DNA Repair 12 (2013) 1043–1052

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Coordination of MYH DNA glycosylase and APE1 endonuclease activities via physical interactions

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ARTICLE INFO

Article history:
Received 3 May 2013
Received in revised form 30 August 2013
Accepted 20 September 2013
Available online 24 October 2013

Keywords:
Base excision repair
Regulatory protein interactions
DNA glycosylase
AP endonuclease
Checkpoint clamp

ABSTRACT

MutY homologue (MYH) is a DNA glycosylase which excises adenine paired with the oxidative lesion 7,8-dihydro-8-oxoguanine (8-oxoG, or G°) during base excision repair (BER). Base excision by MYH results in an apurinic/apyrimidinic (AP) site in the DNA where the DNA sugar–phosphate backbone remains intact. A key feature of MYH activity is its physical interaction and coordination with AP endonuclease I (APE1), which subsequently nicks DNA 5′ to the AP site. Because AP sites are mutagenic and cytotoxic, they must be processed by APE1 immediately after the action of MYH glycosylase. Our recent reports show that the interdomain connector (IDC) of human MYH (hMYH) maintains interactions with hAPE1 and the human checkpoint clamp Rad9–Rad1–Hus1 (9–1–1) complex. In this study, we used NMR chemical shift perturbation experiments to determine hMYH-binding site on hAPE1. Chemical shift perturbations indicate that the hMYH IDC peptide binds to the DNA-binding site of hAPE1 and an additional site which is distal to the APE1 DNA-binding interface. In these two binding sites, N212 and Q137 of hAPE1 are key mediators of the MYH/APE1 interaction. Intriguingly, despite the fact that hHus1 and hAPE1 both interact with the MYH IDC, hHus1 does not compete with hAPE1 for binding to hMYH. Rather, hHus1 stabilizes the hMYH/hAPE1 complex both in vitro and in cells. This is consistent with a common theme in BER, namely that the assembly of protein–DNA complexes enhances repair by efficiently coordinating multiple enzymatic steps while simultaneously minimizing the release of harmful repair intermediates.

1. Introduction

Reactive oxygen species of endogenous and environmental origin continuously cause single-base modifications in genomic DNA [1]. Base excision repair (BER) is the major pathway that protects the genome from the mutagenic and cytotoxic effects of such nucleobase damage [2]. Damage-specific DNA glycosylases initiate BER by cleaving the *N*-glycosidic bond between the damaged base and the sugar-phosphate backbone, creating an apurinic/apyrimidinic (AP) site in the DNA [3]. Universal repair enzymes take over to complete repair with synthesis of either a single-nucleotide (SN) patch [4] or a long-patch (LP), consisting of 2–13 nucleotides [5]. AP endonuclease 1 (APE1) is an essential multifunctional enzyme that cleaves the phosphodiester bond 5′ to an AP site generated by DNA glycosylases in both the SN- and LP-BER subpathways [3].

7,8-dihydro-8-oxoguanine (8-oxoG, or G°), generated by the oxidation of guanine, is one of the most prevalent oxidative lesions and is repaired by BER [6]. If DNA replication occurs prior to the repair of the G° lesion, replicative DNA polymerases frequently misincorporate adenine opposite G°. Human MutY homologue (hMYH) is the DNA glycosylase responsible for excising misincorporated adenines to initiate repair of A/G° lesions. Notably, germline mutations of *hMYH* cause the colorectal cancer predisposition syndrome,

Abbreviations: 8-oxoG or G°, 7,8-dihydro-8-oxoguanine; 9–1–1, Rad9–Rad1–Hus1; AP, apurinic/apyrimidinic; APE1, AP-endonuclease 1; BSA, bovine serum albumin; BER, base excision repair; BstMutY, *Bacillus stearothermophilus* MutY; Ec-cMutY, *Escherichia coli* MutY catalytic domain; EtBr, ethidium bromide; F, tetrahydrofuran abasic site analog; GST, glutathione *S*-transferase; h, human; HSQC, heteronuclear spin quantum coherence; IDC, interdomain connector; LP, long-patch; MAP, MYH-associated polyposis; MEF, mouse embryonic fibroblast; MYH or MUTYH, MutY homologue; ODN, oligodeoxynucleotides; *S. cerevisiae*, *Saccharomyces cerevisiae*; *S. pombe*, *Schizosaccharomyces pombe*; SN, single-nucleotide patch.

