at 213 K the anion signal has almost the same width as that at 193 K, whilst the cation signal has broadened by some 50%. Hence the broadening of both cation and anion peaks over the entire temperature range shows that this broadening does not arise from a classic two-site exchange process.

Solutions which contain mixed alkali metals CsM (M = Na, K, and Rb) in crown ether (L)-THF solvent mixtures show ^{133}Cs NMR spectra which consist of only a single NMR line due to the complex cation $Cs^+(L)_2$. Furthermore, ^{23}Na, ^{39}K, ^{85}Rb, and ^{87}Rb NMR studies of the same solutions show the presence of the Na^-, K^-, and Rb^- ions. This clearly reflects the greater thermodynamic stability, in these solutions, of each species $Cs^+(L)_2M^-$ compared with $M^+(L)_2Cs^-$. The temperature dependences of the NMR characteristics (δ, $\Delta\nu_{1/2}$) of the Cs^+ signals in these mixed-metal solutions are consistent with the assignment to $Cs^+(L)_2$. Increasing the temperature leads to a substantial increase in the line width of the cation signal, coupled with a very small diamagnetic shift and decrease in the spin-lattice relaxation rates. For example, the $Cs^+(12C4)_2$ NMR signal observed from CsNa solutions in a 12C4/THF solvent mixture has δ = -22.4 ppm, $\Delta\nu_{1/2}$ = 95 Hz, and T_{1n} = 8 ms at 198 K changing to δ = -26.4 ppm, $\Delta\nu_{1/2}$ = 720 Hz, and T_{1n} = 34.5 ms at 243 K. No signal could be detected above 250 K. The decrease in the cesium nuclear spin-lattice relaxation rate at higher temperatures is consistent with quadrupolar relaxation for the cation-based signal in the system. However, the substantial increase in the line width suggests the presence of exchange processes. Clearly, direct exchange between Cs^- and, for example, Na^+ is ruled out in these mixed-metal solutions.

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The Characterization of Abasic Sites in DNA Heteroduplexes by Site Specific Labeling with ^{13}C

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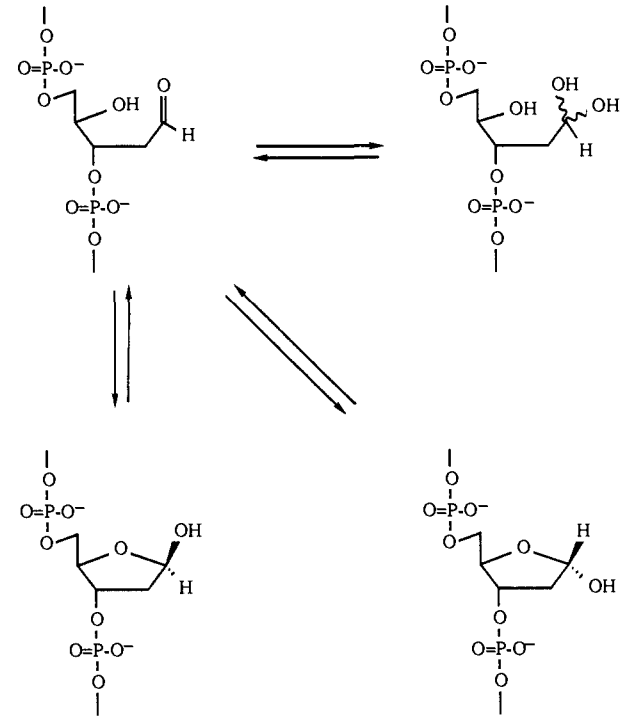
Damage to a base in DNA duplexes is followed by either chemical or enzyme-catalyzed hydrolysis of the N-glycosidic bond to yield a baseless site.[2,3] For example, the spontaneous hydrolysis of the 4-amino group of cytosine to yield uracil occurs at a genetically significant rate. Since this lesion is mutagenic, the cells of all organisms contain the enzyme uracil-DNA glycosylase which hydrolyzes the N-glycosidic bonds of deoxyuridine residues to release uracil. The resulting mixture of open chain aldehyde and hydrate and cyclic hemiacetals (Scheme I) is termed an abasic site. The abasic site is then repaired by the action of several additional enzymes. Despite the intermediacy of abasic sites in the repair of damaged DNA and their reported mutagenicity during transcription,[4,5] no detailed information is available about

Scheme I



their structure, and controversy exists regarding the chemical reactivity of such sites.[6,7] We have prepared heptameric heteroduplexes containing a single abasic site (with each of the four bases opposite the abasic site) in which the 1- and 3-carbons of the abasic site are labeled with ^{13}C, thereby enabling direct observation of the ^{13}C resonances associated with the aldehydic carbon of the abasic site.

