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Modulation of the processive abasic site lyase activity of a pyrimidine dimer glycosylase

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ABSTRACT

The repair of *cis–syn* cyclobutane pyrimidine dimers (CPDs) can be initiated *via* the base excision repair (BER) pathway, utilizing pyrimidine dimer-specific DNA glycosylase/lyase enzymes (pdgs). However, prior to incision at lesion sites, these enzymes bind to non-damaged DNAs through charge–charge interactions. Following initial binding to DNA containing multiple lesions, the enzyme incises at most of these sites prior to dissociation. If a subset of these lesions are in close proximity, clustered breaks may be produced that could lead to decreased cell viability or increased mutagenesis. Based on the co-crystal structures of bacteriophage T4-pdg and homology modeling of a related enzyme from *Paramecium bursaria Chlorella* virus-1, the structure–function basis for the processive incision activity for both enzymes was investigated using site-directed mutagenesis. An assay was developed that quantitatively measured the rates of incision by these enzymes at clustered apurinic/apyrimidinic (AP) sites. Mathematical modeling of random (distributive) versus processive incisions predicted major differences in the rate and extent of the accumulation of singly nicked DNAs between these two mechanisms. Comparisons of these models with biochemical nicking data revealed significant changes in the damage search mechanisms between wild-type pdgs and most of the mutant enzymes. Several conserved arginine residues were shown to be critical for the processivity of the incision activity, without interfering with catalysis at AP sites. Comparable results were measured for incision at clustered CPD sites in plasmid DNAs. These data reveal that pdgs can be rationally engineered to retain full catalytic activity, while dramatically altering mechanisms of target site location.

1. Introduction

Although human cells possess DNA glycosylases to initiate base excision repair (BER) for many forms of base damage, they lack enzymes that initiate BER on the major form of ultraviolet (UV) light-induced damage, *cis–syn* cyclobutane pyrimidine dimers (CPDs) [1–3]. In most organisms, repair of these lesions occurs *via* the nucleotide excision repair (NER) pathway. However, subsets of bacteria, bacteriophages, and eukaryotic viruses also use DNA glycosylases with an associated abasic site lysase activity to initiate repair of CPDs *via* the BER pathway. While the most extensively studied glycosylase with UV-damage specific activity is encoded by the *denV* gene from the bacteriophage T4 (reviewed in [4]), another closely related enzyme is produced by *Paramecium bursaria Chlorella* virus-1 (Cv-pdg) [5–9]. Interest in the Cv-pdg enzyme stems from data demonstrating that relative to the T4-pdg, it has broader substrate specificity [8,9] and an ~10-fold increased catalytic efficiency [8]. Both of these enzymes utilize the α-NH_2 group of the amino terminal threonine residue as the active site nucleophile to initiate chemistry at the C1′ of an abasic site or a CPD [10–12]. These reactions proceed through an imino intermediate that can be reduced to a stable DNA–protein complex. Structure–function analyses of T4-pdg include the crystal structure of the native enzyme [13] and two co-crystal structures of the protein in complex with CPD- or apurinic/apyrimidinic (AP) site-containing DNA [14,15]. These structures have revealed that the charge distribution on the surface of the comma-shaped T4-pdg is highly asymmetric with the positively charged amino acids clustered on the concave, DNA-binding surface, while the convex backside surface contains predominantly acidic residues. These structures also reveal the identity of residues that may be

Abbreviations: AP, apurinic/apyrimidinic; BER, base excision repair; CPDs, cyclobutane pyrimidine dimers; NER, nucleotide excision repair; pdg, pyrimidine dimer DNA glycosylase/AP site lyase; UV, ultraviolet.

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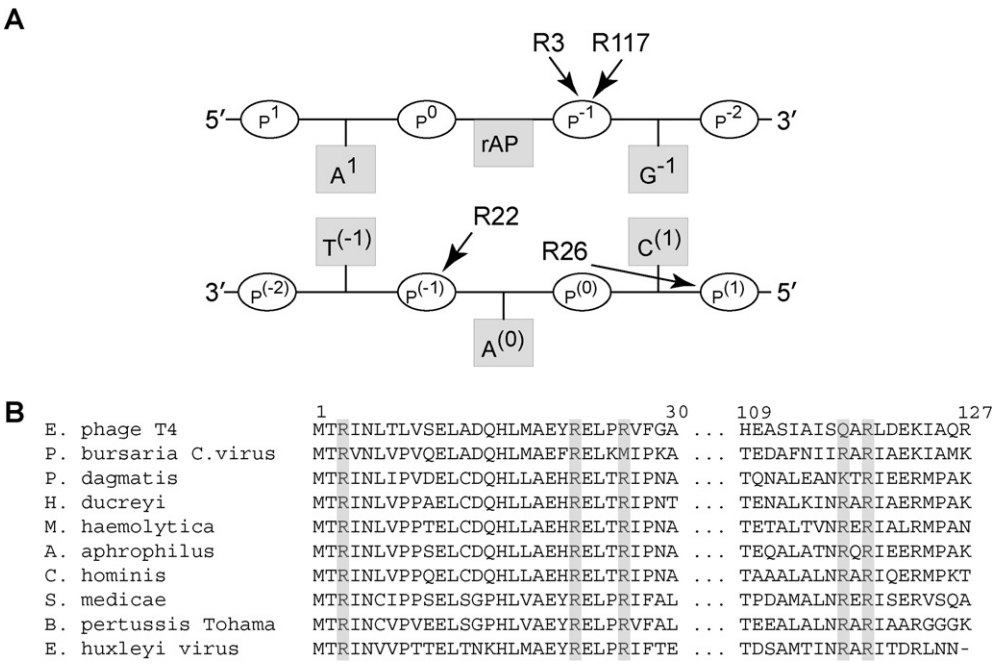

