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Discrimination against major groove adducts by Y-family polymerases of the DinB subfamily

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ABSTRACT

Y-family DNA polymerases bypass DNA adducts in a process known as translesion synthesis (TLS). Y-family polymerases make contacts with the minor groove side of the DNA substrate at the nascent base pair. The Y-family polymerases also contact the DNA major groove via the unique little finger domain, but they generally lack contacts with the major groove at the nascent base pair. *Escherichia coli* DinB efficiently and accurately copies certain minor groove guanosine adducts. In contrast, we previously showed that the presence in the DNA template of the major groove-modified base 1,3-diaza-2-oxophenothiazine (tC) inhibits the activity of *E. coli* DinB. Even when the DNA primer is extended up to three nucleotides beyond the site of the tC analog, DinB activity is strongly inhibited. These findings prompted us to investigate discrimination against other major groove modifications by DinB and its orthologs. We chose a set of pyrimidines and purines with modifications in the major groove and determined the activity of DinB and several orthologs with these substrates. DinB, human pol kappa, and *Sulfolobus solfataricus* Dpo4 show differing specificities for the major groove adducts pyrrolo-dC, dP, N^6-furfuryl-dA, and etheno-dA. In general, DinB was least efficient for bypass of all of these major groove adducts, whereas Dpo4 was most efficient. DinB activity was essentially completely inhibited by the presence of etheno-dA, while pol kappa activity was strongly inhibited. All three of these DNA polymerases were able to bypass N^6-furfuryl-dA with modest efficiency, with DinB being the least efficient. We also determined that the R35A variant of DinB enhances bypass of N^6-furfuryl-dA but not etheno-dA. In sum, we find that whereas DinB is specific for bypass of minor groove adducts, it is specifically inhibited by major groove DNA modifications.

1. Introduction

The Y-family DNA polymerases were first identified as a class of DNA polymerases in 2001 [1] and are conserved throughout all domains of life [2–4]. They are characterized by their ability to copy damaged DNA in a process known as translesion synthesis (TLS). The polymerase domains of Y-family polymerases resemble a right hand, with domains identified as the thumb, palm, finger, and little finger [2,3]. The finger domains in Y-family polymerases tend to be smaller than those of replicative DNA polymerases, leading to virtually no major groove contacts at the nascent base pair [4]; the little finger, which is unique to the Y-family, makes contacts on the major groove side of the DNA, although not at the nascent base pair. Thus, major groove DNA adducts are not expected to be subject to the same steric constraints as minor groove adducts, as they seem to protrude into a solvent-accessible area between the little finger and the fingers domains [5–11]. In general, replicative DNA polymerases are also tolerant of major groove modifications [12,13].

Y-family DNA polymerases bypass a wide variety of DNA damage [4,5,7,14–21] including interstrand DNA crosslinks [22], protein–DNA crosslinks [23], other bulky adducts [5,7,24], thymine–thymine dimers [8], and damage induced by reactive oxygen species [25–28]. *Escherichia coli* Y-family DNA polymerase IV (DinB) is proficient in its ability to bypass minor groove N^2 adducts of guanosine, in particular DinB bypasses N^2-furfuryl-dG ~15-fold more efficiently than it bypasses templates containing the natural nucleotide dG [16]. Human pol κ, a DinB ortholog whose polymerase domain is 38% identical to that of DinB, is also proficient in bypassing bulky adducts such as benzo[a]pyrene at the N^2 position of dG [24,29–32]. Substitution of F171 with Ala in human pol κ led

Abbreviations: pol, polymerase; *E. coli*, *Escherichia coli*; *S. solfataricus*, *Sulfolobus solfataricus*; fdA, N^6-furfuryl-dA; εdA, 1,N^6-ethenodeoxyadenosine.

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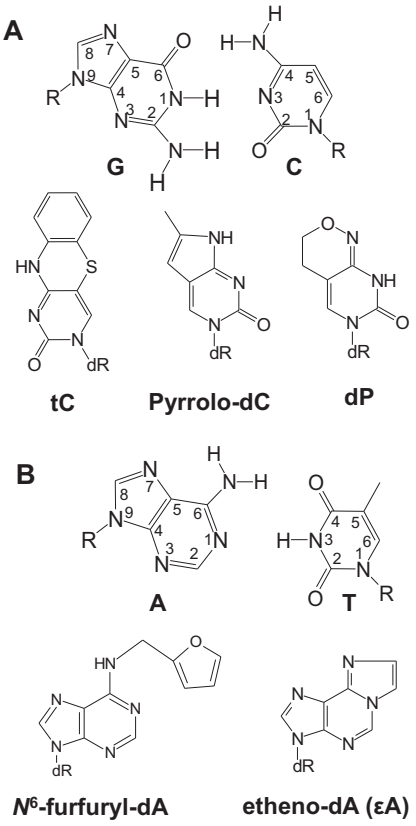

Fig. 1. Structures of modified bases. (A) The canonical Watson–Crick base pair G:C and cytosine analogs pyrrolo-dC and dP. (B) The canonical Watson–Crick base pair A:T and adenosine analogs N^6-furfuryl-dA (fdA) and etheno-dA (εdA).

