Evidence for tautomerism in nucleic acid base pairs. ^{1}H NMR study of ^{15}N labeled tRNA

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ABSTRACT

The imino proton resonances of ^{15}N labeled tRNA appear as asymmetric doublet signals, the asymmetry being dependent on the applied magnetic field strength. Assuming a tautomerism of the type N–H···N $\rightleftharpoons$ N···H–N in the base pairs the line shapes can be simulated. The most important parameters fitted in the simulation are the rate constants of the proton transfer and the mole fractions of either tautomeric state. The rate constants are of the order of $100s^{-1}$ and the mole fractions of the non dominant tautomer about 0.1 depending on the temperature and on the nature of the base pairing. The observations are attributed to a double proton transfer in the base pairs. The unexpectedly slow rates of the double proton transfer process may be connected with a concomitant conformational change of the duplex structure.

INTRODUCTION

Since the discovery of the DNA double helix structure [1] the tautomerism of nucleo bases has been widely discussed [2-4]. It has been proposed that small amounts of unusual tautomers in DNA, leading to wrong base pair formation, may be responsible for certain mutations[3]. However, several lines of evidence indicate that the ratio of the enolic to the keto form of monomer thymine or guanine bases is very small [3,4]. This has led to the conclusion that the amount of unusual tautomers in base pairs is equally small [5]. However, there is no experimental background for this assumption. Very recently, DiVerdi and Opella [6] applied solid state NMR techniques to ^{15}N labeled DNA. They found no evidence that the proton in the NH···N hydrogen bonds of GC or AT base pairs is transferred from the guanine N1 or the uracil N3 to the cytosine N3 or the adenine N1. Interpretations of relaxation time measurements also make a proton transfer in the base pair hydrogen bonds unlikely [7].

We wanted to attack the problem of tautomerism by ^{1}H NMR of ^{15}N labeled tRNA. The imino proton resonances of tRNA appear apart from the rest of the proton resonances, between 11 and 15 ppm downfield [8]. Their chemical shifts and

solvent exchange characteristics indicate that they stem from NH protons of the base pair hydrogen bonds. A number of specific tRNA's from various sources have been thoroughly investigated [9]. Some of the imino proton resonances were assigned to distinct secondary or even tertiary base pairs by either chemical modification or the study of the proton exchange behaviour of tRNA fragments with water as a function of the temperature [9]. Since the chemical shifts are influenced by ring currents of adjacent base pairs an attempt was made to assign the resonances by ring current shift calculations using the coordinates of known crystal structures[10]. This procedure was criticized, the main objection being the presumed differences of the structures in solution and in the crystal form [11]. Recently, precise NOE experiments were successfully applied to assign the imino proton resonances [12,13]. Using this technique at least an identification of secondary or tertiary H bond resonances seems possible.

In the ^{1}H NMR spectrum of tRNA's containing ^{14}N in natural abundance (99.7%) the imino proton resonances appear as relatively broad singlets [8,9]. In addition to the usual dipolar interaction, quadrupolar relaxation of ^{14}N nuclei produces considerable line broadening. As a consequence, the resolution of the corresponding ^{1}H NMR spectra of tRNA's can be achieved only at very high field. Replacement of ^{14}N by ^{15}N should reduce the line width because ^{15}N lacks a quadrupole moment. Additionally, the coupling of the ^{15}N nuclei (nuclear spin 1/2) with the H bond protons should lead to a characteristic signal pattern of the imino proton resonances containing information on the location of the H bond protons, rates of tautomerism and of proton exchange with the solvent protons. Finally, selective decoupling of ^{15}N resonances of the bases should help in the identification of secondary or tertiary base pairs. For these reasons we prepared ^{15}N labeled tRNA's of which some spectral features have already been published [14]. Here we report on a detailed NMR lineshape analysis of some of the imino resonances. The signals show an asymmetric doublet structure which cannot be explained by proton exchange with the solvent, conformational changes or the formation of closed and open structures but only by the presence of interconverting tautomers. This explanation follows immediately from previous NMR studies of double proton transfer[15-17] between hydrogen bonded nitrogen atoms. Thus, our results provide the first evidence for the existence of these presumed tautomers as well as kinetic and thermodynamic data of the tautomerism.

