Variation of Nonexchangeable Proton Resonance Chemical Shifts as a Probe of Aberrant Base Pair Formation in DNA†

Lawrence C. Sowers,‡ G. Victor Fazakerley,§ Hyun Kim,∥ Larry Dalton,∥ and Myron F. Goodman*,‡

Molecular Biology Section, Department of Biological Sciences, and Department of Chemistry, University of Southern California, Los Angeles, California 90089-1481, and Service de Biochimie, Bat. 142, Department de Biologie, Centre d'Etudes Nucleaires de Saclay, 91191 Gif-sur-Yvette Cedex, France

Received April 29, 1986

ABSTRACT: Variation of nonexchangeable proton resonance chemical shifts for deoxycytidine and deoxyadenosine as a function of protonation and imino tautomer formation has been determined. Protonation induces downfield shifts of proton resonances whereas formation of the rare imino tautomer induces upfield shifts. Titration curves are constructed on the basis of spectrophotometrically determined pK values. Excellent fit is obtained between theoretical titration curves and experimental data, which indicates that chemical shifts of base protons may be used to quantitatively determine the relative concentrations of either rare imino tautomeric conformations or protonated base forms. These data may be utilized as an aid in the elucidation of the nature of hydrogen bonding between mismatched base pairs in DNA oligomers containing cytosine or adenine residues. These data, in conjunction with the oligonucleotide study of Patel et al. [Patel, D. J., Kozlowski, S. A., Ikuta, S., & Itakura, K. (1984) *Biochemistry 23*, 3218–3226], have been used to rigorously argue the existence of a "protonated" adenine residue in the A-C mismatch. This structure allows reconciliation of the NMR solution data with crystallographic data [Hunter, W. N., Brown, T., Anand, N. N., & Kennard, O. (1986) *Nature (London) 320*, 552–555], which support the protonated base pair.

It is generally believed that transition mutations in vivo proceed via the formation of base mispairs during DNA replication. Several mechanisms have been postulated to explain base mispair formation including rare tautomeric forms (Watson & Crick, 1953; Freese, 1959; Topal & Fresco, 1976; Fresco et al., 1980; Singer & Kusmierek, 1982), ionized bases (Lawley & Brookes, 1961, 1962; Topal & Fresco, 1976; Sowers, 1983; Sowers et al., 1986a), and wobble base pairs (Crick, 1966; Topal & Fresco, 1976; Patel et al., 1982b).

Recent advances in oligonucleotide synthesis and high-resolution NMR spectroscopy have provided significant insights into the nature of hydrogen bonding between complementary and mismatched bases in DNA. In spite of these new advances, examination of exchangeable proton resonances alone may not be sufficient to make unambiguous identification of the hydrogen-bonding interaction. In some cases, several possible hydrogen-bonding structures may be in rapid equilibrium with one another, and further, some of these possible hydrogen-bonding structures may involve ionized bases. Either of these events may result in sufficient line width broadening, which would preclude observation of critical proton resonances and subsequent description of the hydrogen bonding in the base pair in question.

In general, however, nonexchangeable base proton resonances are observable and assignable, and the chemical shifts of these resonances may be determined with a high degree of accuracy. In this paper, we have determined the influence of base protonation and tautomerization on the chemical shifts of the nonexchangeable base protons of dC and dA. Base protonation induces downfield shifts of proton resonances whereas formation of rare tautomers induces upfield shifts. We present evidence that measurements of chemical shifts may be utilized to quantitatively determine the relative proportion of neutral, protonated, or rare tautomeric forms at physiological pH. We present the measurement of nonexchangeable base proton resonances as an additional tool for the elucidation of the nature of base pairing interactions between mismatched bases in DNA. In addition to providing qualitative evidence for the existence of a modified base form, measurement of chemical shifts can be a sensitive indicator of the position of equilibrium between possible conformations.

Materials and Methods

2′-Deoxycytidine and 2′-deoxyadenosine were purchased from Sigma. The N^3-methyl derivative of deoxycytidine was prepared by methylation with dimethyl sulfate in dimethyl sulfoxide according to the method of Brookes and Lawley (1962). The UV spectral characteristics at different values of pH were identical with those reported previously. The proton NMR spectrum at neutral pH was identical with that reported by Leutzinger et al. (1982).

N^3-Methyl-2′-deoxyuridine (N^3mdU)[1] was prepared by hydrolytic deamination of N^3-methyl-2′-deoxycytidine (N^3mdC). The UV and proton NMR spectra were identical with those previously reported (Leutzinger et al., 1982).

The N^1-methyl derivative of deoxyadenosine was prepared by methylation of deoxyadenosine with methyl iodide in N,N-dimethylacetamide (Jones & Robins, 1963). N-Methyl derivatives were isolated as the free deoxynucleosides.

