Biochemical and Biophysical Research Communications 452 (2014) 967–973

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

RhoGDI facilitates geranylgeranyltransferase-I-mediated RhoA prenylation

Zakir Tnimov, Daniel Abankwa [1], Kirill Alexandrov *

Department of Molecular Cell Biology, Institute for Molecular Bioscience, The University of Queensland, 306 Carmody Road, St. Lucia, QLD 4072, Australia

ARTICLE INFO

Article history:
Received 27 August 2014
Available online 16 September 2014

Keywords:
RhoGDI
Rho GTPases
GGTase-I
Protein prenylation

ABSTRACT

Protein prenylation is a post-translational modification where farnesyl or geranylgeranyl groups are enzymatically attached to a C-terminal cysteine residue. This modification is essential for the activity of small cellular GTPases, as it allows them to associate with intracellular membranes. Dissociated from membranes, prenylated proteins need to be transported through the aqueous cytoplasm by protein carriers that shield the hydrophobic anchor from the solvent. One such carrier is Rho GDP dissociation inhibitor (RhoGDI). Recently, it was shown that prenylated Rho proteins that are not associated with RhoGDI are subjected to proteolysis in the cell. We hypothesized that the role of RhoGDI might be not only to associate with prenylated proteins but also to regulate the prenylation process in the cell. This idea is supported by the fact that RhoGDI binds both unprenylated and prenylated Rho proteins with high affinity *in vitro*, and hence, these interactions may affect the kinetics of prenylation. We addressed this question experimentally and found that RhoGDI increased the catalytic efficiency of geranylgeranyl transferase-I in RhoA prenylation. Nevertheless, we did not observe formation of a ternary RhoGDI*RhoA*GGTase-I complex, indicating sequential operation of geranylgeranyltransferase-I and RhoGDI. Our results suggest that RhoGDI accelerates Rho prenylation by kinetically trapping the reaction product, thereby increasing the rate of product release.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Rho GTPases are post-translationally prenylated on their C-termini with either 20-carbon geranylgeranyl or, less commonly, 15-carbon farnesyl groups. This protein modification is catalyzed by the geranylgeranyltransferase type I (GGTase-I) and farnesyltransferase (FTase) enzymes, respectively [1]. Prenylation is critical for the functioning of Rho proteins, as it enables them to reversibly associate with intracellular membranes [2] and promote binding to their key regulators, such as GDP exchange factors (GEFs) [3] and Rho GTPase-activating proteins (GAPs) [3,4]. An additional level of Rho regulation is brought about by the Rho GDP dissociation inhibitors (RhoGDIs) [5]. Three human RhoGDIs have been identified with the first isoform, RhoGDI-1 (here, RhoGDI), being most ubiquitously expressed and associating with most of the Rho proteins. RhoGDI forms tight complexes with prenylated Rho GTPases, thereby withdrawing them from the membrane and association with GEFs [6,7], GAPs [8], and downstream effectors.

Recently, it has become evident that the function of RhoGDI goes beyond its originally postulated role. It has been shown that the absence of RhoGDI leads to depletion of the cytosolic fraction of prenylated Rho proteins, where the highly hydrophobic isoprenoid moiety functions as a signal for degradation by intracellular proteases [9]. As a result, competition between prenylated Rho proteins for limiting amounts of RhoGDI [10] results in RhoGDI-mediated regulation of their intracellular levels [9,11]. Thus, it may be a physiological requirement for the prenylated Rho proteins to be in complex with RhoGDI after dissociation from the membranes or following their prenylation. The latter assumption is supported by the fact that impairment of the Rho:RhoGDI interaction leads to concentrations of RhoA, Cdc42, and Rac1 in the putative protein prenylation sites: the endoplasmic reticulum [9] and Golgi apparatus [12].

Further, RhoGDI's ability to bind both unprenylated and prenylated forms of Rho with physiologically relevant affinities [5,13] suggests that it may be involved in Rho pre- and postprenylation processing. This may be similar to the function of Rab escort protein (REP), which binds newly synthesized Rab proteins and presents them to the RabGGTase [14,15]. This function, however, cannot be attributed to RhoGDI, as GGTase-I does not require an accessory protein for its activity. Yet, RhoGDI may accelerate

* Corresponding author.
E-mail address: k.alexandrov@uq.edu.au (K. Alexandrov).
[1] Current address: Turku Centre for Biotechnology, Åbo Akademi University, Tykistökatu 6B, 20520 Turku, Finland.

http://dx.doi.org/10.1016/j.bbrc.2014.09.024
0006-291X/© 2014 Elsevier Inc. All rights reserved.

prenylation by kinetically trapping the product of the reaction and delivering it to the membrane compartment, similar to REP.

In this study, we assessed a possible role of RhoGDI in the prenylation reaction. We found that RhoGDI accelerates GGTase-I-mediated RhoA prenylation *in vitro* with a fluorescent analog of farnesyl pyrophosphate or with geranylgeranyl pyrophosphate by increasing the enzyme's processivity. This was not accompanied by formation of a RhoGDI∗RhoA∗GGTase-I ternary complex, indicating sequential operation of the proteins. Together with previous findings, these results strongly suggest that RhoGDI may be an essential component of the Rho prenylation machinery *in vivo*.

2. Materials and methods

2.1. DNA cloning

The C-terminal eGFP-RhoGDI and N-terminal mCherry fusions of RhoB and Cdc42 were cloned using the in-fusion protocol into the pOPINE vector [16] at the Queensland University Protein Expression Facility. The constructs for expression of RhoA, RhoGDI, and GGTase-I are reported elsewhere [5].

