INTRODUCTION

The ability of the genome to support normal cellular homeostasis and to transmit genetic information is directly dependent upon how well its covalent structure is preserved. Inside the cell, DNA is under constant attack by exogenous environmental toxins and cellular metabolites, free radicals, oxidizing agents etc. Damage to a base in DNA duplexes is followed by either chemical or enzyme catalyzed hydrolysis of the N-glycosidic bond to yield a baseless site (1, 2). Thus the removal of a heterocyclic base by the selective hydrolysis of an N-glycosidic bond produces either an apurinic or an apyrimidinic (AP/abasic) site in a DNA sequence (3). For example, the spontaneous hydrolysis of the 4-amino group of cytosine to yield uracil occurs at a genetically significant rate. Since this lesion is mutagenic, the cells of all organisms contain the enzyme uracil-DNA glycosylase which hydrolyzes the N-glycosidic bonds of deoxyuridine residues to release uracil (Figure 1).

![Figure 1](image1.png)

The resulting mixture of open chain aldehyde and hydrate and cyclic hemiacetals (Figure 2) is termed as an abasic site. This DNA lesion, generally represented is referred to as abasic/AP site.

![Figure 2](image2.png)
In solution, they exist predominantly as a mixture of ring-closed $\alpha$- and $\beta$-hemiacetals with a minor amount (<1%) of ring-opened aldehyde and aldehyde hydrate (4, 5). The aldehyde form is susceptible to base-catalyzed $\beta$-elimination that leads to a DNA strand break (6). The half-life of an abasic site in oligomeric duplex DNA has been measured by Stubbe group (7) and the Sheppard group in five different sequence contexts and ranges from 200 to 900 h under physiological conditions of pH 7.5, 150 mM NaCl at 37 °C (8, 9).

**LITERATURE SURVEY OF DNA ABASIC SITES**

**BIOCHEMICAL STUDIES**

A wide range of mutagenic, carcinogenic and antitumor agents, which damage DNA, have abasic and related sites as a common intermediate in repair. Progress in chemical and biophysical methods have significantly contributed to our understanding of the structure and reactivity of abasic sites. Molecular, biological and biochemical methods have advanced our knowledge concerning the importance and fate of these sites.

Abasic sites are produced as an initial step in DNA repair by the protective action of N-glycosylases, which remove damaged or abnormal bases, including those resulting from reactions of DNA with environmental carcinogens (10-11). Most of the abasic sites are believed to result directly from spontaneous depurination (12), or indirectly from deamination of cytosine to uracil, which is then eliminated by uracil glycosylases (13). Abasic sites also result from hydrolysis of oxidized or alkylated bases by lesion-specific glycosylases (14-16). The abasic sites are common DNA lesions. Lindahl has estimated that there is a constant level of approximately 10000 abasic sites for a typical human cell (12). For a typical *Escherichia coli*, there are about 40-400 such events per cell division and in a typical mammalian cell 4000-40000 uracil formed per cell division (1, 12, 17-20).

To prevent the deleterious effect of damage such as mutagenesis, diseases and cell death, all organisms studied to-date have evolved a variety of DNA repair processes specific of the type of DNA damage (21, 22). The modifications of the heterocyclic DNA base that are caused by agents involved in normal cellular metabolism resulting in oxidation, alkylation, deamination or base loss, in turn resulting in the formation of an abasic (AP) site, are principally addressed by the Base Excision Repair (BER) pathway (23). The DNA glycosylases, the key enzymes of the BER pathway, specifically recognize and excise the damaged base by the activity of DNA glycosylase. *In vivo*, the
abasic sites are thought to be repaired via the base excision repair pathway in humans, which initially involves apurinic/apyrimindic endonuclease/APE 1 (24, 25). Unrepaired abasic sites are mutagenic. AP sites that remain in DNA undergoing replication present a blockade to continued synthesis by the replicative polymerases (Pols). Replication through the AP sites, however, can be mediated by the action of translesion synthesis (TLS) or DNA Pols, could occur by other means, such as a copy-choice type of DNA synthesis or recombination.

Because an AP site lacks a base, it is the ultimate non-instructional DNA lesion, and any replication through it would be highly mutagenic. In both prokaryotes and mammalian cells, an A-residue is predominantly inserted opposite the AP site, and biochemical studies have shown that replicative polymerases from prokaryotes as well as eukaryotes preferentially insert an A opposite the AP site (11, 26-31). In addition, NMR studies have indicated that DNA duplex containing an A opposite the AP site retains all features of B-form DNA, and both the unpaired A and AP residue lie inside the helix, intra-helically. These observations have led to the formulation of an A-rule, which posits that DNA Polymerases preferentially insert an A opposite a non-instructional DNA lesion, such as an AP site, because that confers the least amount of helix distortion.

