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DNA Repair 4 (2005) 1295–1305
DNA REPAIR
www.elsevier.com/locate/dnarepair

The mechanism of base excision repair in *Chlamydiophila pneumoniae*

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Received 30 November 2004; received in revised form 16 June 2005; accepted 24 June 2005
Available online 8 August 2005

Abstract

Repair of damaged DNA is of great importance in maintaining genome integrity, and there are several pathways for repair of damaged DNA in almost all organisms. Base excision repair (BER) is a main process for repairing DNA carrying slightly damaged bases. Several proteins are required for BER; these include DNA glycosylases, AP endonuclease, DNA polymerase, and DNA ligase. In some bacteria the single-stranded specific exonuclease, RecJ, is also involved in BER. In this research, six *Chlamydiophila pneumoniae* (*C. pneumoniae*) genes, encoding uracil DNA glycosylase (CpUDG), endonuclease IV (CpEndoIV), DNA polymerase I (CpDNApolI), endonuclease III (CpEndoIII), single-stranded specific exonuclease RecJ (CpRecJ), and DNA ligase (CpDNALig), were inserted into the expression vector pET28a. All proteins, except for CpDNALig, were successfully expressed in *E. coli*, and purified proteins were characterized in vitro. *C. pneumoniae* BER was reconstituted in vitro with CpUDG, CpEndoIV, CpDNApolI and *E. coli* DNA ligase (EcDNALig). After uracil removal by CpUDG, the AP site could be repaired by two BER pathways that involved in the replacement of either one (short patch BER) or multiple nucleotides (long patch BER) at the lesion site. CpEndoIII promoted short patch BER via its 5′-deoxyribophosphodiesterase (5′-dRPase) activity, while CpRecJ had little effect on short patch BER. The flap structure generated during DNA extension could be removed by the 5′-exonuclease activity of CpDNApolI. Based on these observations, we propose a probable mechanism for BER in *C. pneumoniae*.

Keywords: *Chlamydiophila pneumoniae*; Base excision repair; DNA repair

1. Introduction

Many types of genomic DNA damage occur frequently in all organisms. They include deamination of cytosine, methylation and oxidation of bases, misincorporation during DNA replication, and DNA strand breaks [1–3]. To counteract the deleterious effects of DNA damage, almost all organisms have evolved several strategies to repair damaged DNA. These strategies include direct reversion, translesion synthesis, recombination repair, nucleotide excision repair, mismatch repair and base excision repair [4–11].

Base excision repair (BER) is initiated by a damage-specific DNA *N*-glycosylase that is responsible for the recognition and removal of an altered base through cleavage of the *N*-glycosylic bond [12]. These characterized glycosylases include uracil DNA glycosylase, adenine DNA glycosylase mutY, 3-methyladenine DNA glycosylase AlkA, endonuclease III, and formamidopyrimidine DNA glycosylase [13–17]. After removal of specific damaged bases by DNA glycosylases, the apurinic/apyrimidinic (AP) sites were generated. The generated AP sites can be cleaved by AP endonuclease and AP lyase [18,19]. AP endonuclease cleaves 5′ of AP sites through hydrolysis of phosphodiester bonds, yielding single-stranded breaks (SSBs) with 3′-hydroxy and 5′-deoxyribose-5-phosphate (5′-dRP) ends [20]. AP lyase cleaves 3′ of AP sites via a β-elimination reaction, generating SSBs with 3′-α,β-unsaturated aldehyde and 5′-phosphate ends. Except

Abbreviations: *C. pneumoniae*, *Chlamydiophila pneumoniae*; CpUDG, *C. pneumoniae* uracil DNA glycosylase; CpEndoIV, *C. pneumoniae* endonuclease IV; CpEndoIII, *C. pneumoniae* endonuclease III; CpDNApolI, *C. pneumoniae* DNA polymerase I; CpRecJ, *C. pneumoniae* RecJ; CpDNALig, *C. pneumoniae* DNA ligase; EcDNApolI, *E. coli* DNA polymerase I; EcDNALig, *E. coli* DNA ligase; 5′-dRPase, 5′-deoxyribophosphodiesterase; BER, base excision repair; AP site, apurinic/apyrimidinic site; SSB, single-stranded break; 5′-dRP, 5′-deoxyribose-5-phosphate; IPTG, isopropyl thiogalactoside; β-ME, β-mercaptoethanol; PMSF, phenylmethylsulfonyl fluoride; TCA, trichloracetic acid

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doi:10.1016/j.dnarep.2005.06.014

for cleavage of AP site, AP endonuclease can also remove blocked 3′ termini, such as 3′-α,β-unsaturated aldehydes, producing 3′-hydroxy ends for DNA polymerase repair synthesis [21–24].

