well with published data for $AgNO_3$ in chelating solvent mixtures.[10] However, the $\delta^{103}Rh$ value for II (+46 ppm relative to the accepted Ξ = 3.16-MHz reference) is one of the highest ever reported for a Rh(I) complex,[11] a situation resulting from nitrogen coordination at the rhodium center.[12]

Finally, since polarization-transfer experiments are very easy to set up (no triple irradiation attachment needed as for ^1H-{M} INDOR nor precise determination of proton-transition frequencies as in SPI/SPT experiments) we anticipate a widespread use of this technique in metal NMR spectroscopy, since many organometallic complexes show long-range metal proton scalar couplings.[13]

A full paper on the chemistry, NMR parameters, and dynamic behavior of these silver(I) and rhodium(I) complexes will be published shortly.

Acknowledgment. We thank Dr. D. M. Grove and Professor Dr. K. Vrieze for helpful discussions.

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Structural and Dynamic Information about Double-Stranded DNA from Nitrogen-15 NMR Spectroscopy

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Nitrogen-15 NMR resonances have been observed for unfractionated yeast tRNA at natural abundance in solution[2] and ^{15}N-enriched *B. licheniformis* cells.[3] However, no one previously has reported observation of the ^{15}N spectrum of DNA. This communication describes our initial studies of the ^{15}N NMR spectral properties of sonicated native DNA obtained from ^{15}N-enriched *E. coli*.

Lack of any previous ^{15}N NMR experiments with DNA can be attributed to the low sensitivity of natural abundance ^{15}N NMR spectroscopy,[4] which would be aggravated by the extremely broad resonances one might anticipate from a molecule the size of DNA. Our recent ^{31}P and ^{13}C NMR relaxation studies of nucleic acids showed that the nucleic acid backbone is relatively mobile.[5-8] Internal motions in nucleic acids lead to line narrowing with consequent improvement in spectral sensitivity and resolution. In fact Rill et al.[9] have recently obtained a high-field, natural abundance ^{13}C NMR spectrum of DNA in which nearly all peaks are resolved. This background led us to believe the ^{15}N NMR spectrum of DNA could be obtained if the DNA was enriched in ^{15}N.

Consequently, we isolated DNA from *E. coli* cells grown on a medium containing [^{15}N]ammonium chloride enriched 90-95% in ^{15}N.[10] The isolated DNA is therefore enriched with ^{15}N in all positions. The high-purity sample[11] used for the NMR studies was sonicated, yielding a double-stranded DNA sample with a median length of 300 base pairs.[12]

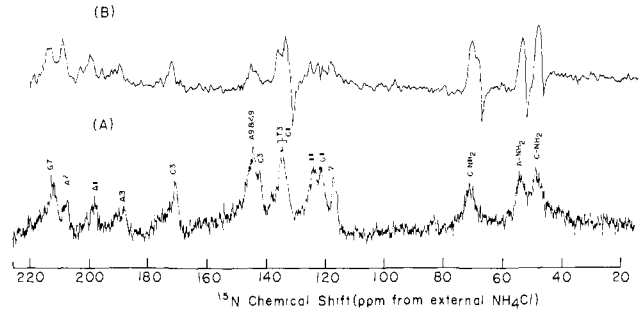

Figure 1. (A) ^{15}N NMR spectrum (30.4 MHz) obtained at 35 ± 2 °C of ^{15}N-labeled DNA (55 mg/mL) in 50 mM sodium cacodylate, pH 7.5, and 50 mM NaCl;[12] 16 412 transients were accumulated with 70° pulses by using an acquisition time of 1.16 s, a pulse recycle time of 4.16 s, and broadband proton decoupling on only during signal acquisition. The Fourier transform spectrum displayed was the result of exponential multiplication equivalent to 4-Hz line broadening. (B) 30.4-MHz spectrum obtained at 45 ± 2 °C of DNA (45 mg/mL) in 50 mM sodium cacodylate, pH 7.5, and 50 mM NaCl;[12] 170 304 transients were accumulated with 90° pulses using an acquisition time of 0.073 s, a pulse recycle time of 0.3 s, and broadband proton decoupling on during acquisition. Exponential multiplication equivalent to 8 Hz was used.