**STRUCTURAL STUDIES**

First structural study on a DNA with abasic lesion was accomplished by Fazakerley and group (1987) with restrained Molecular Mechanics calculations on a nonamer DNA duplex (32, 33). First study on DNA duplex with an abasic furan lesion was furnished by Kalnik et al. (1988) from Patel group using NMR and Molecular Dynamics (MD) simulations. DNA was found to contain both abasic site and adenosine residue in the stacked-in intra-helical orientation (34).

Kumar et al. (1995) discussed the first structure of a DNA hetero duplex containing a natural abasic site where $\alpha$- and $\beta$-hemiacetals were found to be in a ratio of 40:60 (35). Bolton et al. (1997) evaluated the solution structure of the duplex with an A-tract and an abasic site, and studied that the presence of the abasic site in the A-tract region induced changes in the DNA structure up to four base pairs away from the damaged site (36). Bolton et al. (1998) also determined the detailed structure of a DNA duplex containing an abasic site by investigating apyrimidinic and apurinic duplex and concluded that the abasic site in duplex with pyrimidine-abasic site was predominantly present as a $\beta$-hemiacetal whereas in adenine-abasic site the $\alpha$- and $\beta$-hemiacetals of abasic site were equally
present (37). Bolton et al. (2008) developed a mix and measure fluorescent screening method that utilized multiple reporter molecules that bind to different features of quadruplex DNA (38).

![Tetrahydrofuran](image)

Figure 3

de los Santos et al. (1998) discussed the 3-D structure of a DNA heteroduplex containing a synthetic abasic site (tetrahydrofuran or abasic furan, Figure 3) residue positioned in a sequence that promoted deletion of one base (39). de los Santos et al. (2001) determined solution structure of two oligodeoxynucleotide duplexes containing adjacent tetrahydrofuran residues by NMR spectroscopy and MD simulations to understand the structural properties of clustered DNA lesions (40). de los Santos et al. (2008) also studied the orientation of bi-stranded lesions affects their recognition by DNA repair enzymes and are more toxic than single oxidative lesions. The structure of two DNA duplexes, each containing two stable abasic site analogues positioned on opposite strands of the duplex were compared by NMR spectroscopy and restrained molecular dynamics/r-MD (41).

Stubbe et al. (2001) with the help of 2D-NMR and modeling techniques discussed that both anomic forms in the abasic site were extra-helical without any significant distortion in the backbone opposite the lesion (42). Stubbe et al. (2007) determined the structure of 4’-oxidized abasic site (X) in a defined duplex DNA by 2D- NMR methods and MD simulations. The study revealed that the natural abasic site was conformationally more flexible than the 4’-oxidized abasic site (43). Stubbe et al. (2008) also investigated the structural models and conformational flexibility of duplex DNAs with four different bases (A, G, C or T) opposite an abasic site in the same sequence context (5’-CCAAAGXAXA8CCGGG-3’), where X denotes the abasic site. The results indicated that all four duplexes adopted an overall B-form conformation with each unpaired base stacked between adjacent bases intra-helically. The conformation around the abasic site was more perturbed when the base opposite to the lesion was a pyrimidine (C or T) than a purine (G or A) (7).
Boulard et al. (2003) by using $^1$H- and $^{31}$P-NMR and MD studied the presence of an N-(2-deoxy-b-D-erythro-pentofuranosyl) formamide (F) residue, a ring fragmentation product of thymine, in a frameshift context in a DNA duplex. Two dimensional NMR studies showed that the cis- or trans-isomers of the formamide residue were observed in the ratio 3:2 in solution. The $^{31}$P-NMR data revealed a modification of the phosphodiester backbone conformation at the extra-helical site (44).

Ross et al. (2001) performed time-resolved fluorescence experiments to characterize the states involved in this conformational change and proposed the contribution of altered conformation and dynamics of abasic sites in specificity of repair (45). Leumann et al. (2000) investigated the stability of an 18-mer oligonucleotide duplex containing one abasic site by analyzing UV melting curve and concluded that loss of stability encountered due to removal of nucleobase from the stack cannot be compensated with conformational restriction of abasic site (46).