MATERIALS AND METHODS

^{15}N labeled tRNA's were obtained from E.coli MRE 600 which were grown on a minimal medium containing (^{15}NH$_4$)$_2$SO$_4$, 95% enriched in the ^{15}N isotope (Roh-

stoff-Einfuhr GMBH,Düsseldorf,Germany). The tRNA's were isolated and purified according to the procedures described previously [14]. ^{1}H NMR spectra were obtained with Bruker WM 400 and WM 500 NMR spectrometers using the 2-1-4 Redfield pulse technique to suppress the water signal [18]. Acquisition times were generally 0.5 s with 0.1 s delay. Usually, 6-15 minutes were needed to record each spectrum.

Prior to the NMR measurements the tRNA's were extensively dialyzed against quartz-distilled water and the appropriate buffers. The solutions were 100 mM in NaCl, 15 mM in $MgCl_2$ and 10 mM in Na-cacodylate. The pH was 7.0. 5% ^2H_2O was added for the deuterium lock system. The samples (about 220 µl with 1.5-6 mg tRNA) were transferred to 5 mm NMR sample tubes (Wilmad, Buena, N.J.,USA). Na-3-(trimethylsilyl)-1-propane-sulfonate (TSP) was used as reference.

RESULTS AND DISCUSSION

Typical ^{1}H NMR spectra of the imino proton resonances for three [15]N tRNA's are shown in Fig.1 and Fig.2. Compared to reported spectra of tRNA's with 99.7% [14]N twice the number of signals is observed. It appears that the H bond protons are coupled to one of the two nitrogen bridge atoms leading to doublet structures in the spectra. However, the doublets are asymmetric, i.e., the line widths and the peak heights of each doublet line are different although their intensities are equal. In addition, the observed coupling constants $^1J_{1_H-^{15}N}$ vary widely between 89 and 96 Hz [14]. The asymmetry depends on the strength of the applied magnetic field. At 400 MHz the asymmetry is less pronounced. With broad band decoupling of the ^{15}N resonances the doublets coalesce to singlets (spectra not shown). This result is proof of scalar coupling between the H bond protons and the ^{15}N nuclei. The proton resonances of the decoupled spectra are well separated and resolved owing to a reduction in linewidth by a factor of 2-3 as compared to the spectra of t-RNA's with the natural abundance of [15]N [10].

With increasing temperatures the line widths decrease even more (Fig.2). Following a minimum (for tRNA$_1^{fMet}$ around 55°C) the line widths increase again in the premelting temperature range. In the helix coil transition region the doublet signals simply disappear with no coalescence to singulet structures. Clearly, the exchange of the imino protons of the intact double helix with the water protons is too slow to affect the linewidth more than slightly. A more detailed analysis of the ex-change behaviour of the imino protons is under investigation at present. Also a selective decoupling of the bridge nitrogen resonances is currently being carried out in order to assign the proton resonances to secondary or tertiary base pairs.

The peculiar shape of the doublets is observed for most of the imino proton re-sonances regardless of whether they belong to Watson-Crick type AU and GC base
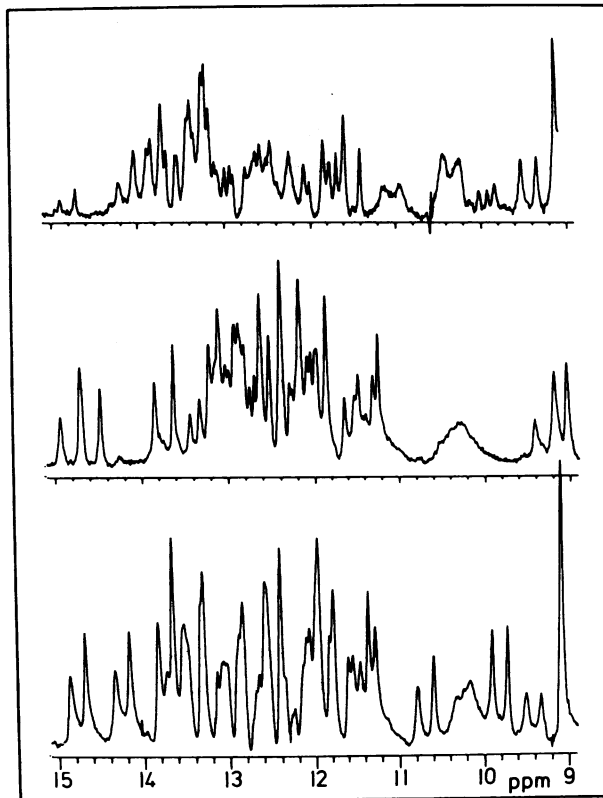
Fig. 1

