Final Report on Grant DE-FG-02ER63350
Damage Recognition, Protein Signaling and Fidelity of Base Excision Repair
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Research funded by grant DE-FG-02ER63350 focused on DNA bending measured NMR spectroscopy and modeled by classical molecular dynamics (MD) simulation. Bending is a structural aspect of DNA that plays a key role in its function. The “architectural” transcription factors do not recognize their response elements in DNA by base sequence; rather, they are attracted to regions of DNA where the magnitude and direction of DNA bending enhances their binding into the minor groove¹. Similarly, intrinsic DNA curvature directs drugs to a compressed minor-groove environment². The most important finding of our research was that oxidation of guanine, a ubiquitous DNA lesion caused by endogenous and environmental oxidative stress, changes DNA bending dynamics in a way that favors binding of glycosylases. Hence, the effect of 8-oxoguanine on DNA bending contributes to its recognition and removal by the base excision repair system³.

Observation of bent DNA structures has been hampered for a long time by the inability of NMR to measure long-range restraints for structural refinement. Research supported by this grant help to overcome this difficulty by pioneering the use of residual dipolar coupling in NMR structure determination⁴. Our theoretical and experimental efforts came together to investigate of an aspect of DNA bending that has been a mystery for the last 20 years. When DNA segments containing A_nT_n sequences are ligated in phase with helical turns, the resulting DNA migrates in polyacrylamide gels electrophoresis (PAGE) much slower than random-sequence DNA. Anomalous PAGE retardation is not exhibited by T_nA_n segments ligated in phase, even though the base sequence is the same as phased A_nT_n segments. Macroscopic bending is widely accepted as the reason for PAGE retardation of phased A_nT_n segments; however, no consensus exists on the question of why phased T_nA_n segments are not equally bent.

We used the technique developed to study bending dynamics in MD simulations³ to quantify bending of the helical axis in NMR-determined structures⁵ of the DNA oligonucleotide $d(CGAGGTTTAAACCTCG)_2$. Due to the length of this DNA fragment and the application of long-range restraints to accurately determine its global structure, we were able to quantify the magnitude and direction of bending in the two halves of the self-complementary sequence as well as the overall curvature. The extent of bending in the two halves was not significantly different (radii of curvature 72 ± 33 nm and 69 ± 14 nm), but the directions of bending was out of phase (approximately into the minor groove at the midpoint of each half. Consequently, the overall curvature of the oligonucleotide was small (radius of curvature 138 ± 23 nm). These data support the conclusion that multiple bends in T_nA_n segments tend to cancel at the TpA step so that overall bending is small and does not greatly affect migration on PAGE when the segments are ligated in phase.

Literature Citations:

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