The synthesis of d(GCGUGCG) in which the deoxyuridine moiety is labeled in its 1'- and 3'-carbons with ^{13}C was previously described;[8] the unlabeled single strand and the four "complementary" single strands d(CGCNCGC), where N = A, G, T, and C (designated the A, G, T, and C strands), were prepared with phosphoramidite chemistry by using an automated DNA synthesizer. The strands were judged homogeneous by HPLC and ^1H NMR spectroscopy at 400 MHz.

Single strands containing the abasic site were obtained by incubation of the deoxyuridine containing strands with sufficient uracil-DNA glycosylase from *Escherichia coli*[9-11] to give complete reaction in approximately 12 h as assessed by HPLC and ^1H NMR spectroscopy.[12] Equal amounts of the abasic strand (designated the D strand for *d*eoxyribose) and each of the four complementary stands were mixed to generate the heteroduplexes studied by NMR spectroscopy.

^1H NMR spectra (at 400 MHz) of the imino region and of the aromatic and anomeric region of mixtures of strands using unlabeled U strand were recorded to assess whether duplex formation

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(12) The incubations were carried out at room temperature in a solution of 10 mM sodium cacodylate, pH 7.0, containing 0.1 M NaCl and 0.1 mM EDTA in D_2O to allow monitoring of the reaction by ^1H NMR spectroscopy; the concentration of the deoxyuridine containing single strand was 1 to 2 mM. The same buffer was used for the NMR studies.

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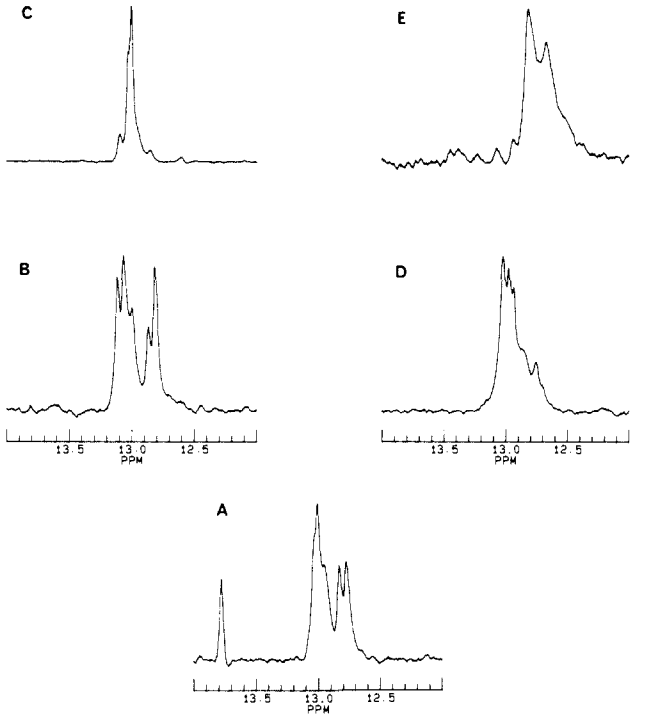

Figure 1. Comparison of the 400 MHz ^1H NMR spectra of the imino proton region of the completely base paired A–U duplex (spectrum A) and of the four duplexes containing the abasic site in 90% H_2O. Spectrum B is that of the A–D duplex, C is that of G–D duplex, D is that of T–D duplex, and E is that of C–D duplex.