Ionization constants (pK values) were determined spectrophotometrically at μ (ionic strength) = 0.1 M, 25 °C, in either

† This work was supported by National Institutes of Health Grants GM21422 and GM33863 and National Institutes of Health NRSA Award 2T32CA09320 to L.S.

* Author to whom correspondence should be addressed.
‡ Department of Biological Sciences, University of Southern California.
§ Department of Chemistry, University of Southern California.
∥ Centre d'Etudes Nucleaires de Saclay.

[1] Abbreviations: N^3mdC, N^3-methyl-2′-deoxycytidine; N^1mdA, N^1-methyl-2′-deoxyadenosine; N^3mdU, N^3-methyl-2′-deoxyuridine; NOE, nuclear Overhauser effect.

0006-2960/86/0425-3983$01.50/0 © 1986 American Chemical Society

Table I: Ionization Constants, Free Energy of Protonation, Tautomeric Equilibrium Constants (K_t), and Free Energy of Tautomerization for dC and dA[a]

		pK	$\Delta G'$(prot.)	K_t	$\Delta G°$(taut.)
dC	dC	4.3	+3.9	3.2×10^{-5}	+6.2
	N^3mdC	8.8	−2.2		
dA	dA	3.8	+4.2	1.0×10^{-5}	+6.9
	N^1mdA	8.8	−2.2		

[a] μ = 0.1, 25 °C. Free energy values reported in kilocalories per mole, pH 7.2.

Table II: Influence of Protonation and Tautomerization on Chemical Shifts (ppm) of Nonexchangeable Base Protons of dA and dC

	protonation	tautomerization
dC H_5	+0.177	−0.209
dC H_6	+0.272	−0.555
dA H_2	+0.202	−0.258
dA H_8	+0.204	−0.168

sodium acetate or sodium phosphate buffers. Spectra were recorded with the aid of a Perkin-Elmer Lambda 3B spectrophotometer.

NMR spectra were recorded at 270 MHz with a Bruker 270SY spectrometer. Nucleoside concentrations were 1 mM in 90% H_2O/10% D_2O, 0.1 M NaCl, and 10 mM phosphate. The solvent peak was suppressed by a 1–t–1 hard-pulse sequence with the carrier placed at ~11.5 ppm (Bleich & Wilde, 1984). Chemical shifts were measured relative to internal tetramethylammonium chloride (3.188 ppm). Values of pH were measured directly in the NMR tubes prior to and after determination of spectra.

Results

The spectrophotometrically determined pK values for dC and dA (μ = 0.1, 25 °C) were 4.3 and 3.8, respectively, consistent with established values. The pK value for N^3mdC was determined to be 8.8. Sun and Singer (1974) previously reported the pK value for N^3-ethyl-2′-deoxycytidine to be 8.6. The pK value for N^1mdA was found to be 8.8 (see Table I). Previously, the pK value for N^1-methyladenosine has been reported as 8.8 (Martin & Reese, 1968) and 8.3 (Macon & Wolfenden, 1968).

The free energy of protonation was determined for a "physiological standard state" ($[H^+] = 1.58 \times 10^{-7}$, pH 7.2) on the basis of the experimentally determined pK values. Tautomeric equilibrium constants were determined by the method of basicity (Angyal & Angyal, 1952), and the free energy of tautomerization was determined from the tautomeric equilibrium constant (Table I). Although these values agree well with accepted tautomeric equilibrium constants (Fresco et al., 1980; Singer & Kusmierek, 1982), the pK values for the N-methyl derivatives of the deoxynucleosides have not been reported previously and, therefore, tautomeric equilibrium constants here represent the first measured values for the deoxynucleoside series (Table I).

Chemical shifts of the nonexchangeable base protons of dC were measured as a function of pH. As shown in Figure 1, protonation results in downfield (higher ppm) shifts of +0.177 and +0.272 ppm for the 5 and 6 protons, respectively. Experimental points in Figures 1 and 2 are displayed as solid circles on a theoretical line constructed from the Henderson–Hasselbach equation and the spectrophotometrically determined pK values. Protonation of dA (Figure 2) similarly induces downfield shifts of the nonexchangeable proton resonances, the magnitudes of which are +0.202 and +0.204 ppm for the 2 and 8 protons, respectively (Table II).

