compared to k_{-e} in all cases), while k_{-e} should follow the order TI ~ TCA > DCA ≥ DCN.[9,10,25] The most plausible explanation for the variation in k_{frag} is that proton loss and fragmentation are synchronous, and thus basicity of the acceptor radical anion plays a major role.[26] The spread of k_{frag} for these acceptors estimated from Φ_{-A} and k_{-e} values spans a range of nearly three orders of magnitude from 3×10^8 s^{-1} (erythro-**1**/TI) to 7×10^5 s^{-1} (erythro-**1**/DCN) for a common donor.

Further evidence supporting the role of acceptor anion radical assisted deprotonation in the fragmentation is the finding of significant deuterium isotope effects when the -OH of the aminoalcohol is replaced by -OD (Table I). In line with the trends in overall reactivity, the isotope increases from 1.3 for TI to ca. 4 with DCN. In summary, the results obtained in this study provide a clear demonstration that radical ion pairs formed by electron transfer in low-to-moderately polar media have properties much like electronically excited states or diradicals such that only relatively rapid reactions can compete with "unimolecular" decay. In the present case the critical matching of reactivity of both acceptor and donor ion radicals allows a rapid and yet highly specific reaction to occur in the relatively narrow time window between formation and decay of the geminate pair.[28]

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(25) Values of k_{-e} show dependence upon both D and A structure as well as exothermicity; thus although exothermicity for DCN/erythro-**1** (2.4 eV) is greater than for DCA/erythro-**1** (2.0 eV), data for the two acceptors fall on displaced parabolic plots (Farid, S., private communication).

(26) Basicity of acceptor anion radicals clearly decreases in the series TI > DCA > TCA. The question of basicity of $DCN^{\bullet-}$ relative to the others is apparently unsettled.[9,27]

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(28) We have found electron-transfer dehydrofragmentation for molecules having the structure H-Z-C-C-Y, where Z = O, N and Y = O, N, or S, to be fairly general provided the acceptor used to mediate the photolysis is strong enough to oxidize the heteroatom Y *and* the corresponding anion radical is basic.[29]

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Coexistence of Conformations in a DNA Heteroduplex Revealed by Site Specific Labeling with ^{13}C-Labeled Nucleotides

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The current bias regarding the conformation of a DNA duplex is that under a given set of environmental conditions each nucleotide unit will assume a rapidly averaged conformation within one of the possible structural families (e.g., A, B, or Z). Time-averaged variations within families have been directly observed by X-ray crystallography, NMR spectroscopy, circular dichroism, and vibrational spectroscopy. However, little information is actually available regarding the rates and mechanisms of interconversion of conformations lying within the same or even different families. In this communication we report the first use of DNA

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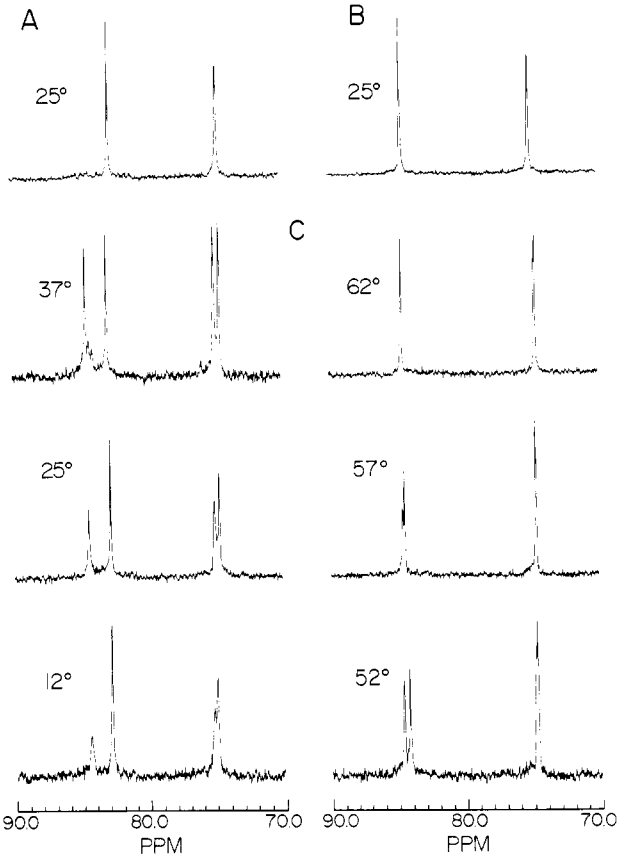

