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Y-box-binding protein 1 as a non-canonical factor of base excision repair

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ABSTRACT

Base excision repair (BER) is a flagship DNA repair system responsible for maintaining genome integrity. Apart from basal enzymes, this system involves several accessory factors essential for coordination and regulation of DNA processing during substrate channeling. Y-box-binding protein 1 (YB-1), a multifunctional factor that can interact with DNA, RNA, poly(ADP-ribose) and plenty of proteins including DNA repair enzymes, is increasingly considered as a non-canonical protein of BER. Here we provide quantitative characterization of YB-1 physical interactions with key BER factors such as PARP1, PARP2, APE1, NEIL1 and pol β and comparison of the full-length YB-1 and its C-terminally truncated nuclear form in regard to their binding affinities for BER proteins. Data on functional interactions reveal strong stimulation of PARP1 autopoly(ADP-ribosyl)ation and inhibition of poly(ADP-ribose) degradation by PARG in the presence of YB-1. Moreover, YB-1 is shown to stimulate AP lyase activity of NEIL1 and to inhibit dRP lyase activity of pol β on model DNA duplex structure. We also demonstrate for the first time YB-1 poly(ADP-ribosyl)ation in the presence of RNA.

1. Introduction

The hallmark of mammalian base excision repair (BER) is the recruitment of several accessory and non-canonical proteins that may not only act as DNA chaperones at lesions thus facilitating their effective detection by damage sensors, but also promote protein-protein interactions to coordinate successive steps during repair process [1].

Multifunctional Y-box-binding protein 1 (YB-1) is considered as one of BER accessory factors, though its precise role in DNA repair is not fully understood [1]. Previously YB-1 was shown to functionally interact with key BER proteins [2–4] and to display increased affinity for damaged DNA [5]. Upon genotoxic stress YB-1 could change its intracellular localization from cytosolic to nuclear [6,7] and undergo specific post-translational modification – partial proteolysis by 20S proteasome [8].

YB-1 structure is partly intrinsically disordered [9], that is also the unique feature of early BER enzymes such as APE1 and NEIL1 [10]. Disordered regions in DNA repair factors serve for interactions with damaged DNA and protein partners to form dynamic repair complexes [11]. These regions are also the primary targets for posttranslational modifications important for DNA repair regulation [10]. Interestingly, YB-1 was identified as poly(ADP-ribose)-binding protein [12] and detected as a component of FLAG-pol β affinity-capture fraction together with PARP1 and PARP2 [13]. Also it was shown recently by us that YB-1 is a target of poly(ADP-ribosyl)ation [14].

PAR-seeded liquid demixing was recently proposed as a general mechanism for coordinating the earliest stages of DNA repair process by acting as transient interaction filter for genome caretakers [15]. It is possible that YB-1 role in BER is based on its high multivalence and intrinsically disordered structure enabling YB-1 involvement in liquid demixing events essential for the assembly of BER machinery on DNA lesions.

In the present work, by using fluorescence-based technique we detect and characterize quantitatively physical interactions of YB-1 with apurinic/apyrimidinic endonuclease 1 (APE1), Nei-like DNA glycosylase 1 (NEIL1), DNA polymerase β (pol β) and poly(ADP-ribose) polymerases 1 and 2 (PARP1 and PARP2). The formation of YB-1-NEIL1, YB-1-PARP1 and YB-1-PARP2 binary complexes has been shown for the first time. Interaction of the C-terminally truncated nuclear form of YB-1 with PARP1, PARP2, NEIL1 and pol β, and DNA-induced modulation of the protein-protein interaction between several pairs were explored as well. In addition to functional interrelations between YB-1 and APE1/YB-1 and NEIL1 revealed earlier in our works [14,16,17], we demonstrate here YB-1 ability to modulate activities of PARP1 and pol β. YB-1 poly(ADP-ribosyl)ation in the presence of RNA has been detected for the first time.

Abbreviations: YB-1, Y-box binding protein 1; PARP1(2), poly(ADP-ribose) polymerase 1(2); PAR, poly(ADP-ribose); PARG, poly(ADP-ribose) glycohydrolase; pol β, DNA polymerase beta; APE1, AP endonuclease 1; NEIL1, Nei-like DNA glycosylase 1; AP site, apurinic/apyrimidinic, abasic site; BER, base excision repair.

