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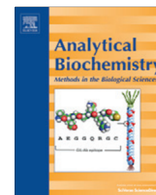

Large-scale preparation and characterization of poly(ADP-ribose) and defined length polymers

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ABSTRACT

Poly(ADP-ribose) (pADPr) is a large, structurally complex polymer of repeating ADP-ribose units. It is biosynthesized from NAD^+ by poly(ADP-ribose) polymerases (PARPs) and degraded to ADP-ribose by poly(ADP-ribose) glycohydrolase. pADPr is involved in many cellular processes and exerts biological function through covalent modification and noncovalent binding to specific proteins. Very little is known about molecular recognition and structure–activity relationships for noncovalent interaction between pADPr and its binding proteins, in part because of lack of access to the polymer on a large scale and to units of defined lengths. We prepared polydisperse pADPr from PARP1 and tankyrase 1 at the hundreds of milligram scale by optimizing enzymatic synthesis and scaling up chromatographic purification methods. We developed and calibrated an anion exchange chromatography method to assign pADPr size and scaled it up to purify defined length polymers on the milligram scale. Furthermore, we present a pADPr profiling method to characterize the polydispersity of pADPr produced by PARPs under different reaction conditions and find that substrate proteins affect the pADPr size distribution. These methods will facilitate structural and biochemical studies of pADPr and its binding proteins.

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Poly(ADP-ribose) (pADPr)[1] is a posttranslational modification made up of ADPr (adenosine 5′-diphosphoribose) units connected linearly or in a branched fashion (Fig. 1) [1]. It is covalently attached to acidic or basic residues of target proteins and can be quite heterogeneous in structure, varying in size (number of ADPr units) and shape (degree of branching) [2,3]. pADPr is biosynthesized from NAD^+ (β-nicotinamide adenine dinucleotide) by enzymes known as PARPs [poly(ADP-ribose) polymerases] and degraded by poly(ADP-ribose) glycohydrolase (PARG) to release ADPr molecules (Fig. 1) [4].

There are 17 types of PARPs but only one PARG gene [5]. The members of the PARP superfamily are all related by the presence of a homologous PARP domain [6]. These enzymes can be subdivided into six groups based on domain architecture (PARP1 subgroup, vault PARP, tankyrases, CCCH PARPs, macro PARPs, and other) and three categories based on enzymatic activity (poly, mono, and inactive) [7]. In this study, we focused on human PARP1 (hPARP1) and human tankyrase 1 (hTNKS1) because both are known to synthesize polymers and are among the best characterized members of the PARP family. It should be noted that hPARP1 and hTNKS1 are also known as ADP-ribosyltransferases 1 and 5 (ARTD1 and ARTD5), respectively, under a recently proposed nomenclature [8].

PARPs and pADPr have been implicated in many cellular processes [1,5,9]. PARP1 is involved in DNA repair/maintenance and transcriptional regulation, whereas TNKS1 has been implicated in telomere maintenance, assembly of the mitotic spindle, and regulation of protein stability [10–17]. pADPr is thought to function through both covalent and noncovalent interactions. Covalent attachment of pADPr can affect the structure and function of the modified protein, in part due to the high density of negative charges on the polymer [18–20]. Two examples are automodification of PARPs, which tend to inactivate enzymatic activity, and heteromodification of histones, which relaxes chromosome packing and increases access to damaged DNA [21].

Noncovalent interactions between pADPr and specific binding proteins are thought to play a role in the DNA damage response

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[1] *Abbreviations used:* pADPr, poly(ADP-ribose); ADPr, adenosine 5′-diphosphoribose; NAD^+, β-nicotinamide adenine dinucleotide; PARP, poly(ADP-ribose) polymerase; PARG, poly(ADP-ribose) glycohydrolase; hPARP1, human PARP1; hTNKS1, human tankyrase 1; HPLC, high-pressure liquid chromatography; bPARG CF, bovine PARG catalytic fragment; GST, glutathione *S*-transferase; Ni-NTA, nickel nitrilotriacetic acid; UV, ultraviolet; FPLC, fast-pressure liquid chromatography; IPTG, isopropyl-β-D-thiogalactopyranoside; PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; CV, column volumes; GF, gel filtration; DTT, dithiothreitol; DHBB column, Dihydroxyboryl Bio-Rex 70 column; MALDI–TOF, matrix-assisted laser desorption/ionization time-of-flight; IMAC, immobilized metal affinity chromatography.