Figure 1. Comparisons of the 100-MHz ^{13}C NMR spectra of AU_I at 25 °C (panel A), of d(CCGUGCC) [the labeled single strand in AU_{II}] at 25 °C (panel B), and of AU_{II} at the indicated temperatures (panel C).

fragments specifically labeled with ^{13}C in a single nucleotide unit to assist the study of duplex conformations by ^1H and ^{13}C NMR spectroscopy; such labeling can be expected to yield unambiguous resonance assignments as well as higher resolution in congested spectral regions. We focus on a comparison of two heteroduplexes labeled with [1',3'-$^{13}C_2$]deoxyuridine: d(CGCACGC) paired with d(GCGUGCG) [AU_I] and d(GGCACGG) paired with d(CCGUGCC) [AU_{II}].[2,3] In low salt, two slowly interconverting conformations of the deoxyuridine are observed for AU_{II} but not AU_I. The populations of the conformers are temperature dependent and approximately equal at 37 °C, demonstrating a previously unrecognized potential for coexistence of multiple, slowly interconverting conformations of a DNA sequence under biological conditions.

Characterization of AU_I and AU_{II} by conventional one-dimensional ^{13}C NMR spectroscopy at 100 MHz revealed unanticipated spectral differences. In low salt[12] at 25 °C, the spectrum

(2) The choice of heteroduplexes containing an A-U base pair was influenced by our interest in the conformational properties and enzymatic processing of DNA duplexes containing structural lesions such as uracil and baseless sugar residues.

(3) [1,3-$^{13}C_2$]Ribose (Omicron Biochemicals) was converted to [1',3'-$^{13}C_2$]deoxyuridine by using standard literature procedures.[4-6] The labeled deoxyuridine was converted to 5'-dimethoxytrityl 3'-*o*-chlorophenylphosphate deoxyuridine[7,8] and incorporated with solution phase phosphotriester chemistry[9,10] into single strands for the duplexes.[11]

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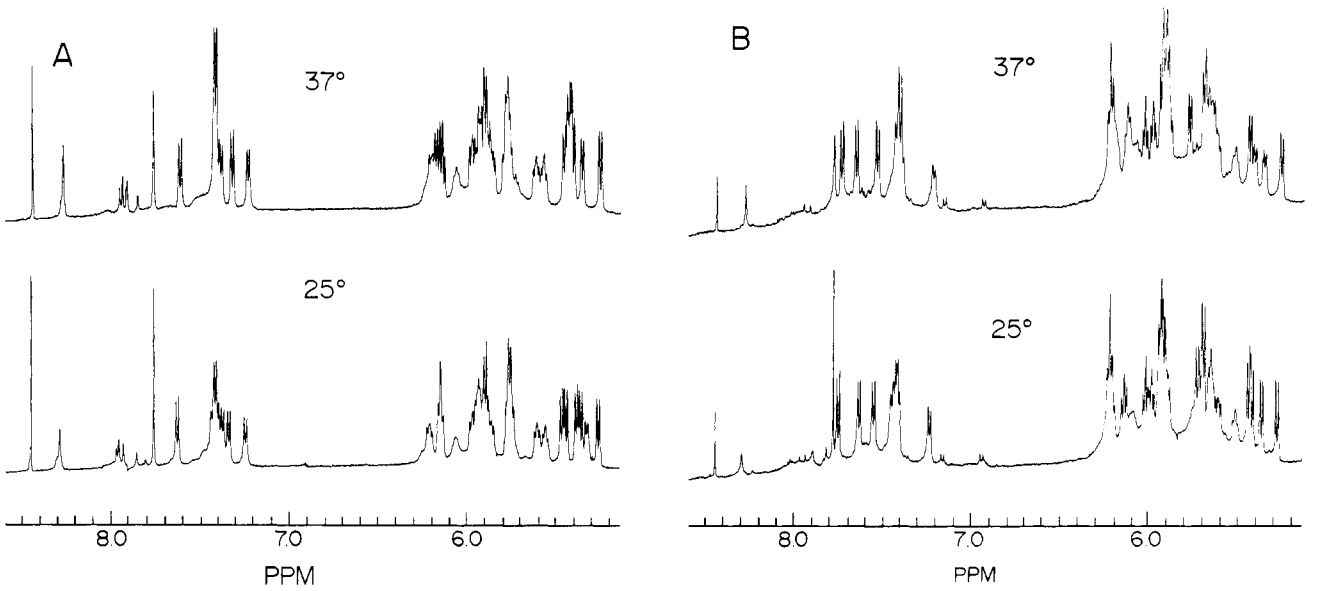

