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Research paper

Poly(ADP-ribosyl)ation as a new posttranslational modification of YB-1

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ABSTRACT

Multifunctional Y-box binding protein 1 (YB-1) is actively studied as one of the components of cellular response to genotoxic stress. However, the precise role of YB-1 in the process of DNA repair is still obscure. In the present work we report for the first time new posttranslational modification of YB-1 – poly(ADP-ribosyl)ation, catalyzed by one of the main regulatory enzymes of DNA repair – poly(ADP-ribose)polymerase 1 (PARP1) in the presence of model DNA substrate carrying multiple DNA lesions. Therefore, poly(ADP-ribosyl)ation of YB-1 catalyzed with PARP1, can be stimulated by damaged DNA. The observed property of YB-1 underlines its ability to participate in the DNA repair by its involvement in the regulatory cascades of DNA repair.

1. Introduction

There is a lot of evidence that multifunctional Y-box binding protein 1 (YB-1) can participate in DNA repair events. Among the prominent facts that corroborate such activity are the translocation of YB-1 from the cytoplasm to nucleus in response to genotoxic stress [1,2], the high affinity of YB-1 to damaged DNA [3–5], and the ability of YB-1 to functionally interact with critical enzymes and proteins involved in different pathways of the DNA repair [5–8]. Moreover, YB-1 was identified as one of the poly(ADP-ribose)-binding proteins [9] and detected together with PARP1 as a component of proposed base excision repair (BER) repairosome [10]. Since PARP1 is known to be one of the key regulation enzymes of DNA repair, it is intriguing to analyze its potential to modulate the activity of YB-1 by means of poly(ADP-ribosyl)ation. Poly(ADP-ribosyl)ation is one of the major cellular responses to DNA damage. Apart from its modulatory activity PARP1 is also known to be a DNA damage sensor [11–13]. In its inactive form ubiquitous PARP1 is associated with nucleosomes [14] while binding to damaged DNA triggers its dimerization [15]. PARP1, activated by DNA damage, utilizes NAD^+ to synthesize poly(ADP-ribose) (pADPr) chains thus post translationally modifying proteins, mainly those bound to chromatin, including histones [16]. It is generally believed that automodification of PARP1 (i.e. autopoly(ADP-ribosyl)ation) leads to its dissociation from DNA apparently due to the anionic character of pADPr [17,18]. In addition, the results of described poly(ADP-ribosyl)ation cycle include reorganization of chromatin in the vicinity of DNA damage, recruitment of repair factors capable of binding pADPr, thus "opening up" damaged spot on DNA and facilitating assembly of the repair machinery [19]. Termination of the signaling cycle and recycling of poly(ADP-ribosyl)ated proteins are performed by poly(ADP-ribose)glycohydrolase (PARG) [20].

In the present study we report the ability of PARP1 to poly(ADP-ribosyl)ate YB-1 protein during its interaction with DNA duplexes containing cluster of oxidative lesions together with double-strand breaks that mimic DNA with multiple damages. We also demonstrate that poly(ADP-ribosyl)ation modifies YB-1 characteristics related to DNA repair events.

Abbreviations: YB-1, Y-box binding protein 1; PARP1(2), poly(ADP-ribose) polymerase 1(2); pADPr, poly(ADP-ribose); AP site, apurinic/apyrimidinic, abasic site; APE1, AP endonuclease 1; BER, base excision repair; OCDL, oxidative clustered DNA lesions.

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2. Materials and Methods

2.1. Proteins and reagents

Recombinant PARP1, YB-1 and mutant form of YB-1 – YB-1*mut*(1-219) – were purified as described [21,22]. The plasmid DNA containing human PARP1 cDNA was a kind gift of Dr. Guy G. Poirier (Université Laval, Québec, Canada). Yeast nicotinamide mononucleotide adenylyltransferase (NMAT), PARG, phage T4 polynucleotide kinase, phage T4 DNA ligase and PARP2 were generous gifts from Dr. Stanislav I. Shram (IMG RAS, Moscow, Russia), Dr. Ekaterina S. Ilina, Dr. Irina O. Petruseva and Dr. Mikhail M. Kutuzov (ICBFM SB RAS, Novosibirsk, Russia), respectively. DNA polymerase β (pol β) and AP endonuclease 1 (APE1) were generous gifts from Dr. Svetlana N. Khodyreva (ICBFM SB RAS, Novosibirsk, Russia). *Escherichia coli* uracil-DNA glycosylase (Ung) was from Biosan (Novosibirsk, Russia). ATP, dGTP and proteinase K were from SibEnzyme (Novosibirsk, Russia). Activated calf thymus DNA was a kind gift from Natalia D. Gayko (ICBFM SB RAS, Novosibirsk, Russia). NAD+ and β-nicotinamide mononucleotide were from Sigma (USA), [α-^{32}P]ATP and [γ-^{32}P]ATP were from ICBFM SB RAS (Novosibirsk, Russia).

2.2. Oligonucleotides

The ***ODN1*** oligonucleotide (Table 1) was from Biosset (Novosibirsk, Russia). ***ODN2*** was from Dr. Timofey S. Zatsepin (Chemistry Department of MSU, Moscow, Russia). 5-formyluracil (5-foU) residue in ***ODN2*** oligonucleotide was obtained according to the procedure published earlier [23] with slight modifications. Briefly, oxidation of 5-(1,2-dihydroxyethyl)-uracil residue by 10 mM $NaIO_4$ was performed in 250 mM sodium acetate buffer (pH 4.0) over 1 h at room temperature. The resulting oligonucleotide containing 5-foU was isolated and annealed at 1.2:1 M ratio to ***ODN1*** oligonucleotide 5′-radioactively labeled by using [γ-^{32}P]ATP with T4 polynucleotide kinase. Final DNA containing oxidative clustered DNA lesions, AP site and 5-foU residues (OCDL DNA), was generated in situ by treating resulting structure by Ung (0.2 u.a. per pmol of DNA) at 37 °C for 25 min.