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http://dx.doi.org/10.1016/j.dnarep.2013.09.007

MYH-associated polyposis (MAP) [7] and the identification of MAP established the first connection between inherited BER defects and cancer [8].

Because AP sites are mutagenic and cytotoxic [8], they must be recognized and processed by APE1 immediately after the action of a DNA glycosylase. A "passing-the-baton" model has been proposed for BER [9] consistent with findings that APE1 interacts with many DNA glycosylases [10–13]. So far, only hMYH has been demonstrated to form a stable complex with APE1 [14], and thus provides an excellent system to study their interaction. A key unanswered question in BER is how the pathway as a whole can be coordinated. Thus far, several candidates have emerged as potential "central coordinators" of BER. In particular, the LP-BER enzymes proliferating cell nuclear antigen (PCNA) and flap endonuclease 1 (FEN1) also physically associate with hAPE1 [15], suggesting that APE1 might be a central coordinator of BER. However, PCNA also interacts with a multitude of enzymes including LP-BER enzymes hMYH [14], DNA polymerases β [16], δ, and ε, replication factor C, FEN1, and DNA ligase I [17,18], implicating it as another potential coordinator and regulator of LP-BER. The heterotrimeric Rad9–Rad1–Hus1 (9–1–1) complex is a DNA clamp that shows striking structural similarity to PCNA [19–21]. 9–1–1 also physically interacts with many LP-BER enzymes including hMYH [22], hTDG [23], hNEIL1 [24], OGG1 [25], hAPE1 [26], polymerase β [27], FEN1 [28,29], and Lig1 [30,31] and therefore is also poised as a likely candidate to coordinate the enzymatic steps of BER.

The physical interaction between MYH and APE1 has been demonstrated by co-immunoprecipitation and GST-pulldown assays [14]. The binding site of APE1 on hMYH has been mapped to residues 295–318 of the flexible interdomain connector (IDC) of hMYH [14,32]. Two groups [13,33] have shown that a large excess of hAPE1 stimulates the glycosylase activity of MYH. However, the origins of this effect remain unclear. In particular, the binding surface(s) used by APE1 to interact with MYH remains to be identified. To address this issue, we used NMR chemical shift perturbation experiments and a synthetic IDC peptide (IDC_{pep}) to identify APE1 residues that contact the IDC region of hMYH. Here, we report that hMYH physically interacts with hAPE1 at the APE1 DNA-binding site and another site distal to the APE1 DNA-binding interface. We hypothesize that the physical interaction between hMYH and hAPE1 is one of several regulatory mechanisms that ensures the BER pathway proceeds to completion to avoid the release of toxic intermediates. We validated these findings by demonstrating that alanine mutants of N212 and Q137, two APE1 residues that exhibit significant IDC_{pep}-induced chemical shift perturbations significantly impair the binding of APE1 to MYH. Moreover, addition of either the IDC peptide or hMYH(65–350) to an APE1 endonuclease reaction generates a modest but reproducible enhancement of APE1 activity. Because APE1 and the Rad9–Rad1–Hus1 (9–1–1) complex both interact with the IDC of MYH [14,22,32], we sought to determine whether these interactions are mutually exclusive. Toward that end, we examined the effects of the hHus1 subunit of the 9–1–1 complex on MYH/APE1 interactions. Surprisingly, hHus1 enhances binding between hMYH and hAPE1. Thus, we propose that 9–1–1 might coordinate safe and efficient BER by assembling hMYH, hAPE1, and other enzymes as a multiprotein complex on damaged DNA.