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2. Materials and methods

2.1. Proteins and reagents

Recombinant YB-1 was produced by expression in *Escherichia coli* BL21(DE3) and purified as described previously [18]. Recombinant PARP1, PARP2, pol β and a 24-kDa fragment of human PARP1 (p24) were purified as described earlier with minor modifications [19–22]. Recombinant human apurinic/apyrimidinic endonuclease 1 (APE1) and its truncated form lacking the N-terminal 35 amino acids (NΔ35APE1) were produced by expression in *Escherichia coli* BL21(DE3)pLysS and purified as described previously [23,24]. p-ET-3-1-YB-1 expression vector was generous gift from Lev P. Ovchinnikov and Dmitry Kretov (Institute of Protein Research RAS, Moscow, Russia). The plasmid DNA containing human PARP1 cDNA was a kind gift of Dr. M. Satoh (Université Laval, Québec, Canada). The plasmid bearing cDNA of mouse PARP-2 was kindly provided by Dr. V. Schreiber (Université de Strasbourg, France). The APE1 and pol β expression vectors were kindly provided by Dr. S.H. Wilson (National Institute of Health, North Carolina, USA). The NΔ35APE1 expression vector was generous gift from Dr. A.A. Ishchenko (UMR 8126 CNRS, Institut Gustave Roussy, France).

Yeast nicotinamide mononucleotide adenylyltransferase (NMAT), NEIL1, PARG, *E. coli* uracil-DNA glycosylase (Ung) and phage T4 polynucleotide kinase were generous gifts from Dr. Stanislav I. Shramm (IMG RAS, Moscow, Russia), Dr. Dmitry O. Zharkov, Dr. Ekaterina S. Ilina, Dr. Svetlana N. Khodyreva and Dr. Irina O. Petruseva (ICBFM SB RAS, Novosibirsk, Russia), respectively. CΔ105YB-1 was a generous gift from Lev P. Ovchinnikov and Dmitry Kretov (Institute of Protein Research RAS, Moscow, Russia). ATP and proteinase K were from SibEnzyme (Novosibirsk, Russia). Activated calf thymus DNA (DNA_{act}) was a kind gift from Natalia D. Gayko (ICBFM SB RAS, Novosibirsk, Russia). HeLa total RNA was obtained according to the protocol for TRIzol® Reagent (from Thermo Fisher Scientific, USA) and treated by DNAse I (New England BioLabs) according to the protocol of the manufacturer to remove contaminating DNA. RNA oligonucleotide was a generous gift from Dmitriy Sharifulin (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). *N*-succinimidyl ester of 5(6)-carboxyfluorescein (FAM-SE) was from Sigma-Aldrich.

2.2. Oligonucleotides

The ***ODN1***, ***ODN2*** and ***ODN3*** oligonucleotides (Table 1) were from Biosset (Novosibirsk, Russia). ***ODN1*** was 5′-radioactively labeled by using [γ-^{32}P]ATP with T4 polynucleotide kinase and annealed at 1:1.2 M ratio to ***ODN2***. Final DNA duplex, containing AP site (***Comp***, Table 2), was generated *in situ* by treating resulting structure by Ung (0.2 u.a. per pmol of DNA) at 37 °C for 25 min.

To obtain ***Comp-F*** DNA duplex, ***ODN1*** was firstly annealed at 1:1.2 M ratio to ***ODN3*** that resulted in ***Overh*** duplex (Table 2). Reaction mixture (total volume 100 μl) contained 50 mM Tris-HCl, pH 8.6, 50 mM KCl, 6 mM $MgCl_2$, 4 μM ***Overh*** duplex, 12 μM Flu-12-dUTP and 8.4 μM pol β. ***ODN1*** extending with Flu-12-dUTP was performed for 30 min at 37°C and stopped by heating for 10 min at 97°C with 100 μl of the loading dye (95% formamide, 0.025% bromphenol blue and 0.025% xylene cyanol). Resulting fluorescent labeled ***ODN1*** (***ODN1-F***) was separated by denaturing polyacrylamide gel electrophoresis followed by electroelution from the gel and annealed to ***ODN2*** at 1:1.2 M ratio. Final DNA duplex, containing AP site within 3′-fluorescent labeled strand (***Comp-F***, Table 2), was generated *in situ* by treating resulting structure by Ung (0.2 u.a. per pmol of DNA) at 37 °C for 25 min.

