed end capping protein

Priyanka Dutta[a,b], Swagata Das[a], Sankar Maiti[a,*]

[a] Department of Biological Sciences, Indian Institute of Science Education and Research, Kolkata, India
[b] Department of Biology, Indian Institute of Science Education and Research, Pune, India

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ABSTRACT

Formins are multi domain proteins present ubiquitously in all eukaryotes from lower fungi to higher vertebrates. Formins are characterized by the presence of formin homology domain-2 (FH2) and formin homology domain-1 (FH1). There are fifteen different formins present in mouse and human. Among these metazoan formins, Delphilin is a unique formin having two PDZ domains at the N-terminus and FH1, FH2 domain at the C-terminus respectively. In this study we observed that Delphilin binds to actin filaments, and Delphilin inhibits actin filament elongation like barbed end capping protein CapZ. *In vitro*, Delphilin stabilized actin filaments by inhibiting actin filament depolymerisation. Therefore, our study demonstrates Delphilin as an actin-filament capping protein.

1. Introduction

Regulating actin dynamics is essential for any organism's survival, a key player in this regulation are formin family of proteins. Formins are multi domain proteins, ubiquitously expressed in eukaryotes. They are actin nucleators, elongation factors, bundlers; characterized by formin homology-2 (FH2) and formin homology-1 (FH1) domain respectively [1]. FH2 domain is dimeric in nature, nucleate actin monomer and have donut-shaped structure linked by flexible linker [2,3]. Compared to other nucleators, they produce unbranched linear actin filaments [4].

In lower eukaryotes there is a small number of formins, like budding yeast has two formin, Bni1 and Bnr1 involved in various functions like actin cable generation and cytokinetic ring formation [5,6]. In fission yeast there are three formins For3, cdc12, Fus1; required for cell polarity, cytokinetic ring and fusion of cells respectively [7,8]. In higher eukaryotes, formins are present in large numbers, such as *Arabidopsis* have 21 formins [9]. Mammals have 15 different formins [1]; research has shown that multiple formins are expressed in a single cell at a time [10,11]. Other than actin nucleation, the specific function of each and every single formin inside the cell is yet to be discovered.

We are interested in the least biochemically characterized formin, Delphilin. Initially Delphilin was discovered as Glutamate δ2 receptor interacting protein [12,13]. So it was named "Del" from delta and "philin" due its affinity for Glutamate delta2 receptor [10]. Delphilin interacts with Glutamate δ2 receptor through PDZ domain present at its N-terminus [13]. The importance of this interaction is yet to be elucidated. Delphilin is reported to be selectively expressed in post synaptic nerve terminals of Purkinje cells (PC) [12]. Delphilin mutation causes induction of long term depression (LTD), though there are no changes in the morphology of PC but the localization of glutamate δ2 receptor is affected [14].

Role of Delphilin in neuronal plasticity and Delphilin mediated actin cytoskeleton dynamics is poorly understood. Our study attempts to take first steps in filling this void. We have performed the biochemical characterization of FH2 domain of Delphilin (Del-FH2). Results showed that Delphilin act as a barbed end capping protein and stabilize actin filaments *in vitro*.

2. Results

2.1. FH2 domain of Delphilin binds F-actin and assembles actin at high concentration:

The Del-FH2 or the FH1 and FH2 domains of Delphilin together (Del-FH1FH2) (Fig. 1A) were purified as N-terminal 6X His tagged protein (Fig. 1B and S1A). Size exclusion chromatography experiments were done using Superose 6 10/300 GL column (GE Healthcare); the molecular weight of the gel filtrated Del-FH2 was determined by the volume at which the elution peak appeared and it validate that Del-FH2 is eluted as a dimer (Fig S1B) [15]. FH2 domain of other formins also get eluted in dimeric form upon gel filtration [16]. To understand the actin binding ability of Del-FH2 we carried out F-actin co-sedimentation assay with purified Del-FH2 and Del-FH1FH2. Our results showed that both Del-FH2 and Del-FH1FH2 fragment co-precipitated in pellet

* Corresponding author.
E-mail address: spm@iiserkol.ac.in (S. Maiti).