Fig. 1. Arginine residues in pyrimidine dimer glycosylases that may be important for DNA binding and processivity. (A) Schematic representation of the sites of interaction between T4-pdg and AP site-containing DNA as revealed by a co-crystal structure of a reduced imine covalent complex (derived from [14]). The reduced AP site-containing strand to which the N-terminal threonine of T4-pdg is linked is shown as the upper strand in which both arginine (R) 3 and 117 bind to the p^{-1} phosphate immediately 3′ to the site of covalent linkage. In the complementary strand (lower) the extrahelical nucleotide ($A^{(0)}$) is bound by R22 and R26 at $p^{(-1)}$ and $p^{(1)}$, respectively. (B) Alignment of highly conserved arginine residues within phylogenetically related pdgs. *Enterobacteria phage T4*; *Paramecium bursaria Chlorella virus*; *Pasteurella dagmatis*; *Haemophilus ducreyi*; *Mannheimia haemolytica*; *Aggregatibacter aphrophilus*; *Cardiobacterium hominis*; *Sinorhizobium medicae*; *Bordetella pertussis Tohama*; *Emiliania huxleyi virus*. Biochemical characterization of pdg activity has only been confirmed for *Enterobacteria phage T4* and *Paramecium bursaria Chlorella virus*.

critical for binding to non-damaged, as well as CPD- or AP site-containing DNA.

Under physiologically relevant salt concentrations, wild-type T4-pdg or Cv-pdg encounter DNA *via* a random collision mechanism, followed by association with, and binding to non-damaged DNA, a process that is guided by local charge–charge interactions in the microenvironment of the DNA. This general binding to DNA is maintained over considerable distances such that experimentally, it is observed that these enzymes will incise most, if not all, CPDs in an ~5 kb plasmid containing 5–10 CPDs per DNA prior to dissociation [16]. Analyses of the co-crystal structures of a catalytically inactive mutant, T4-pdg (E23Q), with CPD-containing DNA [15] and the covalent, reduced-imine intermediate of wild-type T4-pdg with AP site-containing DNA [14] revealed multiple sites of contact between basic amino acid residues, primarily arginines, and DNA in the local vicinity of the lesion (Fig. 1A). Protein sequence alignments of genes with homology to Cv-pdg and T4-pdg revealed significant conservation of these sites (Fig. 1B). Molecular modeling of Cv-pdg, using T4-pdg as a template, predicts a similar charge distribution [17]. It was hypothesized that many of these residues are critical for establishing correct pre-catalytic alignment and orientation of the enzyme on non-damaged DNA and thus, facilitate location of sites of damaged DNA. To test this hypothesis, site directed mutants were made at these sites in T4-pdg and Cv-pdg, and analyzed *in vitro*.

Since human cells are unable to initiate BER at sites containing CPDs, numerous studies have investigated the effects of expressing or introducing T4-pdg or Cv-pdg in mammalian cells [18–27], with an ultimate objective being to use these enzymes in human therapeutics [28]. Collectively, these studies demonstrated that introduction of T4-pdg resulted in: (1) accelerated rates of CPD repair; (2) increased unscheduled DNA repair synthesis; (3) suppression of UV-induced mutagenesis in wild-type cells; (4) increased survival of most NER-deficient cells; and (5) significantly reduced survival of normal repair-proficient cells. In human clinical trials, wild-type T4-pdg was encapsulated into a liposomal delivery vehicle and topically used as a treatment for NER deficient xeroderma pigmentosum (XP) patients [28]. In a one year randomized trial, XP patients who received the T4-pdg-containing lotion showed reduced rates of new actinic keratoses and basal cell carcinomas (68% and 30%, respectively) relative to placebo treated patients. These data are very consistent with the survival studies using XP-derived cells in which activation of BER for CPDs was beneficial [24].

However, since expression of wild-type T4-pdg in repair-proficient mammalian cells resulted in decreased, not enhanced survival, these data suggest that the activity of the enzyme can produce cytotoxic repair intermediates, possibly through the formation of double-strand breaks at clustered CPDs. Similar mechanisms of increased cytotoxicity have been postulated for other glycosylases, NTH1, OGG1 and MAG1 due to their overexpression [29–31]. Insights into a biochemical basis for decreased survival of wild-type mammalian cells expressing T4-pdg come from studies that demonstrated the ability of T4-pdg to catalyze a processive nicking activity in duplex DNA. This activity leads to incision of most, if not all, CPDs in plasmid DNA prior to dissociation of the enzyme from the DNA molecule to which it was initially bound, both *in vitro* and *in vivo* [16,32–34]. The current investigation was designed to test the hypothesis that neutralization of selected positively charged residues in the vicinity of the active site of T4- and Cv-pdg could minimize or eliminate the processive nicking activity of these enzymes without affecting the efficiency of catalysis.

2. Materials and methods

2.1. Site-directed mutagenesis

Site-directed mutagenesis at the designated positions in the T4-pdg and Cv-pdg genes was performed using the bidirectional QuickChange Site-Directed Mutagenesis Kit™ (Stratagene, Santa Clara, CA). Primers for mutagenesis were synthesized by