Table 1
Oligonucleotide sequences and designations.

ODN1	5′-CGGTATCCACCAGGTC*U*GAGACAACGATGAAGCCCAAGCCAGATGAA ATGTAGTC-3′
ODN2	5′-GACTACATTTCATCTGGCTTGGGCTTCATCGTTGTCCCAGACCTGGTGGA TACCG-3′
ODN3	5′-AGACTACATTTCATCTG-3′
oligoRNA	5′-GGGAGA AAAAAG AAAGAA AUGUUC UUCUUC UAAGAA GAAAGA AAAGAA AAAGAA AAAAGA CAAAGA CACGAA GGAAGA-3′

Letter ***U*** in nucleotide sequence designates uracil residue.

2.3. Fluorescent labeling of YB-1, CΔ105YB-1, APE1 and NΔ35APE1

The protein to be labeled was dialyzed against a buffer containing 100 mM MES, pH 7.0, 300 mM NaCl and 2 mM DTT (for APE1 and NΔ35APE1), using a Viva-spin microconcentrator, with five washing steps followed by a final step against the same buffer containing no DTT. *N*-succinimidyl ester of 5(6)-carboxyfluorescein (FAM-SE) was dissolved in DMSO and added to the protein solution. The reaction in the mixture containing 100 mM MES, pH 7.0, 300 mM NaCl, 100 μM protein and 160 μM FAM-SE was allowed to proceed for 17 h at 4°C in the dark. The reaction mixture was diluted by addition of four volumes of a solution containing 100 mM MES, pH 7.0, 150 mM NaCl and 10 mM DTT, and centrifuged at 10,000 rpm for 15 min. The supernatant was dialyzed exhaustively against a solution containing 100 mM HEPES, pH 8.0, 200 mM NaCl and 10 mM DTT, to remove the free dye. The labeled proteins were stored at −20 °C in a solution containing 50 mM HEPES, pH 8.0, 100 mM NaCl, 5 mM DTT and 40% glycerol. The extent of protein labeling was quantified by determining dye and protein amounts in the sample. The dye concentration was measured spectrophotometrically using the absorption coefficients of 68×10^3 M^{-1} cm^{-1} at 494 nm for 5(6)-FAM [25]. The protein concentration was determined using the Bradford assay [26].

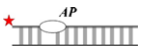
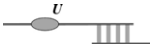
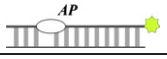
2.4. Fluorescent studies of protein-protein interactions

Binding of YB-1 (or CΔ105YB-1) to BER proteins was examined by fluorescence titration experiments. Fluorescence intensities of solutions of the FAM-labeled protein (at a fixed concentration) in binding buffer were measured in the absence and presence of various concentrations of the potential interaction partner. The binding buffer contained 50 mM HEPES, pH 8.0, 100 mM NaCl and 4 mM DTT. The samples (70 μl) were measured in Corning black 384-well polystyrene assay plates. All the measurements were carried out in duplicates for each specific condition, and the average values of fluorescence were taken. Fluorescence intensity measurements and data analysis were performed using a CLARIOstar multifunctional microplate reader and MARS Data Analysis Software (BMG LABTECH GmbH, Germany). The fluorescent probes were excited at 482 nm (482-16 filter), and the fluorescence intensity was detected at the emission maximum (530 nm; 530-40 filter). The data were plotted (F *versus* C) and fitted by four-parameter logistic equation:

$$F = F_0 + (F_\infty - F_0)/\left[1 + (EC_{50}/C)^n\right],$$

where F is the measured fluorescence intensity of a solution containing the FAM-labeled protein at a given concentration (C) of the binding partner, F_0 is the fluorescence of a solution of the labeled protein

Table 2
DNA duplexes designations and schematic representations.

Oligonucleotides annealed	Duplex designation	Schematic representation
ODN1 + ***ODN2***	***Comp***	
ODN1 + ***ODN3***	***Overh***	
ODN1-F + ***ODN2***	***Comp-F***	

AP – AP site; U – uracil residue; ★ – radioactive label; ☼ – fluorescent label.