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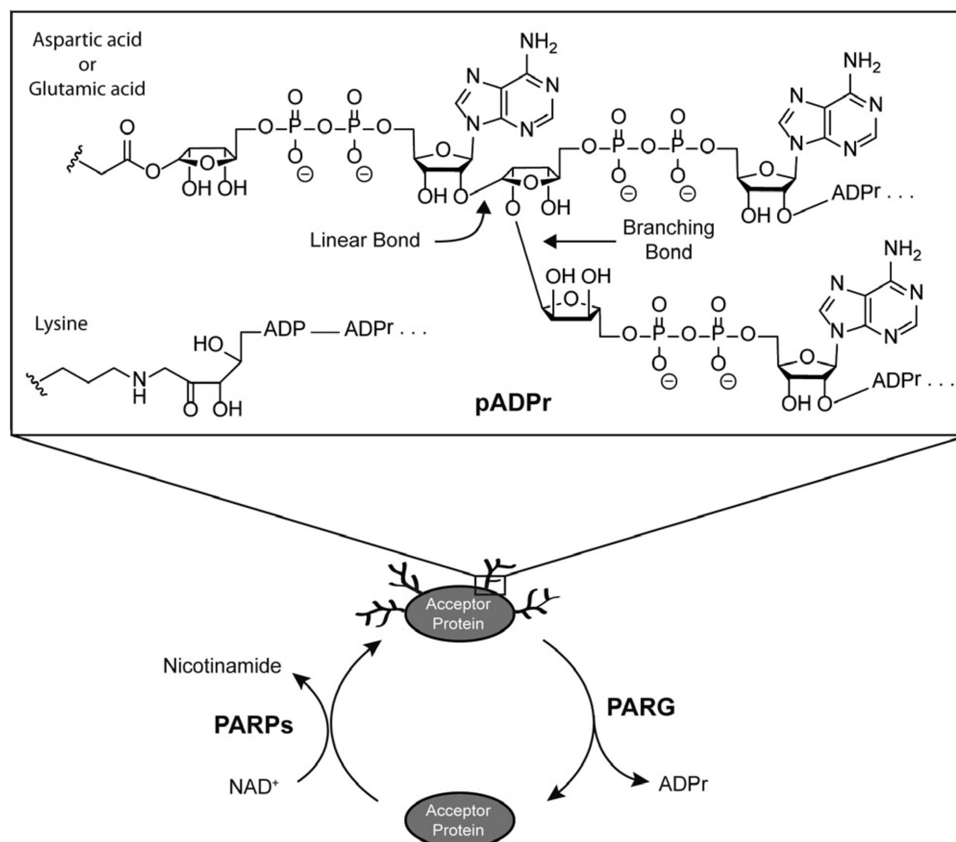

Fig.1. Metabolism of poly(ADP-ribose) (pADPr). PARPs [poly(ADP-ribose) polymerases] biosynthesize pADPr from NAD^+, whereas PARG [poly(ADP-ribose) glycohydrolase] degrades polymer to ADP-ribose (ADPr). pADPr is covalently attached to aspartic acid, glutamic acid, or lysine residues of acceptor proteins, and the ADPr units of the polymer are connected linearly or in a branched fashion.

and in regulation of protein stability [22–27]. The chromatin remodeling enzyme ALC1 is rapidly recruited to DNA damage sites in a pADPr-dependent manner. For the regulation of protein stability, the E3 ubiquitin ligase RNF146 interacts with tankyrase poly (ADP-ribosyl)ated axin and 3BP2 and ubiquitinylates them for degradation. Free pADPr has been reported to be a cytotoxic signaling molecule that causes caspase-independent cell death by inducing the release of apoptosis-inducing factor from the mitochondria [28–32].

Precisely how binding proteins recognize pADPr is unclear, but structural studies of binding proteins interacting with fragments of the polymers have hinted alternative recognition modes. pADPr binding macro domains, which are present in several binding proteins (including some PARPs), are thought to bind to ADPr units at the tips of pADPr, whereas the pADPr binding zinc finger and WWE domains are thought to recognize the PRAMP (phosphoribosyl AMP) units along the length of the polymer [24,26,33,34]. The structure of pADPr itself in solution is also unknown. Spectral analysis on pADPr has suggested a helical structure, but nuclear magnetic resonance studies on ^{13}C- and ^{15}N-labeled polymers showed an absence of any inherent regular structure [35–37]. By electron microscopy, bulk pADPr appeared as "root-like" structures approximately 100 nm long [38,39]. Further characterization is required to fully understand the structure–activity relationship of pADPr.

Detailed structural and biochemical characterization of pADPr has been limited in part because of lack of access to the polymer at a large scale. Polydisperse pADPr is commercially available at a current price of approximately U.S. $175 for 1 nmol (~1 μg). Defined length pADPr is not commercially available. It has been prepared previously, but yields were meager and the scale was insufficient for structural and biochemical studies [40,41]. Here we describe an improved procedure for preparing PARP1 and tankyrase 1 bulk and homogeneous polymers at a large scale. We also present convenient methods to assign pADPr size by anion exchange chromatography using high-pressure liquid chromatography (HPLC).

Materials and methods

hPARP1 (1–1014), hPARP1 (379–1014), and hTNKS1 (1093–1327) constructs were gifts from J. Pascal. hPARP1 (655–1014) and bovine PARG catalytic fragment (bPARG CF, residues 386–977) constructs were generously provided by P. Chang and H. Kleine, respectively [7]. The genes for hPARP1 (1–1014), hPARP1 (379–1014), and hPARP1 (655–1014) were cloned into the pET28 expression vector (Novagen) and hexahistidine tagged at the N terminus [42]. A pET24(+) expression vector (Novagen) was used for hTNKS1 (1093–1327) to generate proteins with the hexahistidine tag at the C terminus. bPARG CF was cloned into a pGEX-2T expression vector (GE Healthcare) with an N-terminal glutathione *S*-transferase (GST) tag.

All reagents were purchased from Sigma–Aldrich, Acros, Alfa Aesar, GE Healthcare, RPI, Roche, Fisher, or VWR and were used without any further purification unless otherwise stated. Nickel nitrilotriacetic acid (Ni-NTA) agarose and glutathione agarose beads were obtained from Qiagen and Sigma–Aldrich, respectively. Gel filtration columns (Superdex 200 and S75) and ECH Sepharose 4B medium were purchased from GE Healthcare. DNA Pac PA100

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