Choi et al. (2000) employed NMR and MD to determine the solution structure of a Dewar-lesion DNA decamer duplex and put forward a study in accordance with the A-rule during trans-lesion replication. (47). MacKerell Jr. et al. (2000) examined the effect of abasic sites on DNA structure. Their main influence was on the effects of the abasic sites on the vicinity of the sites. The study was based on a double helical DNA dodecamers with and without single abasic site in 5’-d(C X T) and results indicated that abasic DNA can be twisted out of shape more easily and in specific ways relative to unmodified DNA (48).

Lavery et al. (1999) used Molecular modeling calculations using JUnction Minimization of Nucleic Acids (JUMNA) on DNA undecamer oligonucleotides to study sequence effects on the conformation of abasic sites within duplex DNA (49). Stivers (1998) with fluorescence emission and excitation spectra studied 10 different DNA duplexes by incorporating the fluorescent nucleotide probe 2-aminopurine, opposite to the site or adjacent to the site on either strand and described the metal-ion and sequence dependent changes in the stacking interactions of bases surrounding abasic sites. (50)

Breslauer et al. (1998) incorporated a tetrahydrofuran abasic site analogue into a family of 13-mer DNA duplexes, with the base opposite the lesion A, C, G, or T and concluded that: (i) the presence of the lesion in all sequence contexts studied does not alter the global B-form conformation characteristic of the parent undamaged duplex; (ii) the presence of the lesion induces a significant enthalpic destabilization of the duplex, with the magnitude of this effect being dependent on the sequence context; (iii) the thermodynamic impact of the lesion is dominated by the identity of the
neighboring base pairs, with the cross strand partner base exerting only a secondary thermodynamic effect on duplex properties (51).

Wilson et al. (1998) shown that (i) the ring structure of an Abasic site, (ii) the base opposite an Abasic site, (iii) the conformation of AP-DNA prior to protein binding and (iv) the F-266 residue of Ape are not critical elements in targeted recognition by AP endonucleases (52). Gao et al. (1996) explored with NMR-restrained computations in the presence of explicit water and determined a solution structure of an anti-parallel triplex (RRY6) containing a site of inversion (53). Recently, Galeone et al. (2009) using NMR and CD studies presented an insight into the influence of inversion of polarity sites on the structural features of quadruplex structures and suggested that the presence of inversion of polarity does not compromise the formation of quadruplexes and in some cases it increases the thermal stability of modified complexes compared with that of the unmodified one (54).

**HYPOTHESIS**

Damage to DNA bases can arise from a number of routes including oxidative stress, the action of various chemical agents, and by radiative processes. The role of the base opposite the abasic site is of interest because there are many routes by which an abasic site can be generated. Because the partner bases opposite abasic site and flanking bases are expected to participate in the interaction with repair enzymes (55-57). Elucidating their role in determining the local conformation of the abasic site is important in understanding the basis of recognition of abasic sites by the apurinic/apyrimidinic endonucleases.

NMR studies have indicated that DNA containing an A-residue opposite abasic site retained all features of B-form DNA, and both the unpaired A and abasic residue lied inside the helix, i.e. intra-helical orientation. Studies of Stubbe and co-workers using 2D-NMR spectroscopic methods on d-(5’-CCAAAGoXAoCTGGG)-3’, where X denotes the abasic site, indicated that the overall DNA structure was predominantly B-form, while the abasic site moiety is partially extra-helical (58).

Stubbe and group (7) have studied the abasic site in their sequence context, where the abasic site is flanked by two purines and A, T, G and C is placed opposite the abasic site. In this study, it was shown that all four duplexes containing abasic site adopted an overall B-form conformation with each unpaired base stacked between adjacent bases in intra-helical orientation. The conformation around the abasic site is more perturbed when the base opposite to the abasic lesion is a pyrimidine (C or T).
than a purine (G or A). This group has confirmed the conformation of abasic site when a purine or pyrimidine is placed opposite to the abasic site.

A fluorescence study by Stivers (50) showed an interesting aspect whether the base facing the AP site was intra-helical or extra-helical. When adenine faced the AP site, it was found to stack inside the helix regardless of the nature of the residues flanking the AP site (33). When guanine faces the site, it is found in a dynamic equilibrium between intra- and extra-helical conformations (33). When a pyrimidine faces the AB site, and the site is flanked by purine residues, then the pyrimidine is found in an extra-helical conformation, or a dynamic equilibrium between intra- and extra-helical conformations (33). Conversely, when the AB site is flanked by two pyrimidines, the pyrimidine opposite the abasic site is found in a stacked conformation (59). Thus, these NMR studies performed in the absence of divalent cations suggested a high degree of flexibility at AB sites, and that the conformation of the site is highly dependent on base facing the site and the stacking interaction energies between nucleotides flanking the site. Thus far, no systematic efforts have been reported on the influence of flanking and unpaired bases on the conformation of abasic sites (7).