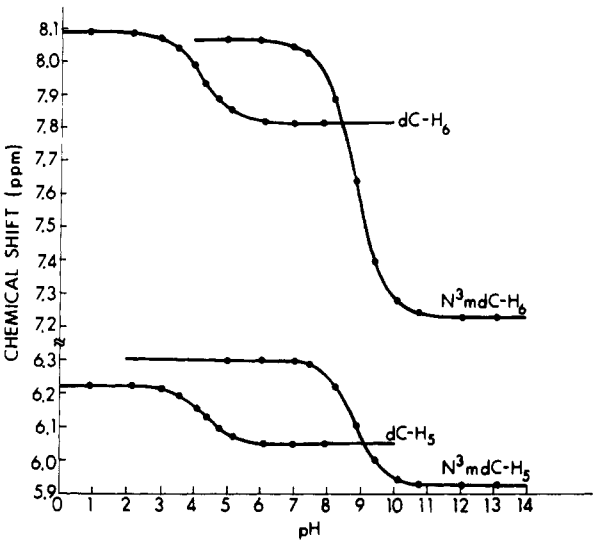

FIGURE 1: Chemical shifts of the nonexchangeable 5 and 6 base protons of 2′-deoxycytidine (dC) and N^3-methyl-2′-deoxycytidine (N^3mdC) as a function of solvent pH. Experimental points are solid circles on a theoretical line.

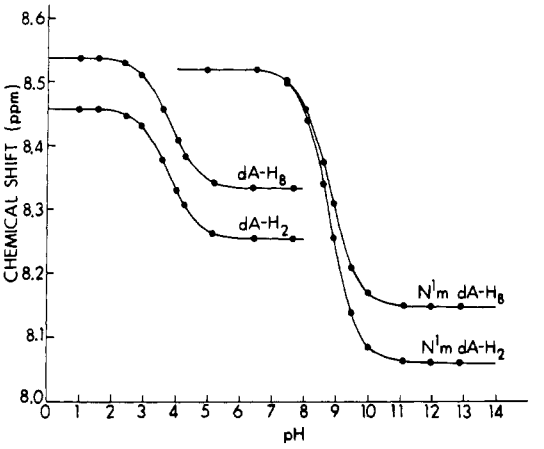

FIGURE 2: Chemical shifts of the nonexchangeable 2 and 8 base protons of 2′-deoxyadenosine (dA) and N^1-methyl-2′-deoxyadenosine (N^1mdA) as a function of solvent pH. Experimental points are solid circles on a theoretical line.

In order to assess the influence of imino tautomer formation, derivatives were "locked" into the imino conformation by methylation of the N^1 and N^3 ring nitrogens of dA and dC, respectively. The N-methyl derivatives are in equilibrium, in aqueous solution, between the protonated and neutral imino tautomeric conformations. For N^3mdC, deprotonation is accompanied by large upfield shifts: −0.836 and −0.386 ppm for the 6 and 5 protons, respectively (Figure 1). Similar upfield shifts were observed for the 8 and 2 protons of N^1mdA: −0.372 and −0.460 ppm, respectively (Figure 2). The assignment of the 2 and 8 protons for the neutral imino tautomer of N^1mdA was established by selective deuteration of the 8 proton as discussed in a recent communication (Sowers et al., 1986b).

Differences in the chemical shifts of the base protons between neutral parent amino and N-methyl imino derivatives are the sum of the influence of tautomerism and the presence of the methyl group. To correct for the influence of the methyl group, the chemical shifts of the protonated parent and N-methyl derivatives were compared. When both are protonated, the derivatives are essentially equivalent electronically, and differences in chemical shifts may be ascribed to the presence of the methyl group.

For the dC series, the presence of the methyl group in N^3mdC shifts the 6 proton upfield slightly (−0.023 ppm) and the 5 proton downfield (+0.079 ppm) relative to the parent compound. The greater influence of the methyl group on the nearer 5 proton can readily be seen by inspection of Figure 1. As an additional indicator of the influence of the methyl group on pyrimidine protons, N^3mdC was hydrolytically deaminated to N^3-methyl-2′-deoxyuridine (N^3mdU). Methylation in the neutral dU series (dU vs. N^3mdU) shifts the 6 proton upfield (−0.034 ppm) and the 5 proton downfield (+0.044 ppm).

Addition of the methyl group to dA has a noticeable downfield effect upon the chemical shift of the nearer 2 proton (+0.064 ppm); however, N-1 methylation has a small upfield effect upon the 8 proton (−0.017 ppm). By subtracting the methyl group effect, the influence of imino tautomer formation can be determined. Conversion to the rare tautomeric form induces upfield shifts [$\Delta\delta$ = δ(imino) − δ(amino)] of all nonexchangeable base protons, the magnitudes of which range from −0.17 to −0.56 ppm (Table II).

Discussion

Protonation and Tautomerization Influences on Chemical Shifts of Nonexchangeable Base Protons for Monomers. On the basis of the p*K* values and tautomeric equilibrium constants presented in Table I, it can be assumed that, at neutral pH, the observed chemical shifts of all nonexchangeable base protons for dA and dC represent the neutral amino conformation and that the minor presence of either protonated or imino tautomeric forms is below the limits of detectability. Further, at 1 mM deoxynucleoside concentration, changes in chemical shift due to base-stacking interactions are negligible (Broom et al., 1967).