Figure 2. Comparisons of the aromatic and anomeric regions of the 500-MHz ^{1}H NMR spectra of AU_I (panel A) and of AU_{II} (panel B) at 25 °C (bottom spectra) and at 37 °C (top spectra). These samples were used for studies of the reversible temperature dependence of ^{13}C NMR chemical shifts, and the purine protons at C-8 have partially exchanged with solvent D_2O.

of AU_I reveals the expected two resonances, with the more downfield signal being assigned to the 1′-carbon of the labeled deoxyuridine residue (Figure 1, panel A). As the temperature is raised,[13] the resonances first broaden and then assume the chemical shifts observed for the labeled single strand. Under identical conditions, *the spectrum of AU_{II} reveals two resonances for each labeled carbon* in an intensity ratio of ca. 1:2 (Figure 1, panel C). In addition, below the T_m for the duplex, the relative intensities of the resonances for each labeled site in AU_{II} are reversibly temperature dependent (Figure 1, panel C): at 12 °C, the intensity ratio is ca. 1:4, and at 37 °C the ratio is ca. 1:1. The splitting of resonances below the T_m and the temperature dependence of their intensities are consistent with an enthalpy difference of approximately 1 kcal/mol for the two conformers. As the temperature is further raised toward the T_m, both resonances for each labeled site broaden and ultimately assume the single chemical shift observed for the labeled single strand. Focusing on the resonances for the 1′-carbon, the temperature dependence of the line widths of the two conformers indicates that the duplex associated with the upfield signal undergoes more rapid exchange with the single strand.[14]

The aromatic and anomeric regions of the 500-MHz ^{1}H NMR spectra of AU_I and AU_{II} recorded at both 25 and 37 °C are compared in Figure 2. Whereas many of the resonances in the spectrum of AU_I obtained at 37 °C are only slightly broadened relative to those at 25 °C, the spectrum of AU_{II} is markedly broadened at the higher temperature. Given the ^{13}C NMR spectral data presented in Figure 1, the line broadening in AU_{II} is best explained by an unexpected exchange between two duplex forms rather than melting of the duplex.[15] The ^{13}C NMR data allow the rate of exchange between the two duplexes to be estimated as on the order of 10 s^{-1}.

The chemical shifts of the ^{13}C resonances of AU_I and of the resonances predominating in AU_{II} at low temperature are characteristic of a nucleotide in a B conformation.[16] Two-dimensional nuclear Overhauser effect spectra of AU_I obtained at 620 MHz indicate that the duplex is in the B family.[17,18] Similar examination of AU_{II} is also consistent with a B-like conformation, although the correlations involving the central A–U base pair have not yet been interpretted. Comparisons of one-dimensional ^{1}H NMR spectra of AU_I and of AU_{II} with spectra of their component single strands demonstrate the absence of excess single stranded species in our samples. Furthermore, integration of the pyrimidine resonances in the ^{1}H NMR spectra of both completely melted duplexes confirm the 1:1 stoichiometry of the component single strands. Finally, spectra of the imino protons of AU_I and AU_{II} confirm the presence of Watson–Crick base pairing and the double helical nature of both heteroduplexes; in both duplexes the terminal G–C base pair imino protons are exchange broadened at 20 °C with the interior five imino protons being observable (though broadened) at 37 °C.

