Supporting Information

hOGG1 removes solution-accessible 8-oxoG lesions from globally-substituted nucleosomes except at the dyad region

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Includes:

Experimental Procedures

Oligonucleotide Synthesis and Purification

145mer oligomers were synthesized on a MerMade 4 (BioAutomation) DNA synthesizer using phosphoramidite chemistry. All synthesis reagents and phosphoramidites were purchased from Glen Research. The sequences of the oligomers are detailed in Scheme S1. For oligomers containing 8-oxoG (lesion strand), incorporation of 8-oxoG was carried out according to Glen Research protocols. We mixed G and 8-oxoG phosphoramidite to obtain 95% of lesion-containing strands with zero or one 8-oxoG lesions as predicted by a Poisson distribution (λ=0.355). As the Widom 601 sequence contains 34 guanines, based on the Poisson distribution we used a 1% molar ratio of 8-oxoG:G amidite in our synthesis. The trityl group was removed on the synthesizer before purification and 2-mercaptoethanol was included during cleavage of the oligomer from the bead. Full-length 145mer synthesis products were purified by 8% denaturing PAGE (0.8 mm thickness). Oligonucleotide concentrations were determined by their absorbance at 260 nm using molar extinction coefficients calculated with OligoAnalyzer 3.1 (www.idtdna.com).

Glycosylase Expression and Purification

His6-tagged hOGG1 was recombinantly expressed in *E. coli* and purified as previously described.[1, 2] SDS PAGE analysis showed hOGG1 purity to be >98%. The concentration of hOGG1 was determined by Bradford assay with γ-globulin standards (Bio-Rad Laboratories).

Nucleosome Core Particle Reconstitution

Expression and purification of canonical *X. laevis* histone proteins (H2A, H2B, H3, and H4) and assembly of the histone octamer were performed according to the published method of Luger, *et al*.[3, 4] NCPs were prepared by stepwise dialysis as reported previously.[2, 5] Briefly, a Slide-a-Lyzer MINI dialysis device was equilibrated in buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, 1 mM dithiothreitol [DTT], 2 M NaCl) at 4 °C. Duplex

radiolabeled on the lesion-containing strand (50 μL of 1 μM) was added to the dialysis device and allowed to incubate for 30 min before addition of histone octamer in a 1:1.05 molar ratio. The concentration of NaCl in the dialysis buffer was progressively lowered at 60 min intervals (1.2 M, 1.0 M, 0.6 M, 0 M). The final dialysis step was carried out for 3 h. Samples were filtered to remove precipitated protein and/or DNA. NCP formation was confirmed by 7 % non-denaturing polyacrylamide (60:1 acrylamide:bisacrylamide; 0.25X TBE) gel electrophoresis (3 h at 150 V, 4 °C) (Figure S1). Only NCP preparations with less than 5 % free duplex DNA were used in the experiments.

Glycosylase Activity Test

Experiments to measure glycosylase activity were based on previously published protocols.[2] Briefly, radiolabeled, lesion-containing substrate and glycosylase were prepared at 2 X experimental concentration (40 nM for duplex and NCP; 1.28 μM for hOGG1) in reaction buffer (20 mM Tris-HCl [pH 7.6], 25 mM NaCl, 75 mM KCl, 1 mM EDTA, 1 mM DTT, 200 μg/ml BSA). Following a temperature pre-equilibration at 37 °C for 2 min, equal volumes of substrate (24 μL) and glycosylase (24 μL) preparations were mixed and incubated for 1 h at 37 °C. Samples contained 20 nM duplex or NCP, 0.64 μM hOGG1, 20 mM Tris-HCl [pH 7.6], 25 mM NaCl, 75 mM KCl, 1 mM EDTA, 1 mM DTT, 200 μg/ml BSA). Reactions were terminated by addition of 1M NaOH quench (48 μL, final concentration 500 mM). A negative control sample (QC) was prepared by adding 1 M NaOH quench (48 μL) to substrate (24 μL) followed by addition of glycosylase (24 μL) before incubation at 37 °C for 1 h. This control serves to reveal any pre-existing damage or incidental damage due to heating or sample work-up. Samples were heated to 90 °C for 2 min after addition of quench to induce a strand break at abasic sites. DNA was isolated from proteins by extraction with 25:24:1 phenol:chloroform:isoamyl alcohol before desalting by ethanol precipitation with the addition of 20 μL co-precipitation reagent (0.5 mg/mL tRNA in 300 mM sodium acetate [pH 8.0], 1 mM EDTA).

