

## Energetic Preference of 8-oxoG Eversion Pathways in a DNA Glycosylase

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 Supporting Information

**ABSTRACT:** Base eversion is a fundamental process in the biochemistry of nucleic acids, allowing proteins engaged in DNA repair and epigenetic modifications to access target bases in DNA. Crystal structures reveal end points of these processes, but not the pathways involved in the dynamic process of base recognition. To elucidate the pathway taken by 8-oxoguanine during base excision repair by Fpg, we calculated free energy surfaces during eversion of the damaged base through the major and minor grooves. The minor groove pathway and free energy barrier (6–7 kcal/mol) are consistent with previously reported results (Qi, Y.; Spong, M. C.; Nam, K.; Banerjee, A.; Jiralerspong, S.; Karplus, M.; Verdine, G. L. *Nature* **2009**, *462*, 762.) However, eversion of 8-oxoG through the major groove encounters a significantly lower barrier (3–4 kcal/mol) more consistent with experimentally determined rates of enzymatic sliding during lesion search (Blainey, P. C.; van Oijst, A. M.; Banerjee, A.; Verdine, G. L.; Xie, X. S. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 5752.). Major groove eversion has been suggested for other glycosylases, suggesting that in addition to function, dynamics of base eversion may also be conserved.

8-Oxoguanine (8-oxoG), one of the most abundant products of oxidative DNA damage in cells, arises from oxidation of guanine by endogenous and exogenous reactive oxygen species.<sup>1</sup> 8-oxoG readily pairs with adenine in a Hoogsteen orientation; during DNA replication, this conformation results in a transversion mutation.<sup>2</sup> Base excision repair (BER) pathways in eukaryote and prokaryote cells excise 8-oxoG to preserve genomic integrity. In prokaryotes, formamidopyrimidine-DNA glycosylase (Fpg), also known as MutM, excises 8-oxoG when the oxidized base is paired to cytosine.<sup>3</sup> Human oxoguanine glycosylase (hOGG1) is a functional homologue of Fpg, but the two enzymes share little structural homology.<sup>4</sup>

The base eversion mechanism is not limited to positioning damaged bases for enzymatic catalysis, and also plays a role in determining selectivity of substrates. Eversion of bases from the helix facilitates recognition of substrates. For example, studies of uracil DNA glycosylase (UDG) using imino proton exchange NMR indicate that UDG has a passive role in trapping spontaneously opened bases, the rates of which are increased for the

substrate.<sup>5</sup> However, the relatively low barrier to sliding of hOGG1 and Fpg (about 2 kcal/mol per base pair), as determined by single-molecule fluorescence studies,<sup>6</sup> suggests these glycosylases do not extrude every base into an extrahelical site, and that substrate recognition takes place at an earlier stage.

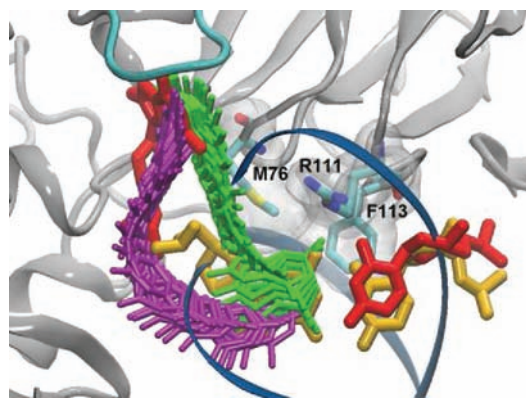
The low energy states of BER DNA glycosylases in general, and Fpg and hOGG1 in particular, have been characterized, primarily by X-ray crystallography. Catalytically inactive Fpg mutants allow trapping of substrates containing 8-oxoguanine in the active site.<sup>7</sup> Intermolecular cross-linking has been used to generate structures of putative intermediates in the catalytic pathway<sup>8,9</sup> as well as an intrahelical structure, interpreted as initial binding of Fpg to 8-oxoG damaged<sup>10</sup> and undamaged<sup>11</sup> DNA. Figure 1 illustrates the initial intrahelical and final extrahelical end points of 8-oxoG in the flipping pathway. Visual inspection suggests the minor groove pathway as the more direct route. However, crystal structures of hOGG1 complexed to DNA have been reported with bases in the major groove at putative intermediate locations along the eversion pathway.<sup>12,13</sup> A UDG crystallographic study also points to a major groove pathway to avoid unfavorable steric clashes with protein side chains in the minor groove.<sup>14</sup>