It was observed that protonation induces significant downfield shifts in all nonexchangeable base protons of both dC and dA as shown in Figures 1 and 2. The direction and magnitude of the observed chemical shift changes are consistent with results of previous studies on free base and ribonucleoside derivatives (Jardetsky & Jardetsky, 1960; Bullock & Jardetsky, 1964; Danyluk & Hruska, 1968; Topal & Warshaw, 1976; Danyluk et al., 1978). In the case of dC, it has been observed that protonation has a greater effect upon the more distant 6 proton, however; this observation is consistent with the calculation of charge density changes as given by Jordan and Sostman (1973). The excellent fit between the experimental data and the theoretical lines (Figures 1 and 2) clearly indicates that chemical shifts may be used to determine the degree of protonation at a given pH.

Formation of the amino tautomeric conformation results in upfield shifts of all nonexchangeable base protons. As discussed previously by Giessner-Pettre and Pullman (1969) and Evans and Sarma (1974), upfield shifts upon rare tautomer formation result from a decrease in the in-plane ring current deshielding. In this study, methylation was used to lock derivatives into the disfavored conformation, and therefore, the magnitudes of chemical shift changes were corrected by subtraction of a contribution attributed to the presence of the methyl group. The presence of the methyl group had the greater influence upon the nearer 2 and 5 protons of dA and dC, respectively.

In order to correct for the methyl group contribution to the observed changes in chemical shift, it was assumed that methyl group effects upon the neutral imino tautomeric conformation would be similar to those observed for the protonated derivatives. The validity of this assumption is supported by observed effects of methyl groups on other related molecules. It was found that substitution of the N^3 proton of dU with a methyl group shifts the 6 proton upfield and the 5 proton downfield. These values are consistent, both in direction and in magnitude, with the correction factors used for the dC series. For purines, Evans and Sarma (1974) have shown that substitution of a methyl group at the N^1 position of inosine has a downfield influence on the 2 proton (+0.15 ppm) and an upfield influence on the 8 proton (−0.01 ppm). Substitution at N^1 of guanosine results in a −0.01 ppm upfield shift of the 8 proton (Evans & Sarma, 1974). In this study, methyl group corrections for dA were +0.064 and −0.017 ppm for the 2 and 8 protons, respectively. It can be concluded that, qualitatively, upfield shifts of nonexchangeable base proton resonances may be diagnostic of imino tautomer formation. Quantitatively, however, the chemical shift of the 6 proton of dC and the 8 proton of dA should be a more accurate indicator of the degree of tautomer formation. For both protons (dC H_6 and dA H_8), chemical shift changes due to tautomer formation are much greater than the methyl group corrections.

The data presented here clearly indicate that upfield changes in chemical shifts are associated with formation of imino tautomers whereas downfield shifts are diagnostic of base protonation. The fit of the experimental data to theoretical lines further indicates that measurement of chemical shifts may permit quantitation of the degree of tautomerization or protonation at a given pH. Previously, changes in chemical shifts have been used to determine complex p*K* values in proteins (Giralt et al., 1983). In order to utilize these data for probing the nature of hydrogen bonding in potentially illegitimate base pairs in DNA, however, base-stacking contributions to shift changes need also be considered.

Chemical Shift Changes and DNA Helix Formation. Upon helix formation, the chemical shifts of nonexchangeable protons of bases involved in Watson–Crick base pair formation shift upfield. The observed upfield shifts are a consequence of base-stacking interactions, and this effect is attributed to neighboring base diamagnetic ring current anisotropy (Arter & Schmidt, 1976; Giessner-Pettre, 1984; Keepers et al., 1984). For the four DNA bases, the magnitudes of these shifts are sequence-dependent and range from near zero for the 8 proton of dG to −0.8 ppm for the 2 proton of dA (Patel et al., 1982a,b, 1984a,b; Fazakerley et al., 1984). It is important to note that, for a given base sequence, the magnitude of the stacking-induced change in chemical shift is determined by the *position* of a proton relative to the neighboring bases and is independent of the nature of the proton. For example, the 6 proton of a thymine residue and the 6 proton of a dC residue in the imino tautomeric conformation would experience a *stacking*-induced upfield shift of the same magnitude if both protons were in the same position, relative to the neighboring bases. For potentially aberrant base pairs, observed chemical shift changes between single- and double-stranded conformations should, to a first approximation, be the sum of base stacking and changes due to base tautomerization or protonation, as given by eq 1.

$$\Delta\delta(\text{obsd}) = \Delta\delta(\text{stacking}) + \Delta\delta(\text{prot./taut.}) \quad (1)$$