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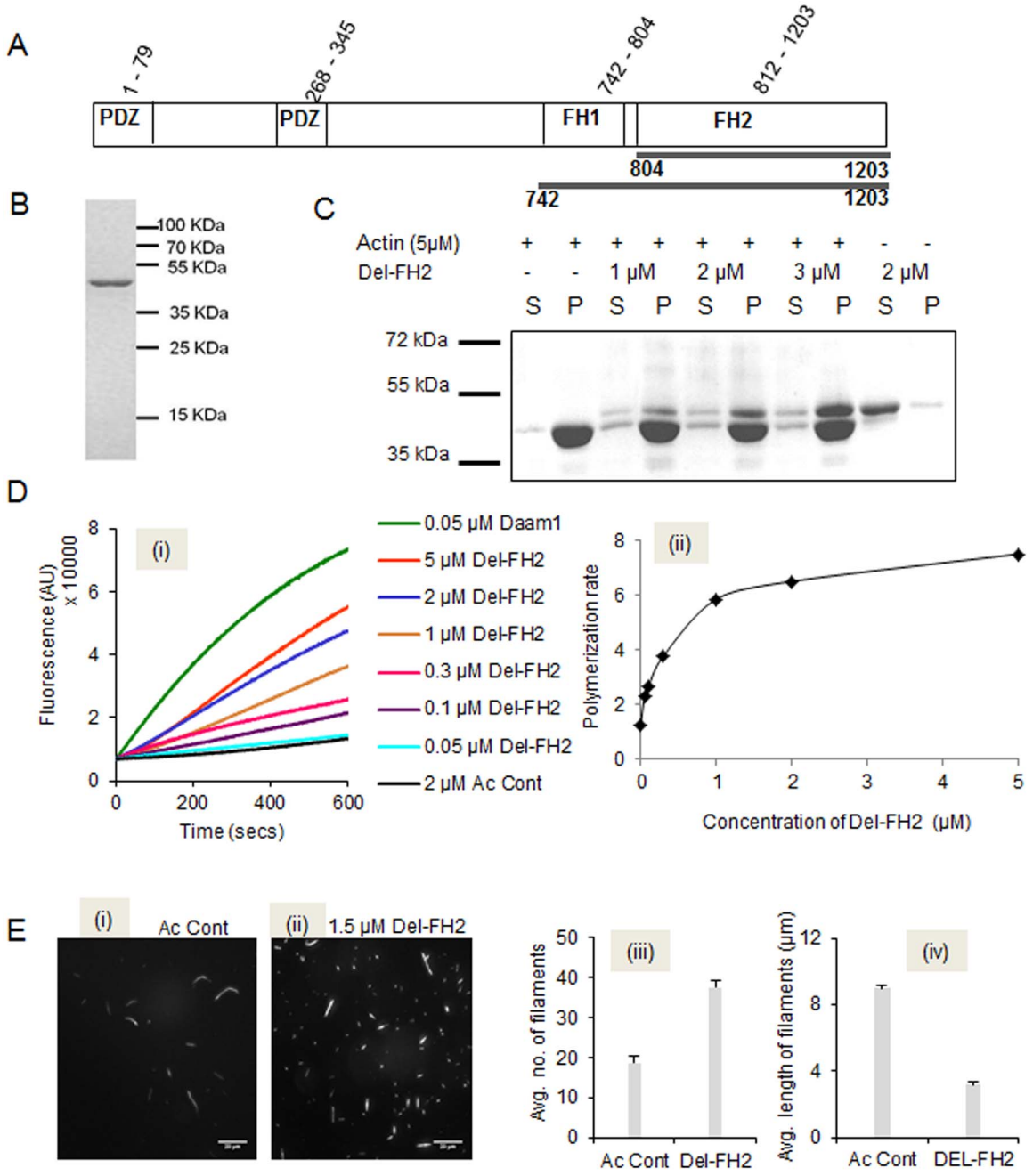

Fig. 1. FH2 domain of Delphilin binds to F-actin and assemble actin at slow rate: (A) Schematic illustration of FH1FH2 and FH2 domain of Delphilin. (B) Coomassie stained 12% SDS-PAGE of purified Del-FH2. (C) F-actin co-sedimentation assay of Del-FH2. S and P indicate supernatant and pellet fractions respectively. The actin concentration was 5 μM with varying concentration (1 μM, 2 μM and 3 μM) of Del-FH2. (D) (i) Pyrene-actin spontaneous assembly assay; time course polymerization of the indicated concentration of Del-FH2 (0.05 μM, 0.1 μM, 0.3 μM, 1 μM, 2 μM and 5 μM) and Daam1 (positive control 0.05 μM). For *in vitro* kinetics 2 μM actin (10% pyrene labelled) and indicated (μM) concentration of corresponding protein was used. (ii) Plot of actin assembly rate on the concentration of Del-FH2 when half of the actin monomers were polymerized. (E) Fluorescence microscope images of rhodamine phalloidin stained actin filaments in absence (i) and presence (ii) of 1.5 μM Del-FH2. 0.65 μM rhodamine phalloidin stained actin filaments were observed under microscope. Scale bar 20 μm. Magnification is 60×. (iii) and (iv) Statistical representation of the number of filaments and the length of the filament (μm) respectively. Error bars represent standard error from 200 filaments for each bar graph.

fraction along with F-actin in a concentration dependent manner. Results indicated that Del-FH2 and Del-FH1FH2 was able to bind F-actin as they remained in the supernatant fraction of the protein control reaction (Fig. 1C and S1C). Binding to actin filaments confirmed that Del-FH2 behaved similar to FH2 domain of other formins with respect to F-actin binding [17]. Del-FH2 did not have any actin bundling property (Fig S1D). To determine the binding affinity of Del-FH2 with actin, we had performed the co-sedimentation assay of Del-FH2 with varying concentration of actin (Fig S1E). The binding affinity had been checked using the co-sedimented Del-FH2 with actin. The fraction bound Del-FH2 was plotted against varying concentration of actin and the slope of the linear fitting curve was taken as dissociation constant (Fig S1F). The dissociation constant was found to be 0.2 μM which indicated the strong affinity of Del-FH2 for actin.

FH2 domain of formins generally shows *in vitro* actin nucleation activity except Fhod1 [18]. For3 of *Schizosaccharomyces pombe* does not show *in vitro* actin nucleation at low concentration [7]. *In vitro* actin assembly assay of purified Del-FH1FH2 and Del-FH2 with 2 μM actin monomers (10% pyrene labelled) showed that it was able to assemble actin when present in high micro molar concentrations