2.2. Protein expression and purification

GGTase-I, RhoA, and RhoGDI were expressed and purified as described previously [5].

2.3. Preparative enzymatic prenylation of RhoA

Purified RhoA was prenylated *in vitro* by GGTase-I using geranylgeranyl (Sigma) or NBD-geranyl pyrophosphate as described earlier [5].

2.4. Preparation of RhoA-GG∗RhoGDI-TMR complex

RhoA was prenylated in the presence of RhoGDI labeled with TMR as reported earlier [5].

2.5. Labeling of RhoGDI with maleimide dye

Bovine wild-type RhoGDI was labeled with tetramethylrhodamine maleimide (TMR) (Life Technologies, USA) according to the manufacturer's protocol.

2.6. Fluorescence anisotropy measurements

The time course of RhoA prenylation reaction by GGTase-I was performed at 37 °C in 1-ml quartz cuvettes (Hellma, Germany) on a Spex Fluoromax-4 spectrofluorometer fitted with polarizers of L-geometry (Jobin Yvon Inc., USA). The experiments were carried out in FA buffer containing 25 mM Hepes–NaOH, pH 7.2, 40 mM NaCl, 2 mM $MgCl_2$, and 1 mM TCEP. The NBD fluorophore was excited at 479 nm, and emission was observed at 560 nm. Fluorescence anisotropy $\langle r\rangle$ was determined as:

$$\langle r\rangle = \frac{I_{VV} - G \times I_{VH}}{I_{VV} - 2 \times G \times I_{VH}}$$

where I is the fluorescence intensity and the first subscript letter indicates the direction of exciting light and the second shows the emitted light; the "G-factor" G was defined as:

$$G = \frac{I_{HV}}{I_{HH}}$$

Primary data analysis was performed with Grafit 5.0 (Erithacus software) and the "Fluorescence" implementation (Jobin Yvon Inc., USA) of Origin 7.0 (Originlab Corporation, USA).

2.7. Steady-state enzyme kinetics and data analysis

After setting up a preincubation reaction mixture containing 0.1 μM GGTase-I and 1 μM NBD-GPP with or without RhoGDI in FA buffer with 20 μM $ZnCl_2$ (FAZn) at 37 °C for 2 min and measuring its fluorescence anisotropy, the reaction was started by the addition of RhoA. Initial velocities were calculated from the linear phase of the reaction. Then, apparent initial rates and the Michaelis–Menten (K_m) constant were calculated as described previously with some changes [17]. In brief, the apparent initial rates were defined as:

$$v' = \frac{\Delta\langle r\rangle}{\Delta t} \times A_0$$

where $\Delta\langle r\rangle$ is fluorescence anisotropy change over time Δt and A_0 is the initial concentration of a substrate (RhoA). K_m was obtained from a fit to the Hill Eq. (1):

$$v' = \frac{V'_{max} \times A_0^h}{K_m^h + A_0^h} \quad (1)$$

where V'_{max} is the apparent maximal prenylation velocity and h is the Hill coefficient.

2.8. SDS–PAGE-based NBD-geranylation assay

The assay was carried out as described previously with minor modifications [18]. In brief, RhoA (4 μM) was incubated with GGTase-I (0.4 μM) at RT, and NBD-GPP (4 μM) was in FAZn buffer in a final volume of 10 μl. Then, the reaction was quenched by the addition of 2× Laemmli SDS–PAGE sample buffer and analyzed by electrophoresis. Subsequently, the gels were scanned on a Typhoon Trio fluorescent scanner (GE Healthcare; λ_{ex} = 488 nm; cutoff filter 520 nm), followed by staining with SimplyBlue (Invitrogen) and documentation with Odyssey (LI-COR Biosciences, USA).

2.9. HPLC-based geranylgeranylation assay

GGTase-I mediated RhoA geranylgeranylation was carried out in a 96-well 2-ml plate in a water bath. The reaction mixture, containing 0.25 μM GGTase-I and 1–16 μM RhoA with or without RhoGDI in FAZn buffer, was preincubated at 37 °C for 2 min before the addition of 2 mM GGPP solution (Sigma–Aldrich) to a final concentration 100 μM and mixed; 50-μl aliquots were withdrawn from the reaction and mixed with 50 μl of ice-cold stop solution (20% glycerol, 5 mM EDTA, 0.05% TFA) and flash-frozen. Samples were analyzed via HPLC (Prominence, Shimadzu) on a 150 × 2 mm C4 Jupiter column (300-Å pore size, 5-μm diameter beads) and a 40–61.5% acetonitrile gradient in 0.05% TFA. Obtained chromatograms were analyzed in Shimadzu LabSolution software and Grafit 5.0 (Erithacus software).

2.10. Microscale thermophoresis measurements

Thermophoresis was used to measure the affinity between GGTase-I and RhoGDI-TMR or between GGTase-I and RhoA-GG∗RhoGDI-TMR complex in a setup similar to that reported elsewhere [19]. Protein mixtures were loaded into standard treated glass capillaries. Measurements were carried out at room temperature in a Monolith NT.115 instrument (Nanotemper Ltd., Germany). The TMR-labeled RhoGDI was illuminated with a green LED (540–560 nm), and fluorescence was detected with a 585–615-nm cut-off filter. Data analysis was performed with NT Analysis 1.5.37 software (Nanotemper Ltd., Germany) as well as with Dynafit 4.0 (Biokin, USA) [20].

Download English Version:

https://daneshyari.com/en/article/10754354

Download Persian Version:

https://daneshyari.com/article/10754354

Daneshyari.com