had occurred. The spectra of the imino regions that were obtained at 15° are shown in Figure 1 (spectra B–E) along with that of the completely base paired heteroduplex prepared from the A and U strands (spectrum A). These spectra demonstrate both the extent of excision of the uracil residue (compare the A–U duplex with the A–D duplex) as well as duplex formation (note the presence of imino protons associated with G–C base pairs in spectra B through E); the spectra of the aromatic and anomeric regions (not shown) also demonstrate duplex formation between the abasic and each complementary strand. Significant broadening of the imino resonances in all four duplexes was observed at 25°. Since the melting temperature of the A–U duplex is about 60° [8] and significant broadening of the imino resonances of this duplex is observed only at temperatures in excess of 37°, the abasic lesion appears to reduce the stability of the A–D duplex relative to the A–U duplex.[13] However, duplex formation between the C and D strands and between the G and D strands is of interest since mixtures of either the C strand or the G strand with the U strand do not form duplexes as assessed by the imino region of the ^1H NMR spectrum and melting behavior monitored by changes in UV hyperchromicity (data not shown). ^{31}P NMR spectra (data not shown) revealed the absence of resonances shifted from the region expected for a B-duplex and suggest that no gross distortion of the helical structure is present in the duplexes.

The ^{13}C NMR spectra (at 100 MHz) of the duplexes prepared with the labeled D strand were recorded at 15°, and the regions containing the resonances associated with the 3-carbon (the upfield signals) and the sp^3 forms of the 1-carbon (the downfield signals) are shown in Figure 2. Focussing on the resonances associated with the 1-carbon, the conclusion is that the abasic site in each duplex is populated by approximately equal amounts of both anomers of the hemiacetal. Resonances associated with the open chain aldehyde and its hydrate could not be distinguished from the resonances associated with the natural abundance ^{13}C at other sites in the duplexes; therefore acyclic structures make structurally (but not chemically) insignificant contributions.[14]

(13) Alternatively, the broadening of the resonances of the heteroduplexes containing abasic sites might be explained by the abasic site mimicking the end of a duplex with accompanying fraying of the adjacent base pairs.

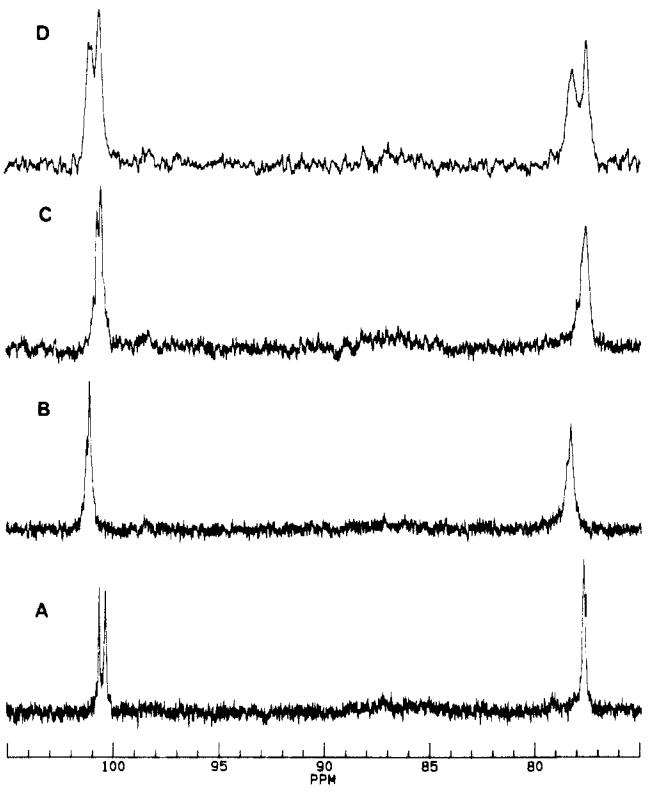

Figure 2. Comparison of the 100 MHz ^{13}C NMR spectra of the four duplexes containing the abasic site. Spectrum A is that of the A–D duplex, B is that of the G–D duplex, C is that of the T–D duplex, and D is that of the C–D duplex. The resonances at approximately 101 ppm are associated with the 1-carbon and the resonances at approximately 78 ppm are associated with the 3-carbon in the abasic site.

Our results indicate that the conformational features of the duplex are dependent upon the identity of the base opposite the abasic site. The ^1H NMR chemical shift differences observed between the four duplexes in both the imino and aromatic regions cannot be attributed solely to the ring currents from the base opposite the abasic site. Similarly, the ^{13}C NMR results may suggest that the ratio of anomeric forms of the abasic site depends upon the identity of the base in the complementary strand; we plan to fully characterize the conformational differences between these duplexes.

