Progress in Biophysics and Molecular Biology 117 (2015) 134–142

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

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

Review

Structural basis for incision at deaminated adenines in DNA and RNA by endonuclease V

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ARTICLE INFO

Article history:
Available online 28 March 2015

Keywords:
Endonuclease V
Deamination
Hypoxanthine
Inosine

ABSTRACT

Deamination of the exocyclic amines in adenine, guanine and cytosine forms base lesions that may lead to mutations if not removed by DNA repair proteins. Prokaryotic endonuclease V (EndoV/Nfi) has long been known to incise DNA 3′ to a variety of base lesions, including deaminated adenine, guanine and cytosine. Biochemical and genetic data implicate that EndoV is involved in repair of these deaminated bases. In contrast to DNA glycosylases that remove a series of modified/damaged bases in DNA by direct excision of the nucleobase, EndoV cleaves the DNA sugar phosphate backbone at the second phosphodiester 3′ to the lesion without removing the deaminated base. Structural investigation of this unusual incision by EndoV has unravelled an enzyme with separate base lesion and active site pockets. A novel wedge motif was identified as a DNA strand-separation feature important for damage detection. Human EndoV appears inactive on DNA, but has been shown to incise various RNA substrates containing inosine. Inosine is the deamination product of adenosine and is frequently found in RNA. The structural basis for discrimination between DNA and RNA by human EndoV remains elusive.

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1. Introduction

Three of the four nucleobases in DNA can be deaminated at their primary amine positions, whereby the amino group is replaced by a carbonyl moiety. Thus, the deaminated bases will have altered base pairing properties compared to the unmodified variants. Adenine is deaminated to form hypoxanthine, guanine to xanthine or oxanine, and finally cytosine is deaminated to form uracil (Fig. 1a). Deamination can be caused by spontaneous hydrolysis (Lindahl, 1993; Shen et al., 1994), by reaction with endogenous or exogenous factors, or by enzymatic reactions. For example, nitrate or nitrite metabolism, as well as chronic inflammation and macrophage

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http://dx.doi.org/10.1016/j.pbiomolbio.2015.03.005
0079-6107/

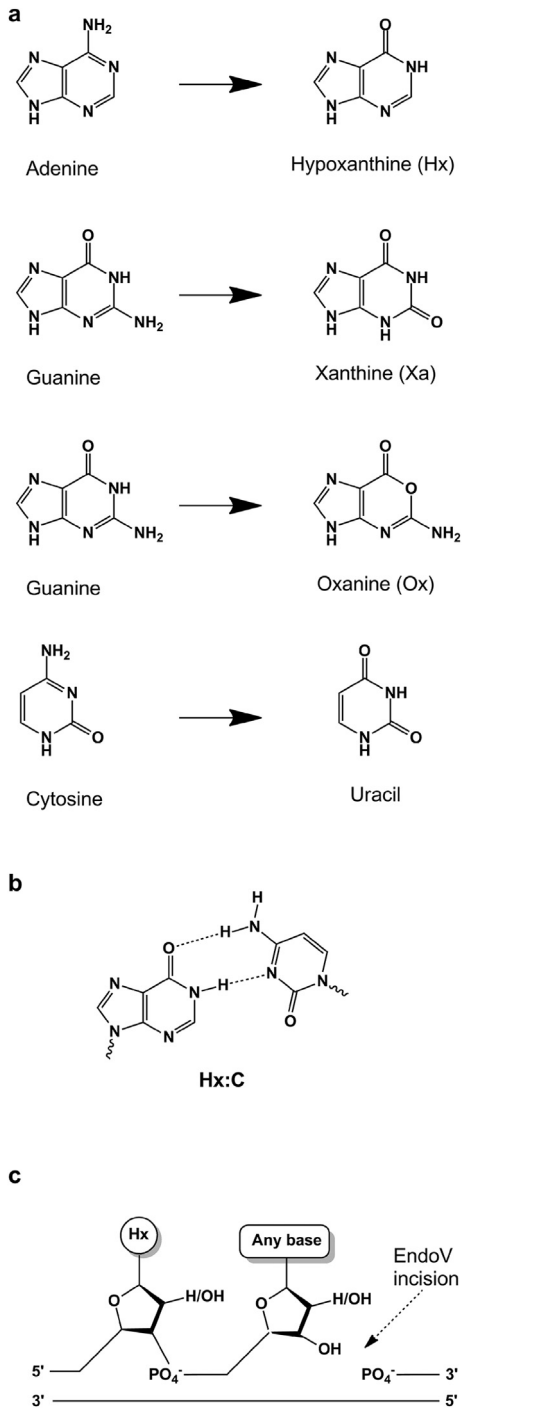

Fig. 1. Base deamination. (**a**) Deamination of adenine, guanine and cytosine produce hypoxanthine (Hx), xanthine (Xa) and oxanine (Ox), and uracil, respectively. (**b**) Hypoxanthine forms base pair with cytosine. (**c**) EndoV hydrolyses the second phosphodiester group 3′ of the deaminated base (Hx).