There are two alternative BER pathways, short and long patch BER. In short patch repair, one nucleotide gap, generated via removal of 5′-dRP by 5′-dRPase or removal of 3′-α,β-unsaturated aldehyde by 3′-phosphodiesterase activity of AP endonuclease, is filled by DNA polymerase and the nick is sealed by DNA ligase. In long patch repair, two to eight nucleotides are incorporated into the repaired DNA strand by DNA polymerase and the flap structure produced during primer extension is cleaved by eukaryotic FEN-1 or the 5′-exonuclease activity of prokaryotic DNA polymerase I [25]. Short patch BER is believed to be the preferred BER pathway and is favored by the presence of an intact nucleotide residue at the 5′ side of SSBs, such as that generated by the AP lyase activity of a bifunctional DNA glycosylase [26,27]. However, the presence of 5′-dRP residue at the 5′ side of SSBs, generated by the sequential actions of a monofunctional DNA glycosylase and an AP endonuclease on the damaged base, inhibited the short patch BER. The 5′-exonuclease activity on the flap structure produced during primer extension are required for long patch BER.

Both short and long patch BERs have been studied extensively in *E. coli*. However, no effort has been expended to study BER in *Chlamydiophila*, the obligate intracellular pathogens distinguishable from other gram-negative bacteria by a distinctive biphasic developmental cycle, i.e. the elementary body and the reticulate body. Infection by *Chlamydiophila* causes many diseases in humans and animals. For example, *C. pneumoniae* is a pathogen that causes pneumonia, bronchitis [28], atherosclerosis [29], and even Alzheimer's disease [30].

Because it is difficult to culture *Chlamydiophila* and there is no practical gene transfer system set up for *Chlamydiophila*, a better strategy for establishing the molecular biology of *Chlamydiophila* nowadays is to express Chlamydial proteins in a heterologous host, and then characterize the expressed proteins in vitro. This strategy has been applied in our efforts to address the mechanism of Chlamydial BER. Genomic sequencing indicates that most of the proteins involved in BER exist in *Chlamydiophila* [31,32]. In this research, five potential *C. pneumoniae* BER proteins, CpUDG, CpEndoIV, CpDNApolI, CpEndoIII, and CpRecJ, were successfully expressed in *E. coli*, respectively. After characterized individually in vitro, these proteins and EcDNALig were used to reconstitute BER in vitro. Two alternative pathways, short and long patch BER, involved in *C. pneumoniae* BER of uracil in vitro. BER could be partially completed by the combined action of CpUDG, CpEndoIV, CpDNApolI, and EcDNALig. CpEndoIII promoted short patch BER through its 5′-dRPase activity, however CpRecJ had little effect on short patch BER. The 5′-exonuclease activity of CpDNApolI on the flap structure generated during DNA extension would be involved in long patch BER.

2. Materials and methods

2.1. Materials

Pyrobest™ DNA polymerase, restriction endonucleases, T4 DNA ligase, T4 polynucleotide kinase, terminal deoxynucleotidyl transferase, Klenow fragment, *E. coli* DNA polymerase I (EcDNApolI), and *E. coli* DNA ligase were obtained from TaKaRa (Dalian, China). [γ-^{32}P]ATP and [α-^{32}P]dNTPs were purchased from Yahui Biotech Co. (Beijing, China). A pET expression kit, including expression vectors, *E. coli* strains BL21 (DE3), and Ni-NTA His·Bind® Resin, was purchased from Novagen, CM Sepharose®, DEAE Sepharose®, and [α-^{32}P]ddATP were products of Amersham. Oligonucleotides were synthesized by Sangon Biotech Co. (Shanghai, China). *C. pneumoniae* AR39 genomic DNA was kindly provided by Dr. Jin Huang in Dr. Stephen Lory's Laboratory (Harvard Medical School). All other chemicals and reagents were of analytical grade.

2.2. Construction of expression vectors

The six putative genes encoding proteins involved in *C. pneumoniae* BER were amplified from *C. pneumoniae* AR39 genomic DNA by PCR using specific primers (Table 1). After digested with the restriction endonucleases indicated in Table 1, the amplified fragments were ligated with pET28a (pre-digested by the same enzymes) by T4 DNA ligase, respectively. The constructed expression plasmids, pET28a-CpUDG, pET28a-CpEndoIV, pET28a-CpDNApolI, pET28a-CpDNAlig, pET28a-CpRecJ, and pET28a-CpEndoIII, were confirmed by DNA sequencing.

2.3. Protein expression and purification

Expression plasmids, pET28a-CpUDG, pET28a-CpEndoIV, pET28a-CpDNApolI, pET28a-CpDNALig, pET28a-CpRecJ, and pET28a-CpEndoIII, were transformed into competent *E. coli* BL21 (DE3). Ten ml LB media containing 25 μg/ml kanamycin were inoculated with a single colony and incubated at 37 °C overnight. The overnight cultures were added to 1 l fresh LB media containing 25 μg/ml kanamycin, and incubated with shaking (220 rpm) at 37 °C until OD_{600} reached 0.8. Expression of *C. pneumoniae* proteins was induced with 0.5 mM isopropyl thiogalactoside (IPTG) for 3.5 h with shaking at 37 °C. Centrifugation at 5000 × *g* for 10 min was performed to harvest bacteria.

All operations for protein purification were carried out at 4 °C. The chromatography was performed with a flow rate of 15 ml/h. After suspended in 25 ml lysis buffer (20 mM Tris–HCl, pH 7.9, 300 mM NaCl, 10 mM imidazole, 5 mM β-mercaptoethanol (β-ME), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 10% glycerol), the bacteria were lysed by sonication with 100 × 4 s bursts. Centrifugation at 12,000 × *g* for 60 min was performed to clarify the extracts, and the