Values for tautomerization- and protonation-induced chemical shift changes for dC and dA [$\Delta\delta$(prot./taut.)] are given in Table II. Ranges for the sequence-dependent, stacking-induced upfield shifts for dC and dA residues, gathered from the literature (Patel et al., 1982a,b, 1984a,b; Fazakerley et al., 1984, 1985), which represent $\Delta\delta$(stacking) in eq 1, are as follows: Experimentally, for cytosine residues inside oligomers, the 5 proton undergoes greater stacking-induced changes (−0.3 to −0.6 ppm) than the 6 proton (−0.25

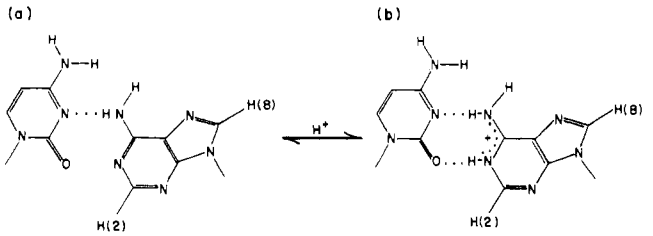

FIGURE 3: Proposed hydrogen-bonding interactions between A and C: (a) the wobble structure and (b) the protonated A wobble structure formed by acquisition of a proton from solvent water.

to −0.45 ppm). For adenine residues inside oligomers, the 2 proton is significantly upfield shifted (−0.4 to −0.8 ppm) whereas the 8 proton undergoes smaller upfield shifts (−0.06 to −0.2 ppm). In general, therefore, rare tautomer formation will induce anomalously high observed upfield shifts, in some cases 3–4 times the magnitude observed for normal base pairs. Generation of protonated base pairs, on the other hand, will induce unusually small upfield, and in some cases significant anomalous downfield, observed chemical shift changes. Careful attention to the sequences selected for study will aid in maximizing the observed differences.

Application of Tautomerization- and Protonation-Induced Chemical Shift Changes to the A-C Mismatch. The data presented in Table II should be useful to both experimentalists and theoreticians for resolving the nature of hydrogen-bonding interactions involving A and C residues. One case of particular interest is the A-C mismatch, which has been investigated by proton NMR (Patel et al., 1984a,b) and crystallography (Hunter et al., 1986). Several possible structures have been proposed for this mismatch: rare tautomers of either A or C (Watson & Crick, 1953; Topal & Fresco, 1976; Fresco et al., 1980), wobble structures (Rein et al., 1983; Topal & Fresco, 1976), and a wobble structure involving a protonated A (Topal & Fresco, 1976; Hunter et al., 1986).

Structures involving the rare tautomers were excluded by Patel et al. (1984a,b) as no imino resonances were observed. Upon helix formation, the 8 proton of the A residue at the mismatch site was observed to shift *downfield* to 8.52 ppm while the chemical shift of the 2 proton did not appear to change significantly (8.05 ppm). No interbase NOE's were observed to the 2 proton of the A residue, indicating that it was much further from the helix axis than in normal A-T base pairs. These data were interpreted as best explained by the wobble structure shown in Figure 3a.

The proposed structure, however, is inconsistent with the data for two reasons. First, it does not explain the anomalous downfield shift of the 8 proton. It was suggested by Patel et al. (1984a) that this observed downfield shift may result from protonation; however, this point was not addressed. Second, the 2 proton of the proposed structure may be far enough from the helix axis to explain the absence of interbase NOE's; however, the observed upfield shifts of the neighboring base protons indicate that the A residue is far enough into the helix to experience some upfield shift. Keepers et al. (1984) have calculated that, in the wobble structure proposed, the upfield shift of the 2 proton of the A residue at the mismatch site should be roughly 25% that calculated for the 2 proton at A(5) of the same oligomer, which was observed to be −0.79 ppm. We suggest that both of these apparent inconsistencies may be resolved by addition of a proton to the structure as indicated in Figure 3b.

Data from our study show that, upon protonation, the chemical shift for the 8 proton of dA moves downfield +0.204 ppm to 8.537 ppm (Table I and Figure 2). The chemical shift of the 8 proton of the A residue at the A-C mismatch site also moves downfield upon helix formation, to 8.52 ppm. Both the direction and magnitude of this downfield shift are consistent with A protonation (Figure 3b), and from the magnitude of the shift, it would appear that the A residue was predominantly protonated.

Assume that structure b, Figure 3, predominates. Subtraction of protonation values in Table II, according to eq 1, from the measured chemical shifts reported by Patel et al. (1984a) gives values for the contribution of *stacking*-induced changes in chemical shifts for the 8 and 2 protons of −0.06 and −0.202 ppm, respectively. These values are much more consistent with expectations when A is paired with C in a wobble geometry. For the 2 proton, the similar magnitude, but opposing direction of the protonation and stacking influences, sums to near zero, resulting in a fortuitous cancellation, and thus the stacking component is apparently "hidden".