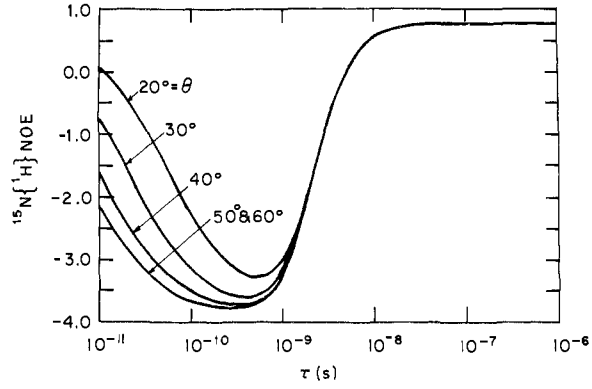

Figure 2. Theoretical dependence of the $^{15}N\{^1H\}$ NOE for ^{15}N at 30.4 MHz assuming only dipolar relaxation, calculated by using a two-state internal jump model appropriate for rotational jumps about the C1′–N bond, as a function of τ_i the lifetime in either jump state. The calculations are made for a series of angular jump amplitudes θ and assume an isotropic reorientation time of 1 μs.

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(11) Diphenylamine and orcinal tests (Schneider, W. C. *Methods Enzymol.* **1957**, *3*, 680-683) indicated that RNA contamination was less than 5%. UV analysis revealed a A_{260}/A_{280} value of 1.9 and a A_{260}/A_{280} value of 2.4, indicating little protein or polysaccharide contamination.

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The 30.4-MHz ^{15}N NMR spectrum of DNA, obtained with gated broadband proton decoupling during acquisition only, is shown in Figure 1. The spectra were obtained on a Bruker WH-300 spectrometer at Hebrew University, Jerusalem. Spectrum A was acquired with a relatively slow pulse repetition rate and gated decoupling to minimize any contributions from the protons to a nuclear Overhauser effect (NOE) for the ^{15}N resonances. As illustrated in Figure 2, and discussed in more detail below, the negative gyromagnetic ratio of ^{15}N can lead to negative signals from the negative NOE or positive signals (although diminished intensity) from a positive NOE, depending on the motional characteristics of the biopolymer. An NOE of zero means the signal will vanish.

Most resonances in the spectrum are resolved. Assignment of the resonances to the various nitrogens of guanine (G), adenine (A), thymine (T), and cytosine (C) bases in DNA are shown in Figure 1. Assignments were made by comparison with published ^{15}N NMR data of mononucleotides and mononucleosides.[13-15] Chemical shifts found for double-stranded DNA are very close to values published for the monomers, with the nitrogens involved in Watson–Crick base pair formation exhibiting the greatest deviation from the monomers' chemical shifts. The chemical shifts observed in double-stranded DNA are guanine N1 (121.8), N3 (142.0), N7 (212), N9 (144.6), NH_2 (49.5); adenine N1 (198.3), N3 (188.2), N7 (207.5), N9 (144.6), NH_2 (54.8); cytosine N1 (134.7); N3 (170.9), NH_2 (71.9); and thymine N1 (124.3), N3 (124.7). The chemical shifts (in parentheses) are in ppm downfield of external 2 M $^{15}NH_4Cl$ in 2 M HCl.