We have also prepared the analogous heteroduplexes with A–T base pairs without site specific ^{13}C labeling: d(CGCACGC) paired with d(GCGTGCG) [AT_I] and d(GGCACGG) paired with d(CCGTGCC) [AT_{II}]. The homologous A–U and A–T pairs have identical optical melting behavior and analogous temperature dependent line broadening effects in their one-dimensional ^{1}H NMR spectra.

The data uniquely available by site specific ^{13}C labeling demonstrate that the sequence of AU_{II} can accommodate either of two slowly interconverting conformations. That AU_{II} but not AU_I can occupy either of two conformations is striking since the sequences of AU_{II} and the "normal" AU_I differ only by an inversion of the base pairs at the ends of the duplexes. Clearly, additional site specific labeling of these and structurally related duplexes will allow a better understanding of the extent of and structural

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(11) The complementary single strands were synthesized on a Systec 1450A DNA synthesizer by using the standard 10 μmol phosphoramidite chemistry program.

(12) The duplexes are formed by mixing 1 μmol of each desalted single strand in 0.5 mL of a solution of 10 mM sodium cacodylate, pH 7.0, 0.1 M NaCl, and 0.1 mM EDTA in D_2O, followed by heating above the T_m and slow cooling.

(13) At duplex concentrations of approximately 0.010 mM the T_m for AU_I is 42 °C and that for AU_{II} is 39 °C; at a duplex concentration of approximately 0.1 mM the T_m for AU_I is 54 °C, and at duplex concentration of 0.13 mM that of AU_{II} is 51 °C. The duplex concentrations used in our NMR experiments are 2.0 mM, suggesting that the T_ms for both duplexes are approximately 60 °C under these conditions.

(14) The upfield signal sharpens and shifts toward the position of the single strand at a lower temperature than sharpening of the downfield signal is observed. The difference in frequency between the downfield signal and the single strand is smaller than the upfield signal.

(15) That line broadening is observed for many resonances and implies that the conformational differences are not strictly localized to the A–U base pair.

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bases for the phenomenon we have observed. Since the coexistence of multiple, slowly interconverting conformations for a DNA sequence under biological conditions is unprecedented,[19] its generality and relevance are of considerable interest.

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(19) Slow equilibration between two conformations of the oligonucleotide d(TCGA) has previously been observed by NMR spectroscopy under conditions of low temperature (<10 °C) and very low ionic strength (no added salt).[20]

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^{1}H NMR Probe for Hydrogen Bonding of Distal Residues to Bound Ligands in Heme Proteins: Isotope Effect on Heme Electronic Structure of Myoglobin

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The ligand-binding properties of heme proteins depend critically on the specific interaction of the axial iron ligands (proximal histidine, bound ligand) with the polypeptide matrix.[2] Steric interplay with distal residues can influence the orientation of bound O_2 or induce tilting of CO or CN^- which would otherwise bind perpendicularly to the heme.[3-6] Hydrogen-bonding interactions can involve either the proximal histidyl imidazole[2,3,7] or a direct interaction between a protic distal side chain ligand and a bound ligand.[2,8,9] The ability to detect the influence of such H-bonding interactions in heme proteins relies primarily on the observation of solvent isotope effects on vibrational frequency[10,11] or ESR line width.[12] While these techniques clearly detect localized effects due to H-bonding, they are generally unable to identify the participating proton. H-bonding by an axial histidyl imidazole or to a bound ligand are detectable by X-ray and neutron diffraction,[3,8] but in the presence of several such interactions, these techniques cannot interpret their functional consequences. ^{1}H NMR is well suited for both the rapid and direct detection of H-bonding and the identification of the participating residue. While the task poses formidable experimental problems for a diamagnetic heme protein, the increased resolution for heme cavity residues in paramagnetic complexes and the exquisite sensitivity of the hyperfine shift to small structural perturbations set ideal conditions for elucidating the H-bonding interactions.[13] We demonstrate herein an isotope effect on the *electronic structure of the heme* in the low-spin, ferric cyanide complex of sperm whale myoglobin, metMbCN, which can be uniquely attributed to an H-bond between the distal (E7) histidyl imidazole and the bound ligand.

