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Self-association of poly(A)-specific ribonuclease (PARN) triggered by the R3H domain

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ABSTRACT

Poly(A)-specific ribonuclease (PARN) is a deadenylase with three RNA-binding domains (the nuclease, R3H and RRM domains) and a C-terminal domain. PARN participates in diverse physiological processes by regulating mRNA fates through deadenylation. PARN mainly exists as a dimer in dilute solutions. In this research, we found that PARN could self-associate into tetramer and high-order oligomers both in vitro and in living cells. Mutational and spectroscopic analysis indicated that PARN oligomerization was triggered by the R3H domain, which led to the solvent-exposed Trp219 fluorophore to become buried in a solvent-inaccessible microenvironment. The RRM and C-terminal domains also played a role in modulating the dissociation rate of the tetrameric PARN. Enzymatic analysis indicated that tetramerization did not affect the catalytic behavior of the full-length PARN and truncated enzymes containing the RRM domain, which might be caused by the high propensity of the dimeric proteins to self-associate into oligomers. Tetramerization significantly enhanced the catalytic activity and processivity of the truncated form with the removal of the RRM and C-terminal domains. The results herein suggested that self-association might be one of the regulation methods for PARN to achieve a highly regulated deadenylase activity. We propose that self-association may facilitate PARN to concentrate around the target mRNAs by restricted diffusion.

1. Introduction

In eukaryotic cells, mRNA turnover is a precisely controlled and highly regulated process. The information determining the fate of an mRNA is predominantly encoded in its gene sequence, which mainly locates at the untranslational region (UTR) at the 5′- and 3′-end of the transcript as well as the promoter region in DNA [1,2]. Most mature mRNAs contain a 7-methylguanylate cap structure at the 5′-end and a long poly(A) tail at the 3′ end. The length of the poly(A) tail has been found to be one of the dominant factors controlling mRNA lifecycle [3,4]. Particularly, the removal of the poly(A) tail, which is also called deadenylation, by deadenylases is the rate-limiting step in the degradation of most eukaryotic mRNAs. Deadenylases (EC 3.1.13.4) are 3′-exoribonucleases with a high substrate preference for poly(A). By regulating the poly(A) tail length and the fate of an mRNA thereby, deadenylases have been found to participate in diverse physiological processes such as early development, cell cycle control and stress response [5,6]. Most eukaryotes have a number of deadenylases with distinct domain organizations, catalytic properties and cellular locations, suggesting that deadenylation is a complex process with multiple regulation sites [6]. Therefore, the identification of novel regulation methods is one of the keys to understand the physiological roles of deadenylases.

Among various deadenylases, poly(A)-specific ribonuclease (PARN) has a unique property of cap-dependent catalysis [7], while the other deadenylases do not interact with the 5′-end of mRNAs directly [6]. PARN is also unique in its domain composition, which contains two additional RNA-binding domains (R3H and RRM) besides the nuclease domain and a C-terminal domain (CTD) with intrinsically disordered sequence features [8,9]. The nuclease domain of PARN belongs to the DEDD type 3′-exoribonuclease and catalyzes the hydrolysis of poly(A) using the two-metal-ion mechanism [10]. The RRM domain interacts with the cap and communicates with the nuclease domain [11–15], which thereby amplifies the processivity of PARN catalysis [16,17]. The nuclease domain and the RRM domain have a high preference to bind with poly(A) [9,13,18], while the R3H domain has no RNA sequence preference [19]. The physiological implication remains unclear for the non-poly(A) binding properties of the R3H domain.

Abbreviations: B-CK, brain-type creatine kinase; CD, circular dichroism; DSG, disuccinimidyl glutarate; IPTG, isopropyl-1-thio-β-D-galactopyranoside; PARN, poly(A)-specific ribonuclease; p74, the full-length PARN; p74dH, p74 with the removal of the R3H domain; p60, the N-terminal fragment of PARN (residues 1–520); p60dH, p60 with the removal of the R3H domain; p54, the N-terminal fragment of PARN (residues 1–470); p46, the N-terminal fragment of PARN (residues 1–446); SEC, size-exclusion chromatography; SDS, sodium dodecyl sulfate

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Nonetheless, the R3H domain is important to the structural integrity and stability of PARN, and the deletion of the R3H domain will dissociate the dimeric molecule into low-activity monomers [19,20].

By modulating gene expression via deadenylation, PARN has been found to participate in diverse physiological and pathological processes including early development in both animals and plants [21–24], carcinogenesis [25], response to various stresses such as osmotic stress [26], serum starvation [27], DNA damage [28,29] and virus infection [30]. To precisely control the fate of a certain mRNA, the activities of the deadenylases need to be highly regulated during the corresponding cellular events. The multi-domain nature of PARN confers it the structural basis of regulation at multiple sites by binding partners or post-translational modifications. For example, the cap-binding ability of the RRM domain can be competed by the other cap-binding proteins such as the nuclear cap binding complex [31]. The activity of PARN can be modulated by monovalent cations such as K^+ and spermidine [15,32,33] and some inhibitors (reviewed in [25]). RNA-binding proteins can recruit PARN or other deadenylases to a certain mRNA target and achieve highly specific deadenylation (reviewed in [6]). Moreover, the activity, mRNA-targeting ability and cellular location of PARN can also be regulated by chemical modifications such as phosphorylation [27,29] and proteolysis [34,35].