The N3 resonance of cytidine has been shown to shift upfield a few ppm in dimethyl sulfoxide solution when the nucleoside is in the presence of guanosine.[16] This was ascribed to the N3 nitrogen being a proton acceptor in a hydrogen bond formed with a cytidine–guanosine dimer. The cytosine N3 resonance is shifted upfield about 10 ppm, relative to the monomer, in double-stranded DNA which is in accord with the N3 being a proton acceptor in a hydrogen bond. The only other nitrogen to perform as a hydrogen bond proton acceptor in Watson–Crick base pairs is the N1 of adenine. Consistent with this notion, we tentatively attribute a resonance 4–7 ppm upfield from its position in the monomer to the adenine N1 in DNA. As explained below, the ^{15}N resonances from hydrogen bond proton donors shift about 1 ppm downfield.

Several signals in the spectrum give evidence of chemical shift heterogeneity with reproducible multiple resonances being observed in some cases. The polydispersity in helix length may be considered as a potential source of chemical shift heterogeneity. However, the sample had been extensively dialyzed to remove small molecular weight species. Furthermore, the chemical shift of largest amplitude in any resonance band (hydrogen bond proton acceptors excepted) agrees well with the value of the monomer. The treatment with S1 nuclease should also remove any single-stranded moieties, leaving only double-stranded DNA. The influence of any paramagnetic ions should also be minimal due to dialysis against EDTA and EGTA. The possibility of DNA aggregation at such high concentrations as a cause should also be considered. The observed line widths provide evidence against extensive aggregation, and the above arguments against size polydispersity also apply to aggregation. A spectrum (not shown) obtained with a solution containing 25% less DNA displayed the same chemical shift dispersion. Since ^{15}N chemical shifts are quite sensitive to the electronic environment of the nigrogen,[4] we conclude that the observed chemical shift heterogeneity is due to a variety of different chemical environments experienced by the nitrogens in DNA.

The chemical shift heterogeneity is exhibited to a variable extent by different resonances, both by those which are and which are not involved in base-pair hydrogen bonding. Especially interesting is the appearance of a peak at 117.4 ppm which is of comparable amplitude and width to the others in the spectrum. That resonance frequency is not appropriate for amide or amine nitrogen resonances. The sample preparation and analysis also militate against attributing it to an impurity. At present, we ascribe this peak to a component of the DNA, possibly to some thymine N1 which is in a different sugar ring conformation. In this regard, we note that X-ray diffraction studies reveal several sugar conformations in a DNA dodecamer.[17] There are other interesting multiple resonances, all reproducible, about whose origin we have no details at present.

Spectrum B in Figure 1 was obtained with a sample containing some thermally denatured DNA; enzymatic, chemical and spectroscopic analysis indicated 15–20% was single-stranded DNA. Spectrum B exhibits five relatively narrow negative peaks which occur 1–1.5 ppm upfield of the resonance positions of protonated nitrogens (all NH_2, G1, T3) in double-stranded DNA. Although full expression of the NOE is not expected to be manifest with the timing of the gated decoupling used to obtain spectrum B, the rapid pulse rate and high duty cycle of the proton decoupling will still enable us to distinguish positive from negative signals. Theoretical NOE curves (Figure 2) were calculated for several rotational jump angles θ by using an isotropic reorientation time τ_o of 1 μs which we found to be appropriate for DNA from ^{31}P, ^{13}C, and ^{19}F relaxation experiments.[5-8,18] The NOE curves, appropriate for protonated nitrogens assuming only dipolar relaxation from bonded protons, are plotted as a function of τ_i, where $1/\tau_i$ is the jump rate between two internal jump states. In fact the curves are not strongly dependent on the exact value of τ_o if $\tau_o \gg \tau_i$ or on the jump amplitude θ if $\tau_i > 2$ ns.

Negative peaks in spectrum B of Figure 1 are attributed to protonated nitrogens on single-stranded DNA which, according to Figure 2, have $\tau_i < 5$ ns (and probably $\tau_o < 1$ μs).[6] The adjacent positive peaks downfield are attributed to protonated nitrogens on double-stranded DNA which have $\tau_i > 5$ ns, according to Figure 2. Hydrogen bonding in native DNA apparently results in the 1–1.5-ppm downfield shift.