Molecular dynamics (MD) provides an unparalleled ability to simultaneously connect structure, dynamics and energy for conformational changes. To generate a minimum potential energy path (MEP) from the intrahelical to the extrahelical end point, the partial nudged elastic band (PNEB)<sup>15</sup> implementation in Amber10<sup>16</sup> was used with all-atom simulations in explicit water. Both paths begin and end with the same intrahelical and extrahelical coordinates, and are continuous in all solute degrees of freedom. The optimized, time-independent paths are shown in Figure 1 (population histograms along each PNEB path are provided in Supporting Information, Figures S1 and S2). The minor groove path rotates around the glycosidic torsion early in eversion to minimize unfavorable steric clashes and form favorable electrostatic interactions with the intercalating triad (M76/R111/F113, see Figure 1). The major groove path shows no steric hindrance for initial breaking of the intrahelical hydrogen bonds.

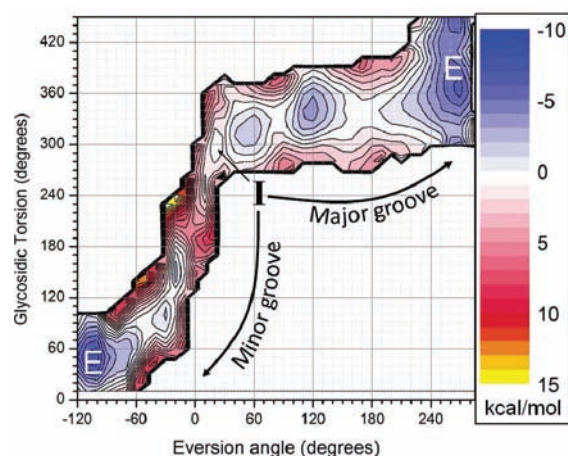
Two-dimensional umbrella sampling was performed to obtain free energy profiles along these paths, using two previously reported reaction coordinates that measure base eversion and the glycosidic torsion rotation.<sup>17,18</sup> The resulting potential of mean force (PMF), shown in Figure 2 (with independent runs

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**Figure 1.** Base eversion paths from NEB calculations. Paths are indicated by snapshots, with major groove shown in purple and minor groove in green. Intrahelical 8-oxoG:C base pair is shown in yellow, extrahelical 8-oxoG:C base pair, in red, catalytic loop, in cyan.



**Figure 2.** Free energy pathways of base eversion via the major (top) and minor (bottom left) grooves. The initial intrahelical state I is in the middle, and the final extrahelical state E is reached on both the right and left sides of the graph. Free energies are given in kcal/mol.

shown in Figure S3), reveals free energy profiles for both pathways. Eversion through the minor groove encounters an energetic barrier of 6–7 kcal/mol; this observation is consistent with the  $\sim 10$  kcal overall barrier to extrusion into Fpg's active site reported in Qi et al. (who reported only a minor groove pmf).<sup>10</sup> Although a small energy penalty is incurred upon breaking intrahelical hydrogen bonds, the main barrier to entering the active site corresponds to initial rotation of the glycosidic torsion.

The major groove PMF indicates a maximum overall barrier of 3–4 kcal/mol, significantly less than through the minor groove. As suggested<sup>14</sup> for UDG, the major groove pathway avoids steric clashes, and instead the smaller barrier arises from eversion past the  $\beta$ -hairpin of the zinc finger motif. 8-oxoG partially rotates to form a stable intermediate (eversion angle =  $120^\circ$ ) that may have an important role in substrate recognition. Crystal structures of hOGG1 indicate a similar *exo* site where undamaged guanine is trapped to avoid processing.<sup>19</sup> The major groove intermediate may provide a similar readout mechanism by Fpg, allowing 8-oxoG to continue along the path to the active site, while discriminating against G. This will be discussed in detail elsewhere.

In summary, we have used MD simulations to show the complete free energy profile of base eversion for Fpg with its 8-oxoG substrate. Our PMF indicates a free energy barrier to flipping into the active site that is significantly lower for major groove eversion (corresponding to  $\sim 2$ – $3$  orders of magnitude faster eversion rate), indicating that this is the preferred path for lesion processing in Fpg. Interestingly, the human functional analogue hOGG1 is also believed to involve base eversion via a major groove pathway, reinforcing the functional conservation between the prokaryotic and eukaryotic enzymes.<sup>19,20</sup> Our data suggest that Fpg not only shares functional similarity to hOGG1, but convergent evolution is also present in dynamic aspects of base recognition by DNA glycosylases.

## ■ ASSOCIATED CONTENT

**S Supporting Information.** Detailed methods, histograms, convergence analysis and complete ref 16. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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