Three recent reports described some biochemical,[15] biophysical,[16] and structural[17] properties of the chemically stable and nearly isosteric reduced 1-carbon analogue of the abasic site we describe in this communication. The observations we have summarized demonstrate that detailed characterization of the actual abasic site is possible. In analogy with our recently described studies of the conformations of heteroduplexes that are labeled with ^{13}C

(14) 5-*O*-Methyl-2-deoxyribose exists in solution as a mixture of the aldehyde (1.1%), the hydrated aldehyde (2.3%), and a mixture of the α- and β-anomers of the cyclic hemiacetal (52% and 44%, respectively): Serianni, A. S., personal communication.

(15) The mutagenic properties of authentic abasic sites (preferential insertion of dA opposite the sites during replication) are mimicked by the reduced analogue: Takeshita, M.; Chang, C.-N.; Johnson, F.; Will, S.; Grollman, A. P. *J. Biol. Chem.* **1987**, *262*, 10171–10179.

(16) In contrast to our observation that heteroduplexes containing authentic abasic sites are more stable than analogous duplexes containing mismatched base pairs, heteroduplexes containing the reduced analogue have been reported to be less stable than analogous duplexes containing mismatched base pairs: Millican, T. A.; Mock, G. A.; Chauncey, M. A.; Patel, T. P.; Eaton, M. A. W.; Gunning, J.; Cutbush, S. D.; Neidle, S.; Mann, S. *Nucl. Acids Res.* **1984**, *12*, 7435–7453.

(17) The solution structure of a heteroduplex containing the reduced analogue of the abasic site has been studied by two-dimensional NMR techniques; the structure is in the B-family, and the abasic site analogue and the opposing base lie within the helix: Cuniasse, P.; Sowers, L. C.; Eritja, R.; Kaplan, B.; Goodman, M. F.; Cognet, J. A. H.; LeBret, M.; Guschlbauer, W.; Fazakerley, G. V. *Nucl. Acids Res.* **1987**, *15*, 8003–8021.

in a site specific manner,[8] the ability to prepare duplexes containing isotopically labeled abasic sites will allow detailed investigation of their structural properties as well as their enzymatic and chemical reactivities.[18]

Acknowledgment. This research was supported by NIH GM-34573 to J.A.G. and P.H.B.

(18) After this research was completed, a report appeared describing the use of uracil-DNA glycosylase to prepare single-stranded oligonucleotides containing an abasic site and the chromatographic characterization of their base-catalyzed hydrolysis products: Stuart, G. R.; Chambers, R. W. *Nucl. Acids Res.* **1987**, *15*, 7451-7462.

FT ESR Study of Photoinduced Electron Transfer

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With Fourier transform ESR (FT ESR) well resolved spectra of organic free radicals can be obtained even if the free induction decay, following a $\pi/2$ microwave pulse, is as short as 1 μs.[1] This makes the technique particularly suitable for the study of short-lived radicals. Results are presented of a FT ESR study of a transient free radical generated in a reversible photoinduced electron transfer reaction. The reaction and spin dynamics have been monitored from the time the radical is generated with nanosecond time resolution. The technique provides data complementary to those provided by flash photolysis.

Figure 1 shows a series of FT ESR spectra of the duroquinone anion radical (DQ^-). The radical was generated by electron transfer from photoexcited zinc tetraphenylporphyrin (ZnTPP, 5×10^{-4} M) to duroquinone (5×10^{-3} M) in ethanol at 245 K.[2] Samples were degassed on a vacuum line by repeated freeze–pump cycles. A Lambda Physik FL2001 dye laser (Rhodamine B, 600 nm, 2 mJ) pumped by a Lambda Physik EMG103MSC excimer laser (pulse width 15 ns, rate 40 Hz) was used as excitation source. Sample volume exposed to the laser beam was approximately 0.1 mL. The FT ESR spectrometer has been described briefly in ref. 1. The spectra represent Fourier transforms of the sum of 10000 (10 μs long) FID's (data acquisition time 4 min) detected in sequential quadrature mode.[1]