^{1}H NMR absorption region of the imino protons of three different [15]N tRNA's of E.coli. The spectra of [15]N tRNA$_1^{Val}$ (below, 27°C) and [15]N tRNA$_m^{Met}$ (above, 40°C) are taken at 500 MHz, the spectrum of [15]N tRNA$_1^{fMet}$ (middle, 45°C) at 400 MHz. Line broadening: 2Hz.

pairs or even to the tertiary base pairs. An explanation of the asymmetry of the doublets follows immediately from results of dynamic ^{1}H NMR studies of Limbach et al.[15-17] on intramolecular proton transfer between hydrogen bonded nitrogen atoms

$$^{15}N-H\cdots^{15}N \quad \rightleftharpoons \quad ^{15}N\cdots H-^{15}N \tag{1}$$

in mesotetraphenylporphine and azophenine, i.e. by assuming the presence of tautomers in the base pairs of tRNA. The ^{15}N atoms are either in the spin state α or ß. As Limbach et al.[15,16] showed the exchange problem is then the sum of four two-site problems, one for each combination of ^{15}N spins, i.e. α α , α ß, ßα , and ßß. The exchange scheme is depicted in Fig.3.
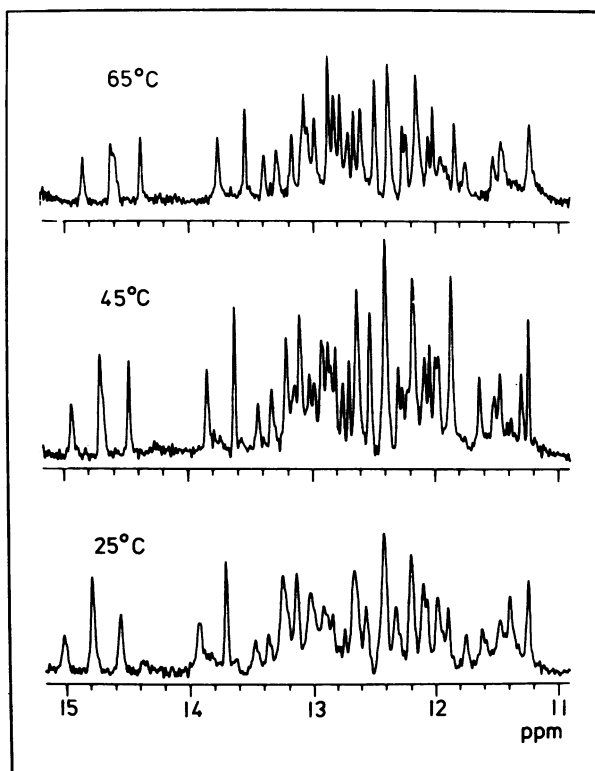
Fig. 2

The low field region of the 400 MHz ^{1}H NMR spectra of E.coli ^{15}N tRNA$_f^{Met}$ at various temperatures. The spectra are resolution-enhanced by Gaussian multiplication (LB=-10Hz, GB=0.17 Hz). Since the original lineshapes are not lorentian, resolution enhancement causes different apparent intensities of the two lines in a doublet. This effect is demonstrated by comparing the doublet at 13.8 ppm with the original lineshapes shown in Fig.5.

The asymmetry arises from the fact that the frequency differences associated with exchanges 1 and 3 are different. These exchange transitions are not allowed in a simple reaction of the type:

$$^{15}NH\cdots^{15}N \ne {}^{15}N-H + {}^{15}N. \tag{2}$$

The asymmetry can also not be explained by proton exchange with the solvent or by relaxation of the ^{15}N nuclei. The proton exchange in the isotopic species ^{14}N-H$\cdots^{15}$N and ^{15}N-H$\cdots^{14}$N contributes the two site transitions 5-6 in Fig.3 so that the total lineshape must be described by a sum of eight two-site problems. The computer program used for the lineshape calculations has been described pre-
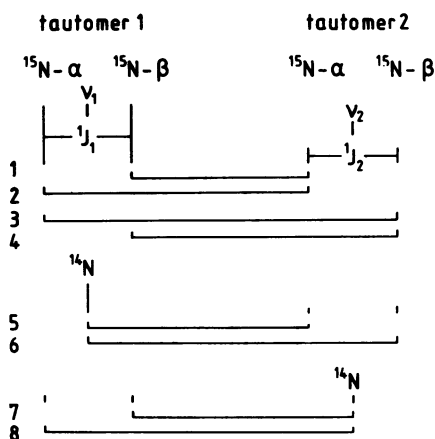
Fig. 3

Spin exchange scheme of the proton transfer reaction described by eq.(1).

viously [15,17]. The lineshape depends on the following parameters for each tautomeric state i=1,2: the chemical shift of the imino proton v_i, the coupling constant $({}^1J_{1_H-15_N})_i$, the static linewidth W_{0i} in the absence of exchange, the mole fraction x_i of the tautomer i and the rate constant $k_{12} = k_{21}x_2/x_1$. During all calculations the good approximation that $({}^1J_{1_H-15_N})_1 = ({}^1J_{1_H-15_N})_2 = {}^1J_{NH}$ and that $W_{01} = W_{02} = W_0$ was made. Further, an ${}^{14}N$ content of 5% was taken into account in all calculations. Since $x_2 = 1-x_1$, the number of independent parameters which describe the spectra were then v_1, v_2, ${}^1J_{NH}$, x_1, W_0, and k_{12}. Fig.4 shows the superposed experimental and calculated lineshapes of the two most downfield shifted doublet signals of E.coli tRNA$^{Val}_1$. These two resonances were previously assigned to the tertiary s^{4}U8–A14 and T54–A58 reverse Hoogsteen base pairs. In the upper and lower part of Fig. 4 the calculated spectra are shown for proton transfer rates which are fast and slow on the NMR time scale. The ${}^{14}N{}^{15}N$ species appear as small singlets in the middle between the doublet lines in the lower spectrum and as doublets in the upper spectrum. The apparent coupling constants in the fast exchange case depend on the ratio of the tautomers, the large coupling being the product $({}^1J_{NH})_1x_1$, the small coupling being $({}^1J_{NH})_2x_2$. Because of this correlation between the mole fractions and the apparent coupling constants it is not surprising that for intermediate exchange rates in the tRNA spectra (Fig.1) ${}^1J_{NH}$ values are observed which vary considerably depending on the extent of the formation of the second tautomer. In general, it will not be possible to observe the fine splitting shown in the upper curve of Fig.4 because the lines are broadened by dipole dipole
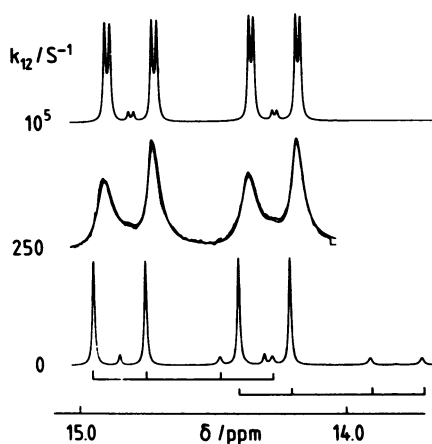
Fig. 4
Superposed experimental and calculated lineshapes of the two most downfield doublet signals of E.coli. ^{15}N $tRNA_1^{Val}$ (W_0= 13 Hz).In the upper and the lower part the calculated spectra are shown for proton transfer rates which are fast and slow on the NMR time scale (W_0 = 5 Hz). The chemical shifts are indicated by the lines of the lower spectrum.

