Expression and purification of intrinsically disordered C-terminal fragments of PARN in fusion with mCherry for FCS and SEC studies

Zuzanna Staszałek, Michał K. Białobrzewski, Anna Niedźwiecka*

Laboratory of Biological Physics, Institute of Physics, Polish Academy of Sciences, Al. Lotników 32/46, PL-02668 Warsaw, Poland

About PARN

Poly(A)-specific ribonuclease (PARN) is a 3'-exoribonuclease that is involved in the degradation of poly(A) tails of various RNAs. PARN takes part in the nonsense-mediated mRNA decay and silencing of mRNAs during the early embryonic development as well as in the maturation of many forms of small noncoding RNAs (miRNAs, snoRNAs, scaRNAs) and of the telomerase RNA component [2, 3]. PARN molecule interacts with both the 3'-terminal poly(A) tail and the 5'-terminal cap structure during processive deadenylation[1, 4]. The protein chain is composed of 639 amino acids (73.4 kDa) which form three globular domains: nuclease, R3H, RRM[1, 4], and a long intrinsically disordered C-terminus that contains a nuclear localization signal (NLS)[5]. PARN protein has been found both in the cytoplasm and the nucleus[1, 3]. In nature, PARN is a homodimer in which the monomers are linked *via* a disulfide bridge[4].

Understanding the role of PARN requires studies of its intrinsically disordered C-terminal region (PARN C IDR) encompassing the NLS, including its structural dynamics, as well as cellular migration and localization.

Protein expression

Sequences coding target proteins were ordered from BioCat GmbH (Heidelberg, Germany). Each of protein construct is carried by pET15b vector. Gene constructs contain three versions of PARN C-terminus: full length C-terminus of PARN with bipartite NLS sequence (500-639 aa of full length protein); truncated C-terminus of PARN with half of first part of NLS and whole second part of NLS sequence (540-639 aa of full length protein); truncated C-terminus of PARN without first part of NLS , so only with second NLS sequence (526-639 aa of full length protein) (Fig. 1). Additionally, constructs include His_6-tag, SUMO protein gene, mCherry fluorescent protein gene, extra HRV 3C digestion site and linker before mCherry gene. The proteins were overexpressed in *Escherichia coli* Rosetta2(DE3)pLysS after addition of 0,5 mM IPTG and overnight incubation in 15°C with constant mixing (Fig. 2).

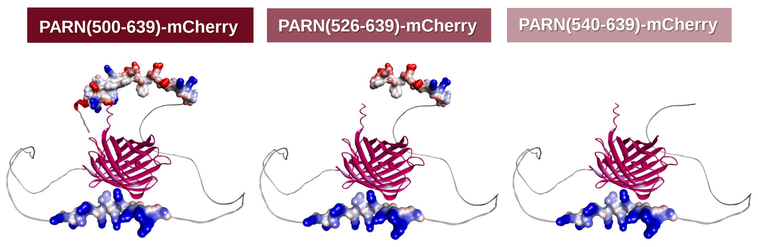

Fig. 1 Probable conformation of PARN(500-639)-mCherry, PARN(526-639)-mCherry and PARN(540-639)-mCherry generated by AlphaFold 2.0 with water accessible surface coloured according to interpolated charge for NLS sequence

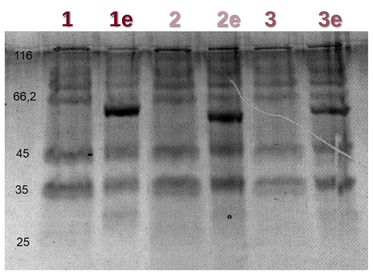

Fig. 2 SDS-PAGE of proteins before (lanes 1-3) and after (lanes 1e-3e) overexpression of His-SUMO-PARN(500-639)-mCherry (M = **57,9** kDa) (lane nr 1), His-SUMO-PARN(540-639)-mCherry (M = **52,9** kDa) (lane nr 2), His-SUMO-PARN(526-639)-mCherry (M = **54,6** kDa) (lane nr 3) (12% polyacrylamide gel after Coomassie blue staining)