Under steady-state conditions, the ESR spectrum of the DQ^- anion radical consists of 13 lines with a binomial intensity distribution and hyperfine splitting of 1.9 G.[4] A maximum of ten hyperfine peaks can be discerned in the spectra presented here. Figure 1 shows that a variation in time delay between laser and microwave pulses affects the overall signal amplitude. The delay also determines the relative intensities and phase of the hyperfine lines. Four distinct time domains can be identified: **[0–1 μs]**. The overall signal amplitude increases with increasing time delay. *In this time period there is also an evolution of the population distribution over available spin states.* At 10 ns delay all hyperfine lines are in absorption, as the delay grows low field peaks become emissive. **[1–10 μs]**. Relative peak intensities stay constant, while the overall signal intensity drops by a factor of 2 to 3. **[10–80 μs]**. The decrease in overall signal amplitude is accompanied by a change of emission peaks into absorption peaks. **[>80 μs]**. No further change in relative amplitudes of hyperfine lines is observed. With a delay of 2 ms a weak DQ^- spectrum can still be recorded.

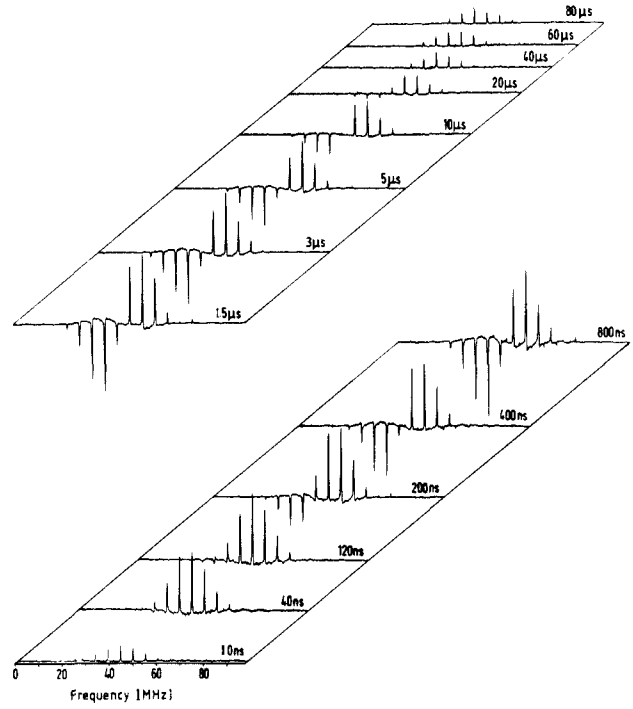

Figure 1. Quadrature-detected FT ESR spectra of photogenerated DQ^- as function of time delay between exciting laser pulse and $\pi/2$ (20 ns) microwave pulse. Absorption peaks point up, emission peaks down. Sample temperature 245 K.

A complete analysis of the data will be presented elsewhere; here a qualitative interpretation will be given. The initial signal growth reflects the kinetics of electron transfer from photoexcited ZnTPP to DQ. The DQ^- concentration reaches a maximum within 1 μs; this is consistent with a reaction rate that is close to diffusion controlled. The change in relative intensities of hyperfine lines in the 0–1 μs domain is due to competition between triplet (TM) and radical pair (RP) spin polarization mechanisms.[5] Spin selective intersystem crossing in photoexcited ZnTPP generates spin polarized triplets.[6] DQ^- and $ZnTPP^+$ formed before spin lattice relaxation obliterates the triplet spin polarization will give enhanced absorption ESR signals. The RP mechanism generates a DQ^- ESR spectrum in which nine low field peaks are in emission and the others in absorption.[7] Both mechanisms are operative, but, as the reaction proceeds, the TM will diminish in relative importance because of triplet spin lattice relaxation ($T_1 \approx 30$ ns). As a consequence, the TM dominated spectrum (10 ns delay) is gradually replaced by a RP dominated spectrum. The signal decay observed from 1 to 10 μs is due to back electron transfer. It is estimated that the initial DQ^- ($ZnTPP^+$) concentration is $\approx 5 \times 10^{-5}$ M. This gives a back electron transfer rate constant of $\approx 2 \times 10^9$ M^{-1} s^{-1}. With longer delays, spin lattice relaxation ($T_1 \approx 10^{-5}$ s) thermalizes the populations. Due to reduction of $ZnTPP^+$ in a side reaction competing with back electron transfer, complete regeneration of DQ also involves a disproportionation reaction.[10] This accounts for the fact that a weak DQ^- signal can be observed milliseconds after the laser pulse.

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