interaction or by proton exchange with the solvent or because the population x_2 decreases with increasing temperature as will be shown below. It should be mentioned that only those spectra could be fitted where the free induction decays were not treated with resolution enhancement procedures prior to the Fourier transformation. The calculations in Fig.4 need some comment concerning the number of parameters which can be extracted from the calculation of the lineshape. In the region $80s^{-1} < k_{12} < 250$ s^{-1} the lineshape does not change very much although v_1, v_2, W_0, and x_1 can be determined without ambiguity. The only significant change in this region is the apparent coupling constant which changes from the value of $^1J_{NH}$ to $^1J_{NH}x_1$. A second characteristic in the slow exchange region is a very weak shoulder on the right side of the signals in Fig.4 which arises from the broadening of the lines of the non-dominant tautomer. It disappears with increasing k_{12}. This shoulder is not easily observed because of the difficulty to find the right phase correction. It is, therefore, difficult to decide whether the rate constant is about 90 s^{-1} or 250 s^{-1}. The spectrum in Fig.4 was calculated with a value of $^1J_{NH}$= 96 Hz and a value of x_1=0.91, the apparent coupling constant being about 91 Hz which is close to what one expects as the true value for the bases. One can conclude then that the interpretation of the apparent constants in terms of $^1J_{NH}x_1$ and the fast exchange case is not correct and that the slow exchange case is probably realized with $k_{12} \approx 90$ s^{-1}. The better fit for k_{12}= 250 s^{-1} is then a result of a small error in the phase correction.

In order to overcome the problem of the phase correction, a resonance assigned previously[9] to the A11–U25 Watson–Crick base pair of E.coli $tRNA_1^{fMet}$ was measured as a function of temperature. The superposed experimental and calculated spectra are shown in Fig.5. All spectra were recorded with the same spectrometer
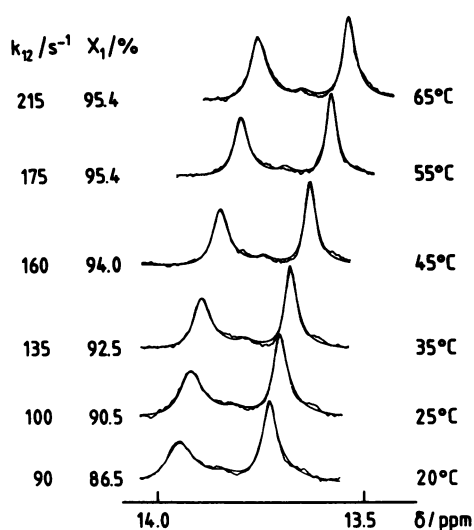
k_{12}/s^{-1}	$X_1/\%$		
215	95.4		65°C
175	95.4		55°C
160	94.0		45°C
135	92.5		35°C
100	90.5		25°C
90	86.5		20°C

14.0   13.5 δ/ppm

Fig.5

Superposed experimental and calculated 400 MHz NMR lineshapes of the A11–U25 imino resonance of E.coli ^{15}N tRNA$_1^{fMet}$ as a function of temperature. No resolution enhancement was applied to these spectra which correspond to an expanded region of spectra shown in part in Fig.2. The rate constants k_{12} and the mole fractions x_1 of the predominant tautomer are given for each spectrum.

adjustment including the same phase correction. Because of the evolution of the signal shape and the peak distances with temperature we know that the slow exchange range is realized below 40°C. In this region the spectra contain enough information for the determination of all 6 of the fitted parameters listed in tab.1 and in Fig.5. By least squares fitting applied the best value for $^1J_{NH}$ was found to be 91.5 Hz in this region, a result consistent with literature values for monomeric hydrogen bonded nucleic acid bases [19]. This value was then held constant in the calculation of the spectra at higher temperatures where the lineshape contains less information. The reason why 5 independent parameters can still be obtained from the lineshape lies in the fact that the doublet lines are not lorentzian and that, therefore, the two linewidths, the two peak heights (i.e.,the asymmetry), and the small intensity between the lines are independent of each other. Fig.5 shows an excellent fit between the experimental and calculated lineshapes; the prediction of our model that the asymmetry must disappear at higher temperatures is particularly

Tab.1

Parameters of the line shape analysis shown in Fig.5.