We believe that all the NMR data on the A-C mismatch can therefore be reconciled by the protonated wobble structure, Figure 3b. The chemical shift data in Table II can also be used to rigorously exclude the presence of rare tautomeric forms. Formation of the rare tautomer by the A residue would have generated significant upfield shifts for both the 2 and 8 protons resulting from the combination of both stacking and tautomerization. The magnitudes of the expected upfield shifts would have been greater than those observed for a normal A-T base pair. As noted, both protons move in the opposite direction. Similarly, formation of the imino tautomer by the C residue would have shifted the 6 proton resonance −0.56 ppm upfield from its observed position (7.26 ppm) in the G-C pair (of the same sequence) to 6.7 ppm. The chemical shift of the 6 proton when the C residue is paired with A is reported to be 7.31 ppm, which is a slight shift in the opposing direction. These data indicate that the C residue has remained electronically unchanged and support the additivity of stacking and protonation/tautomerization influences as indicated in eq 1. The −0.3 ppm downfield shift of the 5 proton of the C residue in the A-C base pair relative to the G-C base pair is explained by a roll of the C into the major groove in order to accommodate the wobble structure.

Although ionized base pairs have historically not been regarded as likely in DNA, several energetic considerations are noteworthy. First, free energy changes associated with base protonation and imino tautomer formation may be determined from the pK values for the derivatives studied here. For both dC and dA, the positive free energy change of protonation at physiological pH is lower than that for tautomerization. In the case of dA, the free energy of protonation at physiological pH is 4.2 kcal/mol, 2.7 kcal/mol lower in energy than the rare tautomer (see Table I).

Second, the positive free energy for protonation of the A residue at physiological pH would be offset by several favorable energetic components. (1) An additional hydrogen bond is formed upon acquisition of a proton from solvent water, and this hydrogen bond replaces the repulsive interaction between lone pairs of electrons on the 2-carbonyl oxygen of the C residue and the ring nitrogen of the A residue. (2) The acidity of the A amino protons is significantly increased upon N-1 protonation, which profoundly increases the strength of the existing N(6)A–N(3)C hydrogen bond (McConnell, 1974). (3) A favorable monopole-induced dipole term is introduced into the stacking interaction. According to the calculations of Jordan and Sostman (1973), the magnitude of an A-C stacking interaction is nearly doubled by A protonation. In the sequence studied by Patel et al. (1984a), the A residue in

the A-C mismatch is between the C's. (4) The presence of the positive charge in the helix would decrease net electrostatic phosphate repulsions along the helix (Topal & Warshaw, 1976).

After this study had been completed, X-ray crystallographic data on a related oligonucleotide containing the A-C mismatch appeared (Hunter et al., 1986). Two close contacts were observed between A and C residues, indicating the presence of two hydrogen bonds. While wobble structures that invoke rare tautomeric forms could not be excluded, these authors suggested that the most plausible structure was also the protonated base pair. The results of our study clearly indicate that, for the A-C mismatch in DNA in solution, the adenine residue is protonated and rare tautomeric forms can be excluded. On the basis of the thermodynamic considerations cited above, we also agree that this is most likely the case in the crystal structure.

Conclusions

In this paper, we have measured the influence of protonation and tautomerization on the chemical shifts of the nonexchangeable base protons of dC and dA. It was found that, for both dC and dA, protonation induces downfield changes in chemical shifts whereas imino tautomer formation induces upfield shifts for all nonexchangeable proton resonances. We suggest that the values reported here may be used to interpret observed chemical shift changes for aberrant base pairs in oligonucleotides. Application of these values to oligonucleotides, however, is based upon the assumption that the influences of protonation tautomerization and base stacking will be roughly additive. To a first approximation, this assumption should be valid; however, if base protonation or tautomerization significantly distorts helix geometry or alters the distance between stacked bases, interpretation of observed chemical shift changes will be more complex. On the basis of this assumption, however, we were able to provide a "best fit" to the structure of the A-C mismatch, which is in accord with crystallographic data (Hunter et al., 1986).

In addition to providing a best fit to oligonucleotide proton NMR data, measurement of chemical shift changes may allow determination of the position of equilibrium between possible structures. Equilibrium constants, including the "complex pK value" for the A residue in the A-C mismatch, may be determined by measuring chemical shifts as a function of pH. Due to potential line broadening of exchangeable proton resonances as a consequence of (1) the rapid equilibrium of possible structures and (2) the presence of protonated species, measured chemical shifts of nonexchangeable base protons may prove to be critical for the characterization of aberrant hydrogen-bonding interactions in DNA.

References

Angyal, S. J., & Angyal, C. L. (1952) *J. Chem. Soc.*, 1461–1466.