activation produce reactive nitrogen and oxygen species (RNS/ROS) such as nitric oxide and superoxide (Dedon and Tannenbaum, 2004; Thomas et al., 2008). The RNS and ROS may further react with the exocyclic amines of DNA nucleobases. RNS have been proposed to cause cytotoxic and mutagenic DNA damage via direct reaction with DNA or via formation of DNA adduct-forming electrophiles from reactions with lipids, proteins and carbohydrates (Nguyen et al., 1992; Wink et al., 1991). Deamination of bases may also be catalysed by enzymes such as AID (activation-induced cytidine deaminase) in order to form uracil in DNA for antibody diversification (Muto et al., 2000; Revy et al., 2000; Muramatsu et al., 2000).

All the deaminated bases in DNA may give rise to mutations due to misincorporation and formation of non-canonical base-pairs during replication (Hill-Perkins et al., 1986; Wuenschell et al., 2003; Yasui et al., 2008). The deaminated DNA bases can cause mutations and cancer predispositions (Demple and Linn, 1982; Schouten and Weiss, 1999; Hussain et al., 2003; Nguyen et al., 1992; Wink et al., 1991). Particularly, for deaminated adenine, an A/T to G/C transition mutation may arise as hypoxanthine mispairs with cytosine (Fig. 1b) (Hill-Perkins et al., 1986; Schouten and Weiss, 1999). Related to this, members of the large APOBEC family, which convert cytosine to uracil in RNA or DNA, are believed to be the source for a large fraction of mutations found in tumors (Beale et al., 2004; Nik-Zainal et al., 2012; Roberts et al., 2013; Roberts and Gordenin, 2014).

Another mechanism for introduction of deaminated bases in DNA is misincorporation of the nucleotides dUTP and dITP (deoxyinosine triphosphate; hypoxanthine is the base of deoxyinosine and inosine in DNA and RNA, respectively; Fig. 1a) by DNA polymerases (Budke and Kuzminov, 2006; Mathews, 2006; Pang et al., 2012). This misincorporation is mainly a non-mutagenic process since the DNA polymerases inserts adenine opposite uracil and cytosine opposite hypoxanthine.

To avoid the mutagenic effect, the deaminated bases in DNA must be detected and removed. The most common deamination product, uracil, is removed via the Base Excision Repair (BER) pathway by way of uracil DNA glycosylases UNG and SMUG1 (Haushalter et al., 1999; Kavli et al., 2002; Olsen et al., 1989). Hypoxanthine and xanthine are also removed by BER glycosylases such as AlkA, Nei and Mug in *Escherichia coli* (Lee et al., 2010b; Saparbaev and Laval, 1994; Terato et al., 2002), and AAG in human cells (O'Brien and Ellenberger, 2004; Saparbaev and Laval, 1994). In addition to excision of hypoxanthine and xanthine bases by BER glycosylases, endonuclease V (EndoV/Nfi) has also been implicated in removal of these lesions in DNA.

In contrast to DNA where deamination of bases is normally regarded a damage caused by spontaneous hydrolysis, nitrosative stress or misincorporation, bases in RNA are frequently edited, with the adenine-to-inosine modification being a frequent event (reviewed in e.g. (Bass, 2002; Gray, 2012)). In RNA, the enzymes ADAR (adenosine deaminase acting on RNA) and ADAT (adenosine deaminase acting on tRNA) deaminate adenosines in mRNA/non-coding RNAs and tRNAs, respectively, to form inosine as a way to generate transcriptome diversity (Keegan et al., 2004; Tsutsumi et al., 2007). The role of hypoxanthine/inosine in DNA and RNA has recently been discussed (Alseth et al., 2014). In short, within DNA, hypoxanthine is a premutagenic lesion, while in RNA, it is an essential, enzymatically generated modification introduced to give transcriptome variety.

2. Endonuclease V – activity and role in DNA/RNA metabolism

2.1. Discovery, activities, mechanism and role of EndoV in prokaryotes

The DNA incision activity of *E. coli* EndoV, encoded by the *nfi* gene, was first reported for DNA substrates containing uracil, apurinic/apyrimidinic sites, and various products formed by treatment of DNA with UV light, X-rays, acids or OsO_4 (Demple and Linn, 1982; Gates and Linn, 1977a, 1977b). Later, *in vitro* studies revealed that *E. coli* EndoV incised a plethora of DNA substrates, including