2.3. NAD+ synthesis

Reaction mixture (50 μl) containing 2 mM β-nicotinamide mononucleotide, 1 mM ATP, 0.5 mCi of [α-^{32}P]ATP, 5 mg/ml NMAT, 25 mM Tris–HCl (pH 7.5) and 20 mM $MgCl_2$ was incubated for 1 h at 37^{0}C. The enzyme was then denatured at 97 °C for 3 min and precipitated by centrifugation.

2.4. Protein separation and analysis

Reaction mixtures were analyzed by denaturing polyacrylamide gel electrophoresis according to Laemmli [24] with modifications. Briefly, 4.5% polyacrylamide stacking gel (37:1 acrylamide:bisacrylamide ratio, pH 6.8) and step gradient separating gel of 4% and 13.5% (37:1 acrylamide:bisacrylamide ratio, pH 8.8) were used. Samples were supplemented with Laemmli loading buffer and heated for 1.5 min at 97^{0}C. Positions of protein bands were visualized by colloidal Coomassie brilliant blue R-250 (RAMA) staining [25] and phosphorimaging with FX phosphorimager (BioRad, USA). Alternatively, samples were analyzed by semi-dry Western blotting using antibodies specific to pADPr (clone 10H, Abcam, UK) or YB-1 (custom-made and described previously [26]) and chemiluminescent "SuperSignal West Pico Substrate" for HRP (Thermo Scientific, USA).

2.5. Poly(ADP-ribosyl)ation by PARP1 and PARP2

Reaction mixture (total volume 10 μl) contained reaction buffer RB (50 mM Tris–HCl, pH 7.5, 40 mM NaCl, 2 mM EDTA and 1 mM DTT), 8 mM $MgCl_2$, 0.1 μM PARP1 (or 0.5 μM PARP2), 0–400 nM YB-1 (or YB-1*mut*(1–219), where indicated), and 40 nM DNA structure (OCDL DNA) or calf thymus DNA activated by DNAse I treatment (DNA_{act}) [27]. Blending of the reaction components was performed on ice. Reaction was started by addition of NAD+ or [^{32}P]-radioactively labeled NAD+* to a final concentration equal to 0.4 mM. Mixtures were incubated at 37 °C for indicated period of time or for 5 min if not stated. If the removal of pADPr tail was required, the samples were supplemented with 3-aminobenzamide to the final concentration of 1 mM to inhibit PARP1 activity. Then PARG was added to a final concentration of 0.1 μM followed by incubation at 37 °C for 2–60 min. Eventually, reaction mixtures were supplemented with Laemmli loading buffer, and heated for 1.5 min at 97 °C. Samples were analyzed as described previously. Experiments were performed at least 3 times.

2.6. Gel-mobility shift analysis

Reaction mixtures (total volume 10 μl) contained reaction buffer RB, 8 mM $MgCl_2$, 40 nM radioactively labeled OCDL DNA structure, 0–400 nM YB-1 and 0.1 μM PARP1, where indicated. Selected samples contained additionally 0.4 mM unlabeled NAD+. Blending of the reaction components was performed on ice. Mixtures were incubated for 5 min at 37 °C, supplemented with 2.5 μl of loading buffer (reaction buffer RB, 20% glycerol, 0.025% bromophenol blue), chilled on ice and loaded onto cooled and equilibrated 7.6% polyacrylamide native gel (76:1 acrylamide:bisacrylamide, 25 mM Tris-borate buffer, pH 8.3). Electrophoresis was conducted at 10 V/cm at 4 °C by using 25 mM Tris-borate buffer pH 8.3 as electrode buffer. Gels were dried, positions of protein-DNA complexes were visualized, and their radioactivity corresponding to the labeled material was counted using FX phosphorimager (BioRad, USA). Experiments were performed 3 times.

2.7. AP site cleavage reaction

Reaction mixtures (total volume 4 μl) contained 25 mM Tris–HCl, pH 7.5, 100 mM NaCl, 2 mM EDTA, pH 8.0, 1 mM DTT, 5 mM $MgCl_2$, 40 nM radioactively labeled OCDL DNA structure, 0 or 400 nM YB-1, 0.4 mM unlabeled NAD+, where indicated, and 0.1 μM PARP1, where indicated. Blending of the reaction components was performed on ice. Mixtures were incubated for 5 min at 37 °C, chilled on ice and then incubated for 1.5 min with APE1 (final concentration 0.05 nM). Reaction was stopped by heating for 2 min at 97 °C with 1 μl of 200 mM $NaBH_4$. After brief chilling of mixtures on ice they were supplemented with proteinase K (final concentration 0.16 mg/ml), incubated for 10 min at 60 °C, heated for 2 min

Table 1
Designations and sequences of oligonucleotides.

ODN1	5′-CGGTATCCACCAGGTC***U***GAGACAACGATGAAGCCCAAGCCAGATGAAATGTAGTC-3′
ODN2	5′-GACTACATTTCATCTGGCTTGGGCTTCATCGTTGTC***X***CAGACCTGGTGGATACCG-3′

U and ***X*** in the sequence stand for the uracil and 5-(1,2-dihydroxyethyl)-uracil residues.