2. Materials and methods

2.1. DNA purification

The oligodeoxynucleotides (ODN) used to make a DNA duplex containing a guanine opposite an abasic site analog (F/G) for the APE1 repair assay were synthesized (trityl-on) at the Keck Foundation Biotechnology Resource Laboratory, Yale University. The ODNs were purified with Glen-Pak cartridges (Glen Research) and their concentration was determined by absorbance. Duplex DNA was hybridized by heating to 80 °C followed by slow cooling to room temperature. The duplex DNA is composed of 5′-AGTGCGTCC*F*CGACGAC, where *F* is a tetrahydrofuran abasic site analog, and its complement, 5′-GTCGTCGGGGACGCACT. Oligonucleotides from Integrated DNA Technologies were also used to create a DNA duplex with a thymine base opposite a tetrahydrofuran abasic site analog (F/T). The duplex DNA is composed of 5′-GCTCA*F*GTACAGAGCTGC and its complement, 5′-GCAGCTCTGTACTTGAGC. The DNA strands were annealed as described [34].

2.2. Creation of expression constructs

The sequences of all constructs were verified before undertaking subsequent experiments.

hAPE1$^{\Delta N38}$: The APE1$^{\Delta N38}$ expression plasmid encodes for truncated human APE1, lacking the first 38 residues of the intact protein. The plasmid was constructed as described [35]. In summary, PCR was used to amplify the gene sequence corresponding to residues 39–318 of human APE1 from an expression plasmid harboring the intact gene. The PCR product was then subcloned into the *Nhe*I and *BamH*I sites of a pET-28 plasmid (Novagen).

hMYH(*65–350*): The hMYH(65–350)-pET19b-pps expression construct has been described [32].

hAPE1, hAPE1-N212A, hAPE1-Q137A: hAPE1 in the pET-28 expression plasmid was a kind gift to the laboratory of Dr. Alex Drohat from Professor Ian Hickson of the University of Oxford. The N212A and Q137A mutants were constructed and their sequences confirmed by Genscript (Piscataway, NJ).

GST-hMYH(*1–350*): The cDNA fragments containing residues 1–350 of hMYH fused to the GST gene were obtained by PCR and ligated into the pGEX-4T-2 vector (GE Healthcare) as described [22].

GST-hMYH(*1–350*)*-V315A*: The V315A mutant of hMYH(1–350) was constructed by the PCR splicing overlap extension method as described [22,36].

hHus1: The hHus1-pET-21a expression plasmid was constructed as described [22]. The hHus1 gene is fused with a gene sequence that encodes for a C-terminal hexahistidine tag.

hHus1-N1: The hHus1 deletion construct containing residues 1–146 was obtained by PCR using pET-21-hHus1 plasmid [22] as template and the primers Chang390 (5′-GGTCGCGGATCCATGAAGTTTCGGGCCAAGATC-3′) and Chang576 (5′-CGCTCTCGAGTAAGTCCTTCCACAATTTCCTTGG-3′). The PCR product was cleaved by BamHI and XhoI into two fragments due to the internal XhoI site at the hHus1 gene. The larger fragment containing residues 1–90 was ligated into pET-21a (EMD Biosciences) to obtain the clone pET21a-hHus1-N1. The hHus1-N1 protein was tagged with His at its C-terminus.

2.3. Protein expression and purification

2.3.1. ^{15}N-labeled APE1$^{\Delta N38}$, and unlabeled APE1, APE1-N212A, and APE1-Q137A

To produce uniformly ^{15}N-labeled protein, APE1$^{\Delta N38}$ was expressed as previously described [35]. Briefly, BL21(DE3) cells (Novagen) were transformed with the pET-28 expression plasmid harboring APE1$^{\Delta N38}$, plated, and incubated overnight at 37 °C. A 50-ml starter culture with Luria broth (LB) medium was grown at 37 °C to an $A_{600} \approx 0.6$–0.8 before transferring cells to 2 l of 3-(N-morpholino)propanesulfonic acid (MOPS) minimal media supplemented with Uniform-[^{15}N]-NH_4CL (1 g/L). The cells were grown at 37 °C until the optical density reached an $A_{600} \approx 0.6$.