Mass spectrometry

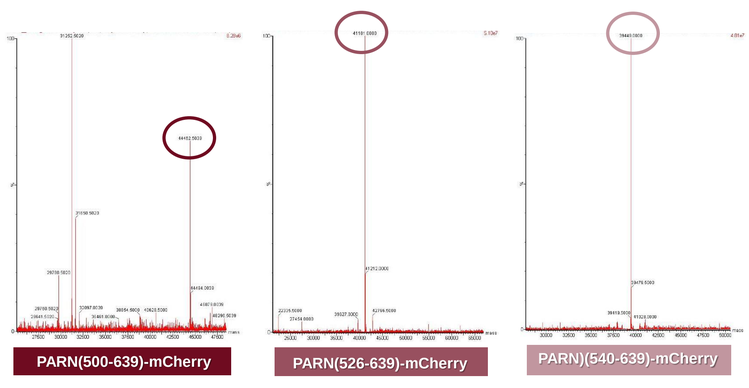

Fig. 7 Mass spectrometry of PARN(500-639)-mCherry, PARN(540-639)-mCherry, PARN(526-639)-mCherry

Molecular masses of purified proteins were checked using mass spectrometry. The obtained masses were comparable with masses of three target proteins predicted by ProtParam tool (Expasy). Spectra from mass spectrometry show also masses of other proteins which marginally contaminate fractions of target proteins (Fig. 7).

Protein purification and stability

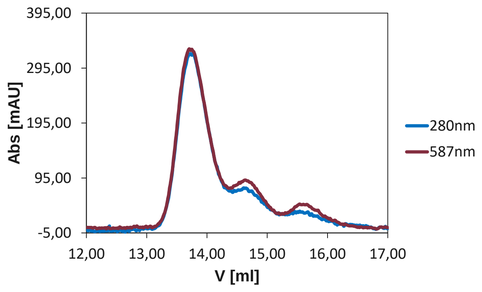

Fig. 3 Chromatogram after SEC purification of His-SUMO-PARN(500-639)-mCherry

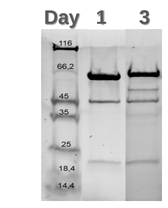

Fig. 4 SDS-PAGE of the same fraction of His-SUMO-PARN(500-639)-mCherry (M = **57,9** kDa) on day 1 and day 3 after purification by SEC (12% TGX Stain-Free polyacrylamide gel Bio-Rad)

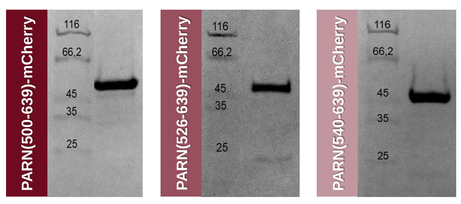

Fig. 5 SDS-PAGE of PARN(500-639)-mCherry (M = **44,5** kDa), PARN(526-639)-mCherry (M = **41,2** kDa), PARN(540-639)-mCherry (M = **39,5** kDa) (12% polyacrylamide gel after Coomassie blue staining)

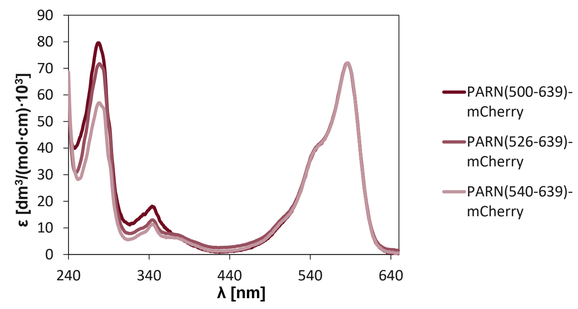

Fig. 6 Ultraviolet absorption of PARN(500-639)-mCherry, PARN(526-639)-mCherry, PARN(540-639)-mCherry

Protein purifications were carried out on HisTrap HP columns and then additional SECs were conducted on Superdex 200 Increase 10/300 GL column (system FPLC ÄKTA) (Fig. 3). After His-SUMO digestion from N-terminus, proteins were purified on HisTrap FF columns (system FPLC ÄKTA). In order to assess the purity and concentration of proteins, the ultraviolet absorption was measured in a range of 650-240 nm.