In addition, hydroxyl radical footprinting (HRF) was performed based on previously published methods.[6, 7] Briefly, 5 pmol of NCPs containing ^{32}P radiolabeled DNA were suspended in 52.5 μL buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) and mixed with 7.5 μL each 10 mM Fe(II)-EDTA, 10 mM sodium ascorbate, and 0.12% w/v aqueous hydrogen peroxide. The reaction was incubated at ambient temperature for 10 min before quenching with the addition of 50 μL 1 mM EDTA in 25% w/v glycerol. The sample was immediately loaded on a pre-running 7% non-denaturing polyacrylamide (60:1 acrylamide:bisacrylamide, 0.25 X TBE) and electrophoresed for 3 h at 150 V at 4°C. The band containing NCPs was excised and NCPs were eluted into buffer (300 mM sodium acetate [pH 8.0], 1 mM EDTA) overnight with gentle shaking (60 rpm at 37°C). The resulting eluent was concentrated (Sartorius Vivaspin Turbo 15, 5 kDa MWCO) and extracted twice against 25:24:1 phenol:chloroform:isoamyl alcohol. An ethanol precipitation was performed as described above.

All samples were dissolved in formamide and electrophoresed on an 8% denaturing PAGE. The gel was dried and exposed before phosphorimaging (BioRad Pharos FX) and quantitation using SAFA gel analysis software.[8] The band intensity of the QC at each band position was subtracted from duplex DNA and NCP substrates. Three

replicates of the experiments were performed, using NCP from separate reconstitutions. Gels were used to assign the relative degree of lesion excision and were in general agreement between replicates. The data in Figure 3 are from a single replicate. For hOGG1 activity on NCPs the data are presented as ratio of integrated band area following hOGG1 cleavage of NCP substrates relative to free duplex DNA. By presenting the data as the ratio of integrated band area the data are normalized to correct for the decreased propensity for small DNA fragments to precipitate during sample workup. Only data for 8-oxoG sites are shown in Figure 3C; data at other sites is not shown because a large band area for NCP reactivity divided by a large band area for duplex reactivity (for example, at 8-oxoG) in some cases ends up equivalent to a small band area in NCP reactivity divided by a small band area in duplex reactivity (i.e., at non-8-oxoG sites) under this operation.

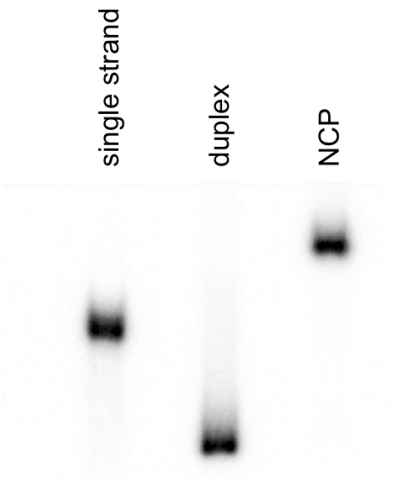

Figure S1. Representative non-denaturing PAGE characterization of NCP with G to 8-oxoG substitutions. Radiolabeled samples were loaded on a 1 mm thick 7% native gel (60:1 acrylamide:bisacrylamide, 0.25X TBE). The gel was run at 4°C for 3 hours at 150 V. Variable migration distances are observed for single strand, duplex, and NCP-incorporated DNA samples.

Scheme S1. Oligonucleotide sequences used in this work
The globally substituted 145mer oligomers were based on the Widom 601 positioning DNA sequence.[9]

145mer lesion strand (numbering based on "I strand" of Vasudevan *et. al.*) [10]

5′- ATC AGA ATC CCG GTG CCG GGG CCG CTC AAT TGG TCG TAG ACA GCT CTA GCA CCG CTT AAA CGC ACG TAC GCG CTG TCC CCC GCG TTT TAA CCG CCA AGG GGA TTA CTC CCT AGT CTC CAG GCA CGT GTC AGA TAT ATA CAT CGA T -3′

145mer complement strand (numbering based on "J strand" of Vasudevan *et. al.*) [10]

5′- ATC GAT GTA TAT ATC TGA CAC GTG CCT GGA GAC TAG GGA GTA ATC CCC TTG GCG GTT AAA ACG CGG GGG ACA GCG CGT ACG TGC GTT TAA GCG GTG CTA GAG CTG TCT ACG ACC AAT TGA GCG GCC CCG GCA CCG GGA TTC TGA T -3′

Supporting References

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