t/°C	W_0/Hz	v_1/Hz	v_2/Hz
20	11.5	5543.0	5419.0
25	11.0	5533.7	5402.0
35	8.0	5525.0	5375.0
45	7.5	5508.5	5322.0
55	7.0	5489.0	5260.0
65	8.0	5475.0	5190.0

well fulfilled. From a logarithmic plot of the equilibrium constant $K=x_1/x_2$ against $1/T$ (Fig.6) values of $\Delta H= 21$ kJmol^{-1} and of $\Delta S= 88$ J K^{-1}mol^{-1} were obtained for the tautomeric equilibrium. From the temperature dependence of the rate constant k_{12} an activation energy of 16 kJmol^{-1} and a frequency factor of log A = 4.75 was derived which corresponds to an activation entropy of $\Delta S^{\neq}_{298K}= -161$ J mol^{-1}K^{-1}. The values of W_0 listed in tab.1 are relatively small, containing an artificial line broadening of 2 Hz. Since we assume a non negligible contribution of dipolar relaxation to W_0 we estimate that even at 55°C the proton exchange rate with the solvent does not exceed a value of about 2–3 s^{-1}. So far we have dealt only with the proton motion in the N-H···N hydrogen bonds. It seems evident that this motion is coupled to the motion of the corresponding proton in the N-H···O hydrogen bond, i.e. that we have monitored not a single but a double proton trans-fer process. A single proton transfer would create electric charges on the nucleic acid bases which seems unfavorable from an energetic point of view. Double and single proton transfer processes can be distinguished from each other by measuring the proton transfer rates as a function of the deuterium mole fraction D of the samples [16,20]. The proton exchange rates are affected by a variation of D only if a double or polyproton transfer process is present as Limbach et al.[16,21] have shown for the HH transfer in meso-tetraphenylporphine, azophenine and the system acetic acid/ methanol/ tetrahydrofuran. In a first attempt to measure the kinetic HH/HD isotope effect of the reaction we measured the signal shown in Fig.5 in a sample with D=0.8. However, though there seem to be some interesting effects the spectra were of insufficiently good quality because of the bad signal-to-noise ratio to obtain a quantitative kinetic isotope effect at present.

We discuss our results in terms of a tautomerism as depicted in scheme I for Watson–Crick base pairs[1-3].

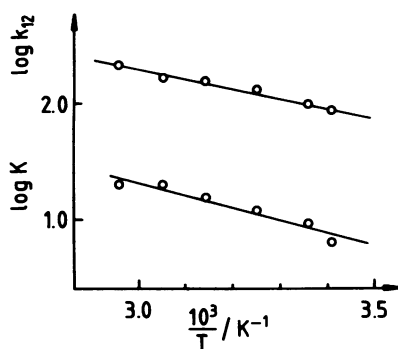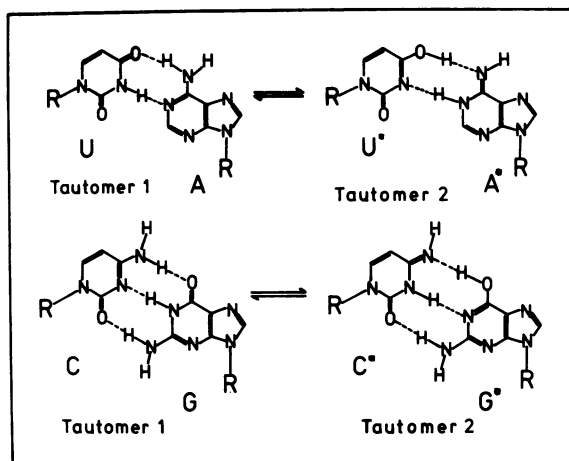We presume that the predominant tautomeric form of the base pairs is the keto

Fig.6
Logarithmic plot of the equilibrium constants $K=x_1/x_2$ (lower trace) and the rate constants k_{12} (upper trace) ver-sus $1/T$. The data were derived from the calulation of the spectra of Fig.5.

SCHEME 1

form although this conclusion cannot be obtained from the lineshape analysis. However, several lines of evidence indicate that this assumption is correct. It has been shown previously that the cytosine C4–NH$_2$ and the adenine C6–NH$_2$ bonds have double bond character, whereas the guanine C2–NH$_2$ bond has not [22]. Because of this sp^2 character the ^{15}N NMR resonances of the amino groups of cytosine and adenine appear more downfield than that of the guanine ring. It would be expected that with base pair formation the ^{15}NH$_2$ resonances of cytosine and adenine would shift further downfield if the enolic tautomer is formed. Indeed this has been found. Watanabe et al.[23] reported a downfield shift of the adenine ^{15}NH$_2$ resonance with increasing concentration of base pairing partners in chloroform. James et al.[24] found an upfield shift of the cytosine and adenine ^{15}NH$_2$ resonances of DNA when the DNA is unfolded. However, these shifts are relatively small. Therefore, a predominant formation of the enolic tautomer seems unlikely. We have some reason to deny the involvement of the H bond between the guanine amino group and the cytosine keto group in the discussed tautomerism. The lack of double bond character of the guanine C2–NH$_2$ bond would exclude a proton transfer. The lack of proton transfer in that H bond would imply that the corresponding ^{1}H NMR lineshape of the NH$_2$ group is symmetric. Indeed, we do observe some symmetric doublet signals, although we are not able at present to identify these doublets clearly as the guanine amino proton resonances. Evidence for the formation of tautomeric equilibria between monomeric nucleic acid bases was reported by Iwahashi and Kyoguku [25,26] from NOE investigations of the base pair H bond ^{1}H NMR resonances.