PARN C proteins are highly unstable because their decay is visible after 3 days (Fig. 4). Even after purification by SEC fractions of 3 proteins with PARNC were slightly contaminated with other proteins (Fig. 5). UV absorption spectra show that proteins were well purified to concentrations in a range of 5-10 mM (Fig. 6).

Protein diffusion

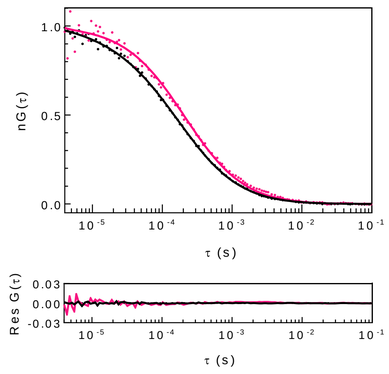

Fig. 8 Example normalized FCS autocorrelation curves with fitting residuals for a folded HSA (66 472 Da, black), and an intrinsically disordered PARN(500-639)-mCherry (44 479 Da, magenta), at 298 K.

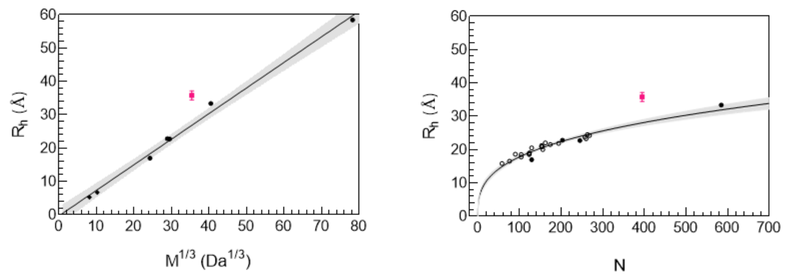

Fig. 9 Dependence of Rh of PARN(500-639) (magenta dots) and standard proteins (dark line) in a function of molar mass (M) or number of amino acids (N)

Hydrodynamic properties of PARN(500-639) were studied by fluorescence correlation spectroscopy (FCS) on Axio Observer LSM 780 Zeiss inverted confocal microscope with ConfoCor3. The diffusion times were determined from autocorrelation curves (Fig. 8) and Rh was calculated from Stokes-Einstien equation.

Rh of PARN(500-639)-mCherry protein is bigger and its diffusion times are longer than it is in the case of reference globular proteins, what indicates that this protein is intrinsically disordered (Fig. 8, 9).

Conclusions and future directions

1. We succeeded in the purification of three PARN C IDRs of different lengths in fusion with mCherry with satisfying purity and confirmed molecular masses.
2. FCS measurements of hydrodynamic radii of PARN(500-639)-mCherry prove intrinsically disordered character of this protein.
3. Purified intrinsically disordered C-terminal regions of PARN will serve to examine detailed cellular localization of PARN and interactions of its NLS region with importins.

Literature

[1] Virtanen A,. et al. Biochem Mol Biol. 48(2):192-209(2013).
[2] Nanjappa D.P. et al. Life Sci. 285:119953(2021).
[3] Niedzwiecka A. et all. Biochim Biophys Acta.1864(4):331-45(2016).
[4] Wu M. et al. Structure.17(2):276-86(2009).
[5] Duan T.L. et al. Cells. 8(8):836(2019).

Acknowledgements

The work was supported by Polish National Science Centre grant UMO-2016/22/E/NZ1/00656 and was performed in the NanoFun laboratories co-financed by ERDF within POIG.02.02.00-00-025/09.