BIOLOGICAL APPLICATIONS OF MAGNETIC RESONANCE

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CONTENTS

HYDROGEN-BONDED PROTON EXCHANGE AND ITS EFFECT ON NMR SPECTRA OF NUCLEIC ACIDS

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I. INTRODUCTION

Nuclear magnetic resonance spectroscopy is one of the few techniques that permits direct observation of protons situated in hydrogen bonds. This possibility had been realized very early in the application of NMR to structural studies of alcohols (Liddel and Ramsey, 1951). Since then numerous studies have been carried out on small molecules from which it became clear that hydrogen bonding results in downfield shifts of the resonances of protons involved in hydrogen bonds. Kearns *et al.* (1971) were the first to observe hydrogen-bonded proton resonances in nucleic acids. These investigators studied the NMR spectra of transfer RNAs and found that the ring N protons of uridine and guanine, when involved in hydrogen bonding, give rise to resonances shifted far downfield to about 14 ppm from the methyl resonance of the reference 2,2-dimethyl-4-silapentane-1-sulfonate (DSS). These findings provided the experimentalists with "a window" through which important junctions in such biological macromolecules could be observed and therefore triggered a number of experiments in which the structure and stability of nucleic acids were studied. This chapter is primarily concerned with the exchange behavior of the hydrogen-bonded protons and its influence on their NMR spectra. Such studies are important for two reasons. First a good understanding of the exchange phenomena of protons in hydrogen bonds is required in order to be able to derive structural information from the hydrogen-bonded proton resonance spectra. For instance, in so-called melting experiments in which the disappearance of hydrogen-bonded resonances is studied as a function

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of temperature, exchange phenomena are to be related to the actual physical disruption of basepaired regions. Second, these exchange processes are interesting per se. The formation and disruption of hydrogen bonds of double helical RNA and DNA structures are key events during a number of biological processes, yet very often they take place far below the thermal helix to coil transition of these hydrogen-bonded structures. For instance, during the replication and transcription of DNA, the two self-complementary strands have to be separated. Physical chemical studies have shown, however, that the spontaneous opening of double-stranded DNA is a very unlikely event under physiological conditions. Stretches of basepairs have little tendency to open up (Gralla and Crothers, 1973; Lukashin *et al.*, 1976). On the other hand, as is known from tritium exchange experiments (Teitelbaum and Englander, 1975a,b) and from chemical modification methods like formaldehyde and mercury binding (McGhee and von Hippel, 1975a,b; Lukashin *et al.*, 1976; Williams and Crothers, 1975), DNA and also RNA possess a conformational motility, which results in fluctuational opening of basepairs. This behavior is likely to play an important role during replication and transcription among other processes. NMR provides a physical method to approach these problems since the hydrogen-bonded proton resonances reflect the fluctuational motility in basepairs well below the thermal melting transition.

Here the influence of exchange on the hydrogen-bonded proton spectra will be discussed after some introductory remarks on the position and assignments of these resonances have been made.

II. LOCATION OF HYDROGEN-BONDED PROTON RESONANCES OF NUCLEIC ACIDS

Protons involved in hydrogen bonds resonate at rather low fields, well resolved from the bulk of proton resonances from the molecule under study. While the resonances of most protons of diamagnetic molecules cover a region of about 10 ppm downfield from the methyl resonance of the reference compound DSS, resonances from protons participating in hydrogen bonds in nucleic acids are found from about 9 to 16 ppm downfield from the reference signal, depending on the type of hydrogen bond involved. This downfield shift is mainly the result of polarization of the N-H bond in one molecule caused by the electric field of atomic charges in the complexing molecule (Berkeley and Hanna, 1964; Slejko and Drago, 1973; Giessner-Prettre *et al.*, 1977). In addition, shifts caused by anisotropic diamagnetic susceptibility terms like the ring current shift of the complexing molecule may also contribute to this downfield shift.

The spectral regions, where hydrogen-bonded ring nitrogen protons and exocyclic aminoprotons participating in basepairs resonate, have been inferred from studies on transfer RNAs and double helical RNA and DNA model systems. In numerous studies, following the discovery of Kearns, Patel, and Shulman, it has been established that resonances from ring N-H protons hydrogen bonded to ring nitrogens, $>N\text{-}H\cdots N<$, are found between 16 to 11 ppm downfield from DSS. This is true for ring N protons in classical Watson-Crick basepairs as well as in nonclassical basepairs. An example is given in Fig. 1, where the spectrum of the double helix formed by oligo A and oligo U is compared with the spectrum of the triple helix formed by oligo A-oligo U-oligo U. One basepair in the triple helix is a normal Watson-Crick combination while the other is a Hoogsteen pair in which the N_3H proton of uridine is complexed to the N_7 ring nitrogen of adenine (see Fig. 1). The ring N protons of AU pairs generally resonate at somewhat lower field values, i.e., between 14.5 and 12 ppm, than the ring N protons of GC pairs, which are found between 13.6 and 11.5 ppm. These numbers should not be considered as exact limiting values, nor are those to be discussed below.