Arter, D. B., & Schmidt, P. G. (1976) *Nucleic Acids Res. 3*, 1437–1447.

Bleich, H., & Wilde, J. (1984) *J. Magn. Reson. 56*, 154–155.

Brookes, P., & Lawley, P. D. (1962) *J. Chem. Soc.*, 1348–1351.

Broom, A. D., Schweizer, M. P., & T'so, P. O. P. (1967) *J. Am. Chem. Soc. 89*, 3612–3622.

Bullock, F. J., & Jardetsky, O. (1964) *J. Org. Chem. 29*, 1988–1990.

Crick, F. H. C. (1966) *J. Mol. Biol. 19*, 548–555.

Danyluk, S. S., & Hruska, F. E. (1968) *Biochemistry 7*, 1038–1043.

Danyluk, S. S., Ainsworth, C. F., & MacCoss, M. (1978) in *Nuclear Magnetic Resonance Spectroscopy in Molecular Biology* (Pullman, B., Ed.) pp 111–124, Reidel, Dordrecht, Holland.

Evans, F. E., & Sarma, R. H. (1974) *J. Mol. Biol. 89*, 249–253.

Fazakerley, G. V., Teoule, R., Guy, A., & Guschlbauer, W. (1984) *FEBS Lett. 176*, 449–452.

Fazakerley, G. V., Teoule, R., Guy, A., Fritzsche, H., & Guschlbauer, W. (1985) *Biochemistry 24*, 4540–4548.

Freese, E. (1959) *J. Mol. Biol. 1*, 87–105.

Fresco, J. R., Broitman, S., & Lane, A. E. (1980) in *Mechanistic Studies of DNA Replication and Genetic Recombination* (Alberts, B., & Fox, C. F., Eds.) pp 753–768, Academic, New York.

Giessner-Prettre, C. (1984) *J. Biomol. Struct. Dyn. 2*, 233–248.

Giessner-Prettre, C., & Pullman, B. (1969) *C. R. Hebd. Seances Acad. Sci. 268*, 115–1117.

Giralt, E., Viladrich, R., & Pedroso, E. (1983) *Org. Magn. Reson. 21*, 208–213.

Hunter, W. N., Brown, T., Anand, N. N., & Kennard, O. (1986) *Nature (London) 320*, 552–555.

Jardetsky, C. D., & Jardetsky, O. (1960) *J. Am. Chem. Soc. 82*, 222–229.

Jones, J. W., & Robins, R. K. (1963) *J. Am. Chem. Soc. 85*, 193–201.

Jordan, F., & Sostman, H. D. (1973) *J. Am. Chem. Soc. 95*, 6544–6554.

Keepers, J. W., Schmidt, P., James, T., & Kollman, P. A. (1984) *Biopolymers 23*, 2901–2929.

Lawley, P. D., & Brookes, P. (1961) *Nature (London) 192*, 1081–1082.

Lawley, P. D., & Brookes, P. (1962) *J. Mol. Biol. 4*, 216–219.

Leutzinger, E. E., Miller, P. S., & Kan, L. S. (1982) *Biochim. Biophys. Acta 697*, 243–251.

Macon, J. B., & Wolfenden, R. (1968) *Biochemistry 7*, 3453–3458.

Martin, D. M. G., & Reese, C. B. (1968) *J. Chem. Soc. C*, 1731–1738.

McConnell, B. (1974) *Biochemistry 13*, 4516–4523.

Patel, D. J., Kozlowski, S. A., Marky, L. A., Broka, C., Rice, J. A., Itakura, K., & Breslauer, K. J. (1982a) *Biochemistry 21*, 428–436.

Patel, D. J., Kozlowski, S. A., Marky, L. A., Rice, J. A., Broka, C., Dallas, J., Itakura, K., & Breslauer, K. J. (1982b) *Biochemistry 21*, 437–444.

Patel, D. J., Kozlowski, S. A., Ikuta, S., & Itakura, K. (1984a) *Biochemistry 23*, 3218–3226.

Patel, D. J., Kozlowski, S. A., Ikuta, S., & Itakura, K. (1984b) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 43*, 2663–2670.

Rein, R., Shibata, M., Garduno-Juarez, R., & Kieber-Emmons, T. (1983) in *Structure and Dynamics: Nucleic Acids and Proteins* (Clementi, E., & Sarma, R. H., Eds.) pp 269–288, Adenine, New York.

Singer, B., & Kusmierek, J. T. (1982) *Annu. Rev. Biochem. 52*, 655–693.

Sowers, L. C. (1983) Ph.D. Thesis, Duke University.