A proton transfer in the N–H$\cdots$N hydrogen bond was excluded from solid state

NMR studies of ^{15}N labeled DNA [6]. It was found that in the GC pairs the guanine N1 position bears a proton whereas the cytosine N3 position does not. However, it seems that either double proton transfer is not possible in the solid DNA or that the method is just not sensitive enough to sense an amount of 5-10% of unusual tautomers. From relaxation time measurements of imino proton resonances of a 12-base-pair DNA restriction fragment it was concluded that unusual tautomeric states are unlikely [7]. We think that the uncertainties inherent in the interpretation of relaxation times with respect to distances do not allow an exclusion of other tautomeric forms.

Although we are not certain whether the tautomerism found for tRNA does occur in the DNA structures it should at least be possible. As we have shown for the s^4U8-A14 and T54-A58 tRNA$_1^{Val}$ imino resonances the tautomerism even occurs in a presumably non-coplanar tertiary base pairing. Hence geometric restrictions apparently do not prevent a tautomerism.

At first sight the double proton transfer rates reported here seem rather slow compared to the rates of the lactim-lactam conversion of o -pyridones [27]. However, double proton transfer reactions can also be very slow even if a tunnelling mechanism applies as, for example, in the case of double proton transfer in tetraphenylporphine or azophenine where the rate constants are only one magnitude higher than the values reported here. The reason for the slow proton exchange rates in tRNA may be a collective rearrangment of more atoms than just the observed protons which reduces tunnelling probabilities. Such a rearrangement may be, for example, a propeller twisting of the base pairs, which was recently observed in a crystallographic study of a double helical dodecanucleotide [29]. We hope to be able to say more about the role of this coupling of the proton and the heavy atom motion by measuring the HH/HD kinetic isotope effects of the tautomerism. Additionally, we are currently investigating ^{15}N labeled DNA restriction fragments in order to know to which extent the tautomerism depends on the molecular structure.

An amount of 5-10% of unusual tautomers can no longer be neglected when discussing the functional properties of DNA or RNA double helix structures. The affinity for hydrogen bonding in the complex between proteins and nucleic acid double helices may be different for either of the tautomeric states and, therefore, the specificity of protein nucleic acid interaction may also depend on the extent of the formation of unusual tautomers. It has recently been shown that some of the imino proton resonances of DNA fragments containing the lac operator sequence shift position when the DNA binding domain of the lac repressor binds specifically to the fragment [30]. These shifts can be interpreted by a change in the geometry of adjacent base pairs but may also be interpreted by a shift in the tautomeric equilibrium.