The observed negative peaks reveal the importance of proton dipolar relaxation for the protonated nitrogens. Lack of additional negative peaks in spectrum B indicates that nonprotonated nitrogens in single-stranded DNA do not have a negative NOE and, consequently, another relaxation mechanism may dominate. On the basis of comparative N–H distances, dipolar relaxation is expected to be 50–100 times less efficient for nonprotonated nitrogens than for protonated nitrogens in DNA. Schweitzer and Spiess[19] showed that the chemical shift anisotropy (CSA) for ^{15}N

(12) DNA (1 mg/mL) in 0.15 M NaCl, 10 mM cacodylate, pH 7.5, was bubbled with nitrogen and sonicated at 20 KHz with an Ultrasonics W-375 sonicator by using a 10-mm probe. Thirty milliliter aliquots, layered with a constant stream of nitrogen and in a methanol–ice bath maintained at <−5 °C, were sonicated for 45 min at an 80% duty cycle with a 5.5 output. The sonicated DNA was treated with S1 nuclease to digest any single-stranded DNA (Wiegand, R. C.; Godson, G. N.; Radding, C. M. *J. Biol. Chem.* **1975**, *250*, 8848–8855), dialyzed, extracted twice with fresh phenol, precipitated with 2-propanol, and redissolved. The sample was dialyzed against 200 volumes (v/v) each of (a) 100 mM NaCl, 20 mM cacodylate, 2 mM EDTA, (b) 1 mM NaCl, 1 mM cacodylate, 2 mM EDTA, (c) 1 mM NaCl, 1 mM cacodylate, 4 mM EGTA, and (d) 1 mM NaCl, 1 mM cacodylate. The sample was then lyophilized and redissolved in doubly glass-distilled water in a 7-mm cellulose nitrate tube which was inserted into a 10-mm NMR tube containing deuterium oxide to provide a deuterium lock. Hyperchromic shift analysis before and following sonication, as well as before and after S1 nuclease digestion, revealed the DNA preparations to have a hyperchromic shift at 260 nm equal to that of the best commercial preparations. Gel electrophoresis on 4% polyacrylamide gel using molecular weight markers derived from Hinf I restriction endonuclease digests of PBR322 phage DNA indicated that 80% of the DNA in the sample had 300 ± 150 base pairs. The samples (1.5 mL) in the NMR tube contained 55 mg/mL of DNA (for spectrum A) or 45 mg/mL (for spectrum B), 50 mM NaCl, and 50 mM sodium cacodylate, pH 7.5.

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is three times larger than that of ^{13}C with a chemical shift anisotropy $\Delta\sigma$ = 673 ppm and an asymmetry parameter η = −0.47 ppm for ^{15}N in pyridine. Since the CSA contribution to relaxation increases with the square of the magnetic field strength, it may dominate relaxation for nonprotonated nitrogens in the 7.05*T* field used to obtain the spectra of Figure 1. Using the above values for the CSA parameters and assuming a single isotropic correlation time τ_{eff}, CSA contributions to the line width of 17 and 150 Hz may be estimated for τ_{eff} = 10 and 100 ns, respectively. Although chemical shift heterogeneity obfuscates line width measurements, it is evident in Figure 1 that line widths for the protonated and nonprotonated nitrogen resonances are similar. Although signal-to-noise ratio was poor, the ^{15}N spectrum of DNA at 9.115 MHz (not shown) was also observed. Line widths for protonated nitrogen resonances were comparable at both field strengths, but nonprotonated nitrogen resonances were roughly three times narrower at the low field strength. Chemical shift heterogeneity may be a factor, but different CSA contributions to line width undoubtedly occur at the two field strengths. The differential behavior of protonated and nonprotonated nitrogen resonances at the two field strengths was consistent with the peak assignments in Figure 1.