Sowers, L. C., Fazakerley, G. V., Eritja, R., Kaplan, B. E., & Goodman, M. F. (1986a) *Proc. Natl. Acad. Sci. U.S.A.* (in press).
Sowers, L. C., Fazakerley, G. V., Kawaja, T. A., & Goodman, M. F. (1986b) (submitted for publication).
Sun, L., & Singer, B. (1974) *Biochemistry 13*, 1905–1913.
Topal, M. D., & Fresco, J. R. (1976) *Nature (London) 263*, 289–293.
Topal, M. D., & Warshaw, M. M. (1976) *Biopolymers 15*, 1755–1773.
Watson, J. D., & Crick, F. H. C. (1953) *Nature (London) 171*, 964–967.

Articles

Bending and Flexibility of Kinetoplast DNA†

Stephen D. Levene,‡ Hen-Ming Wu, and Donald M. Crothers*
Department of Chemistry, Yale University, New Haven, Connecticut 06511
Received October 7, 1985; Revised Manuscript Received March 3, 1986

ABSTRACT: We have evaluated the extent of bending at an anomalous locus in DNA restriction fragments from the kinetoplast body of *Leishmania tarentolae* using transient electric dichroism to measure the rate of rotational diffusion of DNA fragments in solution. We compare the rate of rotational diffusion of two fragments identical in sequence except for circular permutation, which places the bend near the center in one case and near one end of the molecule in the other. Hydrodynamic theory was used to conclude that the observed 20% difference in rotational relaxation times is a consequence of an overall average bending angle of 84 ± 6° between the end segments of the fragment that contains the bending locus near its center. If it is assumed that bending results from structural dislocations at the junctions between oligo(dA)·oligo(dT) tracts and adjacent segments of B DNA, a bend angle of 9 ± 0.5° at each junction is required to explain the observations. The extent of bending is little affected by ionic conditions and is weakly dependent on temperature. Comparison of one of the anomalous fragments with an electrophoretically normal control fragment leads to the conclusion that they differ measurably in apparent stiffness, consistent with a significantly increased persistence length or contour length in the kinetoplast fragments.

It is now generally agreed that DNA molecules can exist in a stably bent conformation, whose character is dictated by special features of base sequence (Marini et al., 1982; Levene & Crothers, 1983; Wu & Crothers, 1984; Hagerman, 1984). This conclusion, stated originally by Marini et al. (1982), is based on the anomalous gel electrophoretic properties of DNA restriction fragments isolated from the kinetoplast body of parasites (Kidane et al., 1984) and the unexpectedly rapid rotational diffusion of these fragments, implying a more compact conformation in solution. Wu and Crothers (1984) used gel electrophoresis to map the bending locus in a fragment isolated from *Leishmania tarentolae* to the sequence -CA_{5-6}T-, repeated at 10 base pair intervals. Because the repeated unit is in phase with the DNA helix screw, the small bends associated with the individual sequence elements add coherently. Levene and Crothers (1983) showed with an appropriate model for sequence-directed bending that computer modeling and molecular graphics predict the position of the bending locus in agreement with the gel electrophoresis experiments.

Our objective in this work is to analyze quantitatively the extent of bending in a DNA restriction fragment containing the *L. tarentolae* kinetoplast (K DNA) bending locus, using rotational dynamics in an experimental approach analogous to that reported by Hagerman (1984). However, we sought to avoid the dubious assumption that the stiffness and contour length of K DNA, as measured by the persistence length P and the rise per base pair h, respectively, are the same as for a DNA fragment of unrelated sequence. Hence, we adopt the strategy used by Wu and Crothers (1984) in mapping the bending locus by gel electrophoresis, namely, to compare the properties of molecules that are identical in sequence except for circular permutation, thus placing the bend either at the middle or near the end of the molecule. As a consequence, comparative measurements refer to molecules of closely related sequence, which are nevertheless quite different in overall shape. While this approach does not eliminate possible problems resulting from variations of P with sequence along a given molecule, it does reduce the error from that source. Primarily because of this difference in experimental approach, our results show that the angular deformation of the helix axis at a bending locus is 2–3 times larger than the bending reported by Hagerman (1984).

Structural interpretation of the observed molecular dimensions from rotational dynamics requires a specific model, of which several have been proposed to account for the sequence-directed bending of DNA in solution. These include deformational anisotropy or wedge bending at ApA dinucleotides (Trifonov & Sussman, 1981; Marini et al., 1982; Levene & Crothers, 1983; Prunell et al., 1984), bending at junctions between regions of different helical structure (Selsing et al., 1979; Levene & Crothers, 1983; Wu & Crothers, 1984), and purine clash (Hagerman, 1984; Zhurkin, 1985), on the basis of Calladine's analysis of DNA structure (Calladine,

†Supported by Grant GM 21966 from the National Institutes of Health.
‡Present address: Department of Chemistry, University of California, San Diego, La Jolla, CA 92093.

0006-2960/86/0425-3988$01.50/0 © 1986 American Chemical Society