REFERENCES

1. Watson, J.D. and Crick, F.H.C. (1953) Nature 171, 737-738.
2. Löwdin, P.O. (1963) Rev.Mod.Phys. 35, 724-732.
3. Ts'o, P.O.P. (1974) in "Basic Principles in Nucleic Acid Chemistry, (Ed.P.O.P. Ts'o), Academic Press, New York, pp.457-461.
4. Grinberg, H., Caparelli, A.L., Spina, A., Maranon, J. and Sorarrian, O.M. (1981) J.Phys.Chem. 85, 2751-2759.
5. Miles, H.T. (1961) Proc.Natl.Acad.Sci.U.S.A. 47, 791-795.
6. DiVerdi, J.A. and Opella, S.J. (1982) J.Am.Chem.Soc. 104, 1761-1762.
7. Early, T.A., Kearns, D.R., Hillen, W. and Wells, R.D. (1981) Biochemistry 20, 3756-3764.
8. Kearns, D.R., Patel, D.J. and Shulman, R.G. (1971) Nature, 229, 338-339.
9. Reid, B.R. (1981) Ann.Rev.Biochem. 50, 959-966.
10. Kearns, D.R. (1977) Ann.Rev.Biophys.Bioeng. 6, 477-513.
11. Jardetzky, O. and Roberts, G.C.K. (1981) NMR in Molecular Biology Academic Press, New York, pp.506-508.
12. Johnston, P.D. and Redfield, A.G. (1981) Biochemistry 20, 3966-4006.
13. Hare, D.R. and Reid, B.R. (1982) Biochemistry 21, 1835-1842.
14. Kaun, E., Rüterjans, H. and Hull, W.E. (1982) FEBS-Letters 141, 217-221.
15. Hennig, J. and Limbach, H.H. (1979) J.Chem.Soc.Far.II 75, 752-766.
16. Limbach, H.H., Hennig, J., Gerritzen, D. and Rumpel, H. (1982) J.Chem.Soc.Far. Disc. No. 74 "Electron and Proton Transfer", in press
17. Limbach, H.H. (1979) J.Magn.Reson. 36, 287-300.
18. Redfield, A.G. and Kunz, S.D. (1975) J.Magn.Reson. 19, 250-254.
19. Poulter, C.D. and Livingston, C.C. (1981) Tetrahedron Letters 9, 755-758.
20. Limbach, H.H., "The Use of NMR Spectroscopy in the Study of Hydrogen Bonding in Solution", (1982), in "Aggregation Processes" (Eds.J.Gormally and E. Wyn-Jones), Elsevier, Amsterdam, chapter 16
21. Limbach,H.H. and Seiffert, W. (1980) J.Am.Chem.Soc. 102, 538.
22. Büchner, P., Maurer, W. and Rüterjans, H. (1978), J.Magn.Reson. 29, 45-63.
23. Watanabe, M., Sugeta, H., Iwahashi, H., Kyoguku,Y. and Kainoska, M. (1981) Eur.J.Biochem. 117, 553-558.
24. James, T.L., James, J.L. and Lapidot, A. (1981) J.Am.Chem.Soc. 103, 6748-6750.
25. Iwahashi,H. and Kyoguku, Y. (1978) Nature 271, 277-278.
26. Iwahashi, H. and Kyoguku, Y. (1980) J.Am.Chem.Soc. 102, 2913-2917.
27. Dreyfus, M., Dodin, G., Bensaude, O. and Dubois,E. (1975) J.Am.Chem.Soc. 97, 2369-2376.
28. Drew, H.R., Wing, R.M., Takano, T., Broka, C., Tanaka, S., Hakura,K. and Dickerson, R.E. (1981) Proc.Natl.Acad.Sci.U.S.A. 78, 2179-2183.
29. Levitt, M. (1978) Proc.Natl.Acad.Sci.U.S.A. 75, 640-645.
30. Buck, F., Hahn, K.D., Zemann, W., Rüterjans, H., Scheek, R., Kaptein, R., Sadler, J., Betz, J. and Hull, W.E. Eur.J.Biochem. submitted for publication.

31. A.G.Redfield, personal communication

NOTE ADDED IN PROOF

According to Redfield[31] there is an alternative explanation of the asymmetry of the doublets shown in fig. 1, 2, 4, 5. The range of frequencies experienced by the proton in a ^{15}N-H dipole during rotation in space depends not only on the dipolar coupling tensor but also on the chemical shift tensor, especially at high fields. This frequency range is smaller for a ^{15}N(α)-H pair than for a ^{15}N(β)-H pair. At a given temperature motional narrowing will then be less effective for the ^{15}N(β)-H line which is the low field line of the doublet.

At present, it is not possible to distinguish experimentally between the proton transfer (PT) and the dipolar coupling/chemical shift anisotropy (DCCSA) mechanism of asymmetric doublet line broadening before more examples of this asymmetry are known. One possible way of distinction between the two mechanisms may be provided by the fact that the DCCSA mechanism predicts always a larger low field line in contrast to the PT mechanism. The observation of an inversed doublet asymmetry with a larger high field line would then be understandable only by the PT mechanism, i.e. by the presence of tautomers.