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Stereochemistry of Lysine 2,3-Aminomutase

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Lysine 2,3-aminomutase catalyzes the reversible interconversion of L-α-lysine (**1a**) and L-β-lysine (**2**).[1] The air-sensitive enzyme purified to near homogeneity from *Clostridium SB4* (*C. subterminale* strain SB4, ATCC 29748) has an equilibrium constant K = (L-β-Lys)/(L-α-Lys) = 6.7 at 37 °C and shows stimulation by *S*-adenosylmethionine, ferrous ion, and pyridoxal phosphate.[2] However, in contrast to most [1,3-8] other known aminomutases, the enzyme neither contains nor is stimulated by coenzyme B_{12}. The interconversion of L-α-lysine and L-β-lysine takes place without exchange of nitrogen or hydrogens with the medium[2,9] and thus, presumably, proceeds with intramolecular (or intermolecular) hydrogen and amino group transfer.[10,11]

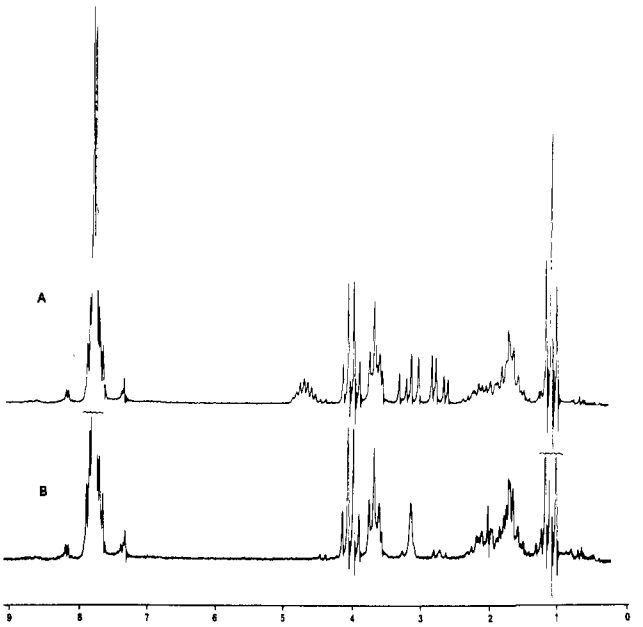

Figure 1. 90-MHz ^1H NMR spectra of **3a** and **3e**: (A) **3a**; (B) **3e** ($CDCl_3$ solutions).

Although the absolute configurations of the substrates of lysine 2,3-aminomutase have long been known, the mechanism of the reaction is still obscure. In an effort to shed some light on this problem, we investigated the cryptic stereochemistry[12,13] of the process and now report evidence which establishes that, in *Clostridium SB4*, the hydrogen and amino group transfers take place with inversion of configuration at both migration termini.[14]

In preliminary experiments, we found that upon incubation of L-lysine-^{14}C with a cell-free extract of *C. SB4* grown on a lysine-rich medium,[15] a reasonably high recovery (ca. 25%) of β-lysine could be obtained after conversion of the crude reaction product into the di-*N*-phthaloyl ethyl ester derivative (**3a**) by heating with phthalic anhydride followed by treatment with diazoethane.[16,17] It was therefore unnecessary to purify the enzyme, and all of our studies were conducted with this cell-free extract. Our ability to isolate β-lysine as the pure derivative **3** in milligram quantities was of extreme importance to our subsequent work. The ^1H NMR spectrum of this compound (Figure 1A) showed an excellent resolution of the signals for the C-2 and C-3 protons.[18]

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(10) Experiments designed to directly examine the question of intra- vs. intermolecular transfer of hydrogen and/or amino group in this reaction are in progress and will be reported elsewhere. For an experiment showing *intramolecular* transfer of the amino group in lysine 2,3-aminomutase in a *Streptomyces*, see the accompanying paper by Gould and Thiruvengadam.[11]
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