It is fundamentally presumed that an A is always placed opposite abasic lesion, since thymidine residue is susceptible to chemical/radioactive damage. An A-residue is oxidative damaged at position 2 or a T-residue is placed opposite abasic lesion? What will be the role of flanking base pairs of AP site in making the abasic site intra-helical or extra-helical? There has not been a comprehensive structural study of such DNA duplexes by 2D-NMR.

**OBJECTIVES**

Hence, we are proposing to study the role of base pairs flanking the abasic site in a duplex DNA where the abasic site is placed opposite Thymidine residue. The abasic site is flanked by i) two purines on either side, ii) two pyrimidines on either side, iii) 5’-purine and 3’-pyrimidine and iv) 5’-pyrimidine and 3’-purine.

1) 5’-U X U/ 3’-Y T Y
2) 5’-Y X Y/ 3’-U T U
3) 5’-U X Y/ 3’-Y T U
4) 5’-Y X U/ 3’-U T Y

Where U = Purine, Y = Pyrimidine, X = Abasic site, and T = Thymine.
Such a study shall furnish the repair mechanism of such AP lesions, especially the role of flanking base pairs. We envisage that stacking energy of purine versus pyrimidine residue will be a determining factor in ensuring a stacked-in (intra-helical) or looped-out (extra-helical) orientation of AP residue and its partner base. Thus, we are proposing to carry out structural studies on following 11-mer oligonucleotide sequences to decipher the role flanking base pairs on the local structural perturbation.

1. DNA-1: 5’-C G G T C A C A T C G-3’
   3’-G C C A G T G T A G C-5’
2. DNA-2: 5’-C G G T C X C A T C G-3’
   3’-G C C A G T G T A G C 5’
3. DNA-3 5- C G G T G X C A T C G 3’
   3’-G C C A C T G T A G C 5’
4. DNA-4 5’-C G G T C X G A T C G 3’
   3’-G C C A G T C T A G C 5’
5. DNA-5 5’-C G G T G X G A T C G 3’
   3’-G C C A C T C T A G C 5’

MATERIALS AND METHODS

SAMPLE PREPARATION: DNA oligonucleotide sequences will be purchased from the commercial sources. The concentration of the solutions containing oligonucleotided sequence will be monitored by UV spectrum of the each solution. The experiments will be carried out in normal physiological pH (=7.0). The pH 7.0 buffer will be prepared with sodium phosphate, sodium chloride and EDTA in water or $^2$H$_2$O appropriately. (8, 9, 35)

NMR PROCEDURES:

The 500 MHz NMR spectra will be obtained using Bruker Spectrometer and the data will be processed using Bruker or appropriate NMR processing software.

One dimensional $^1$H NMR and $^{31}$P NMR spectra with proton decoupling will be obtained.

Heteronuclear $^{31}$P-$^1$H correlation study will be furnished using Bruker 400 NMR spectrometer.
NOESY Experiment in $^2$H$_2$O will be carried out at variable mixing times with a calibration delay with presaturation of the water resonance, using Bruker 500 MHz with Spectral width 5000 Hz in each dimension. Bruker 500 MHz spectrometer will be used for NOESY experiments in 90% H$_2$O and 10% $^2$H$_2$O, with jump and return pulses (35).

DQF-COSY experiment will be used to determine the sugar pucker of deoxyribose sugar of each base residue along the DNA duplex.

Quantitation of NOE Cross Peak Volumes: The volumes in the NOE cross peaks of the data obtained with variable mixing times will be quantified using appropriate FELIX and CNS software.

**FUTURE SCOPE OF THE STUDY**

The number of structural and biochemical studies on Abasic sites has already been conducted by various scientists all over the world. The abasic sites are point of interest as these sites occurs naturally in DNA. These are appropriate removed from the structure of DNA by the reaction of repair enzymes viz. APE enzyme. If the DNA remains unrepaired it may be mutagenic and these sites may have a potential to eliminate such mutagenic effects. A lot of work is still going on these lesions incorporated into a triple helical DNA and quadruplex DNA in order to understand a number of diseases such as Rothmund–Thomson Syndrome (RTS), Bloom Syndrome and Werner Syndrome etc. diseases characterized at the cellular level by chromosomal instability (60).
REFERENCES