In dilute solutions, the purified PARN mainly existed as a homodimer associated via the nuclease domain [9]. However, it has also been noticed that oligomeric forms may also exist as revealed by the oligomeric bands in cross-linked proteins [34], the appearance of 20% oligomeric proteins after lengthy sedimentation equilibrium [9] and 25% level of polydispersity in dynamic light scattering experiments [36]. Considering that oligomerization is a general method to regulate protein functions [37], there is a possibility that PARN oligomerization plays a role in the regulation of its deadenylation action. In this research, we verified the existence of high-order oligomerization and purified the tetrameric PARN. Truncation analysis and spectroscopic experiments suggested that the R3H domain might involve in PARN oligomerization. The tetramers were well-structured and fully active for both the full-length and truncated PARNs. Furthermore, tetramerization significantly increased the catalytic efficiency and processivity in degrading long poly(A) substrate by a truncated form with the removal of the RRM and C-terminal domains. We proposed that the formation of high-order oligomers might facilitate the processive catalysis of PARN when bound to the target mRNAs.

2. Materials and methods

2.1. Materials

Tris were purchased from AMRESCO. Imidazole, sodium dodecyl sulfate (SDS) and polyadenylic acid potassium salts (catalog number: P9403-25MG) were obtained from Sigma-Aldrich, Inc. Dithiothreitol (DTT), kanamycin and isopropyl-1-thio-β-D-galactopyranoside (IPTG) were purchased from INACO. Disuccinimidyl glutarate (DSG) was purchased from Thermo Scientific. All other reagents were local products of analytical grade.

2.2. Protein expression, purification and sample preparation

The construction of expressing plasmids and the purification of the full length human PARN (p74) and the various truncated mutants p60 (residues 1–520 AA), p54 (residues 1–470 AA), p46 (residues 1–446 AA), p74dH (p74 with the removal of the R3H domain), p60dH (p60 with the removal of the R3H domain) and p46dH (p46 with the removal of the R3H domain) were the same as those described previously (Fig. 1) [19]. In brief, the genes were cloned to the vector pET-28a (Novagen) and verified by sequencing. The recombinant proteins were over expressed in *Escherichia coli* BL21 (DE3) or Rosetta (DE3) induced by 0.1 mM IPTG. The proteins in the soluble fractions were purified by Ni^{2+}-affinity chromatography followed by size-exclusion chromatography (SEC) using a Superdex 200 16/60 prep-grade column equipped on an ÄKTA purifier (GE Healthcare). To eliminate the dimeric components, the tetrameric proteins were separated by a two-step protocol. The first step was to collect the tetrameric peak separated by a high load Superdex 200 16/60 prep-grade column, followed by a second step of separation using a Superdex 200 10/300 GL column. The purity of the final products was above 98% as estimated by SDS-PAGE. The protein samples were prepared in buffer A containing 20 mM Tris–HCl (pH 8.0), 100 mM KCl, 0.5 mM DTT, 0.2 mM EDTA and 20% (*v*/*v*) glycerol. The protein concentration was determined according to the Bradford method using bovine serum albumin as a standard [38].

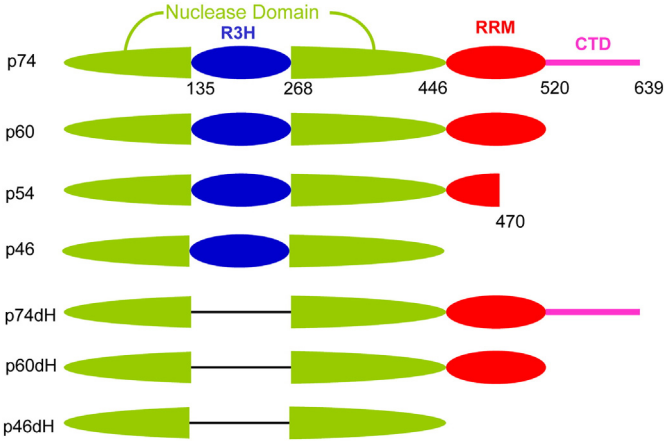

Fig. 1. Domain composition of PARN fragments used in this study. PARN contains three well-folded RNA binding domains (the nuclease, R3H and RRM domains) and a C-terminal extension (CTD). The figure is adapted and updated from [19].

2.3. SEC analysis

SEC analysis was carried out using a Superdex 200 10/300 GL column (GE Healthcare). The column was pre-equilibrated for at least two column volumes using buffer A with a flow rate of 0.4 ml/min. About 120 μl sample was loaded on the injection ring and 100 μl sample was injected into the flow system. The absorbances at 280 nm, 254 nm and 215 nm were monitored simultaneously. The SEC profiles were analyzed and fitted using the software Origin 8.0 (OriginLab Corp.)

2.4. Spectroscopy

Details regarding the spectroscopic experiments were the same as those described previously [19]. In brief, the far-UV circular dichroism (CD) spectra were recorded on a Jasco-715 spectrophotometer using a pathlength of 0.1 cm and a resolution of 0.2 nm. The intrinsic fluorescence was detected by a Hitachi F-2500 spectrophotometer using a 0.2 ml cuvette with a slit-width of 5 nm and an excitation wavelength of 280 nm or 295 nm. The intrinsic fluorescence excited at 280 nm is dominated by both of the Trp and Tyr fluorophores, while that excited at 295 nm is mainly contributed by the Trp fluorophores. All spectroscopic experiments were carried out at ambient temperature using a protein concentration of 0.2 mg/ml in buffer A. All spectroscopic samples were filtered and degassed before use.

2.5. Enzyme assay

The enzymatic activity was measured using the SEC assay as described previously [39]. In brief, the standard reaction buffer contained 20 mM Tris–HCl, pH 7.0, 100 mM KCl, 1.5 mg $MgCl_2$, 0.5 mM DTT, 0.2 mM EDTA and 10% (*v*/*v*) glycerol. The SEC experiments were performed using a Superdex 200 10/300 GL column. The column was pre-equilibrated for two column volumes with the reaction buffer