to more efficient bypass of N^2-benzo[a]pyrene-dG [33]. *Sulfolobus solfataricus* Dpo4, also considered a DinB ortholog and whose polymerase domain is 33% identical to that of DinB, has a similar preference to bypass modified dG as it incorporates dCTP opposite 8-oxo-dG approximately ten times more efficiently than opposite natural G [28]. Dpo4 has been shown to bypass the N^2-dG adducts 1,N^2-etheno-dG and 7-(2-oxoheptyl)-etheno-dG, albeit relatively poorly [34]; similarly, pol κ can bypass heptanone-etheno-dC [35]. Minor groove N^2-dG lesions may block DNA synthesis by replicative polymerases, but can be bypassed with varying efficiencies by these Y-family DNA polymerases. Adducts at the N^6 position of dA, the O^6 position of dG, N^4 of dC or O^4 of dT lie on the major groove side of DNA (Fig. 1). We previously showed that DinB is inhibited by the cytosine analog with a bulky major groove modification 1,3-diaza-2-oxophenothiazine (tC) [6]. On the other hand, Dpo4 has been shown to bypass major-groove modified O^6-methylguanine accurately by incorporating dCTP a majority of the time; however, Dpo4 also misincorporates A and T at frequencies of 20% and 10%, respectively [36]. Human pol κ has also been shown to bypass O^6-methylguanine efficiently [24,37]. When human DNA polymerases κ, η, ι and yeast pol ζ were tested for their ability to accomplish TLS on both stereoisomers of the bulky lesion benzo[a]pyrene at the N^6 position of dA, only pol η was capable of fully extending DNA beyond the (+)-trans-benzo[a]pyrene steroisomer [31].

To extend our previous findings that the major-groove modified base tC blocks DinB activity [6], in this report we investigate the ability of DinB, Dpo4 and pol κ to carry out TLS on two major groove-modified pyridimines, dP and pyrrolo-dC, and two major groove adducts of adenosine, N^6-furfuryl-deoxyadenosine (fdA) and 1,N^6-ethenodeoxyadenosine (εdA) (Fig. 1). Pyrrolo-dC and dP were selected because their major groove modifications are slightly smaller than that of tC (Fig. 1). The modified pyrimidines tested were reasonably good substrates for all three polymerases. The N^6-furfuryl-deoxyadenosine lesion, also known as kinetin, was chosen as one of the major groove purine adducts in this study because it has been observed in both plant and animal tissues [38–40]. The furfuryl moiety is thought to arise from the metabolism of ribose [41,42], as well as from treatment with nitrofurazone, a veterinary antibiotic and suspected carcinogen [43]. Notably, the analogous minor groove lesion, N^2-furfuryl-dG, is considered a cognate lesion of DinB [16] and we reasoned that, although the exact mechanism of formation is unknown, the two adducts could arise through similar routes, as agents that form adducts at the N^2-dG position can also modify the exocyclic N^6 of dA [2]. All three polymerases showed modest bypass of N^6-furfuryl-dA, with Dpo4 being the most efficient. The εdA adduct, a slightly smaller but more rigid major groove modification than N^6-furfuryl-dA, results from exposure to the dangerous carcinogen vinyl chloride [44,45], as well as endogenous aldehyde derived from lipid peroxidation [46]. Human pol κ has been shown to perform very weak TLS with templates containing εdA [47], which we also observe; DinB is inactive on this adduct while Dpo4 is able to bypass it.

2. Materials and methods

DinB was purified as described previously by Beuning et al. [48] and stored in single-use aliquots at −80 °C. Purification of Dpo4 [49,50] and pol κ [24] were carried out as described. The DNA template containing a single N^6-furfuryl-deoxyadenosine was prepared as described from O^6-phenyl-deoxyinosine (Glen Research) [51]. Pyrrolo-dC, ethenodA, dP phosphoramidites and other DNA synthesis reagents were from Glen Research. Etheno-dATP was from Chemgenes; this was the only modification chosen to be assayed as the incoming nucleotide because it was the most inhibitory as the template base and because it is commercially available. DNA sequences used in primer extension assays are shown in Table 1, where template base X = A, C, T, N^6-furfuryl-deoxyadenosine, 1,N^6-ethenodeoxyadenosine, pyrrolo-dC, or dP (Glen Research). We used primers in which the termini were before or after the site of the modified template base [52,53], in order to determine the effect of the modification present in single-stranded DNA ahead of the polymerases or in double-stranded DNA behind the polymerase. DNA containing modifications was synthesized on an Expedite 8909 synthesizer; unmodified DNA was from MWG Operon. DNA primer (running start, standing start, or one of the MatchT series) and each corresponding template were combined to a final ratio of 1:1 (500 nM) and annealed in annealing buffer [20 mM Hepes (pH 7.5) and 5 mM $Mg(OAc)_2$] by heating for 2 min at 95 °C, incubating at 50 °C for 60 min, and then cooling to 37 °C. The reactions were carried out with 100 nM DNA and data were analyzed as described [6]; other reaction details are described in the appropriate figure legends.

3. Results

3.1. Cytosine analogs dP and pyrrolo-dC modestly inhibit DinB

In order to extend our observation that the bulky, major-groove-modified pyrimidine tC blocks replication by *E. coli* DinB [6], we assayed the activity of DinB as well as that of human pol κ and Dpo4 in bypass of other major groove modified pyrimidines pyrrolo-dC and dP. Pyrrolo-dC and dP (Fig. 1) were chosen because they are slightly smaller than tC with only one additional ring in the major groove and because they are commercially available. In primer extension assays with pyrrolo-dC and dP, DinB was moderately active and was more active than on DNA containing tC [6]. DinB