The low field portion of the 500-MHz ^{1}H NMR spectrum of metMbCN in 90% ^1H_2O, 10% ^2H_2O is illustrated in Figure 1A; the previously assigned heme peaks relevant to the work are indicated,[14] with labile proton peaks *A*, *B*, and *C* arising from the distal His E7 ring N_3H, proximal His F8 ring N_1H, and peptide

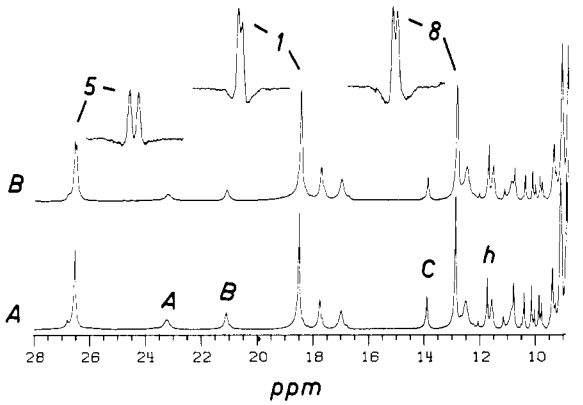

Figure 1. Downfield hyperfine shifted region of the 500-MHz ^{1}H NMR spectrum of sperm whale metMbCN at pH 9.2, 30 °C. (A) In 90:10 $H_2O/^2H_2O$. Peaks are labeled as reported previously.[15-17] *A*, distal His (64) ring N_3H; *B*, proximal His (93) ring N_1H; *C*, proximal His NH; *h*, distal His C_4H. The water resonance was suppressed by using an off-acquisition saturation pulse. (B) In 49:51 $H_2O/^2H_2O$ (pH 9.2). The expanded plots show the three resolved heme methyl resonances after resolution enhancement (sine-bell); the vertical scale is arbitrary, frequency scale magnified 5 times. Asymmetry in the expanded peaks is due to overlapping lines of different width. Note that A, B, C, and h remain single lines.

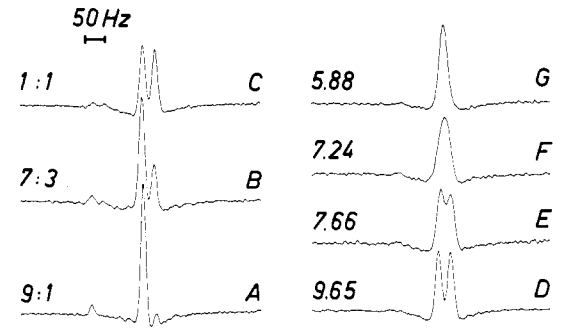

Figure 2. Heme 5-methyl signal(s) of metMbCN at 30 °C. (A–C) At pH 9.2 as a function of solvent composition ($^1H_2O/^2H_2O$): (A) 9:1, (B) 7:3, (C) 1:1. (D–G) In 1:1 $^1H_2O/^2H_2O$ as a function of pH: (D) pH 9.65, (E) pH 7.66, (F) pH 7.24, (G) ph 5.88. The vertical scaling was accomplished by reference to unaltered γ-CH_3 of Ile-99 at −3.2 ppm (not shown). The spectrum at pH 7.24 (F) corresponds to the coalescence of the two component peaks. For clarity, the 5-methyl signals are centered with respect to trace D in D–G in order to compensate for the effect of the titration of His 97 on the chemical shifts.

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