The C-C^+ basepair in acid oligo C solutions gives rise to a resonance at 15.5 ppm, which is assigned to the C^+N_3H proton (Kallenbach *et al.*, 1976). Also at low field, 15.2 ppm, the ring proton resonance of inosine in the basepair I-C (Patel, D. J., 1977, personal communication) is found. Hydrogen bonds of the type $>N\text{-}H\cdots O{=}C<$, i.e., ring N protons participating in hydrogen bonding to a carbonyl oxygen, are found in GU pairs. These protons resonate between 12 to 10.5 ppm. This follows from experiments on yeast $tRNA^{Asp}$ (Robillard *et al.*, 1976) and on poly GT (Kearns, 1977). The same type of hydrogen bonds are present in quadrupole complexes of guanosine monophosphates (Pinnavaia *et al.*, 1975) and of oligo I (Kallenbach *et al.*, 1976). These complexes give rise to resonances at 11.1 ppm and 11.8 ppm, respectively.

The resonances of exocyclic aminoprotons hydrogen bonded to carbonyl oxygen are found upfield from 9 ppm and so far have been accessible to detailed study in a few isolated cases (Patel, 1976, 1977). Exocyclic aminoprotons complexed to ring nitrogens resonate around 9 ppm (Steinmetz-Kayne *et al.*, 1977). An example is provided by the acid form of oligo A (see Fig. 2). At low pH, oligo A forms a double helical complex in which one aminoproton is hydrogen bonded to adenine N_7 and the other aminoproton to the phosphate group of the opposite chain. The resonance at 9 ppm was assigned to the first, the resonance at 8 ppm to the second aminoproton (Geerdes, H. A. M., Kremer, A., and Hilbers, C. W., 1977, unpublished results). These data have been collected in Table I.

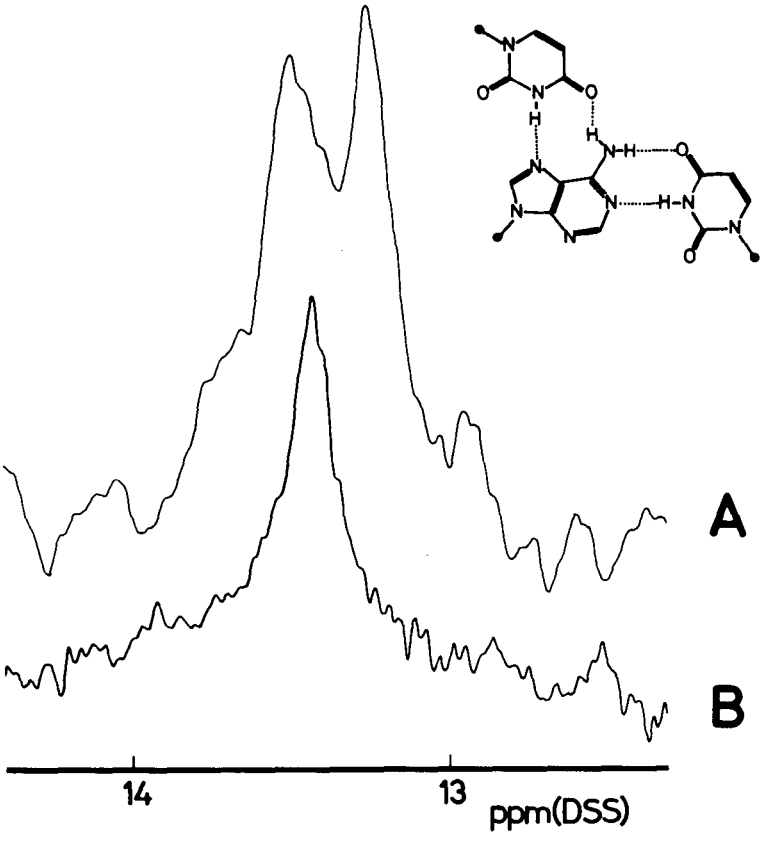


Fig. 1. The 360 MHz spectra of the oligo A-(oligo U)$_2$ triple helix (upper spectrum) and oligo A-oligo U double helix (lower spectrum) in a buffer containing 0.12 M NaCl, 10 mM sodium-cacodylate, 0.5 mM EDTA at pH 7.0, recorded at 5°C. The concentrations (in monomers) were in the triple helix oligo A 8 mM, oligo U 16 mM, and in the double helix both 11 mM. A pairing scheme of the triple combination is given. Note that the resonances are from the ring N_3 protons (Geerdes and Hilbers, 1977, Nucl. Acids Res. 4, 207-221).

In the crystal structure of yeast $tRNA^{Phe}$, also ring N protons have found to be hydrogen bonded to phosphate groups, for instance, the interaction $U_{33}N_3H\text{-}P_{36}$. On the basis of an-

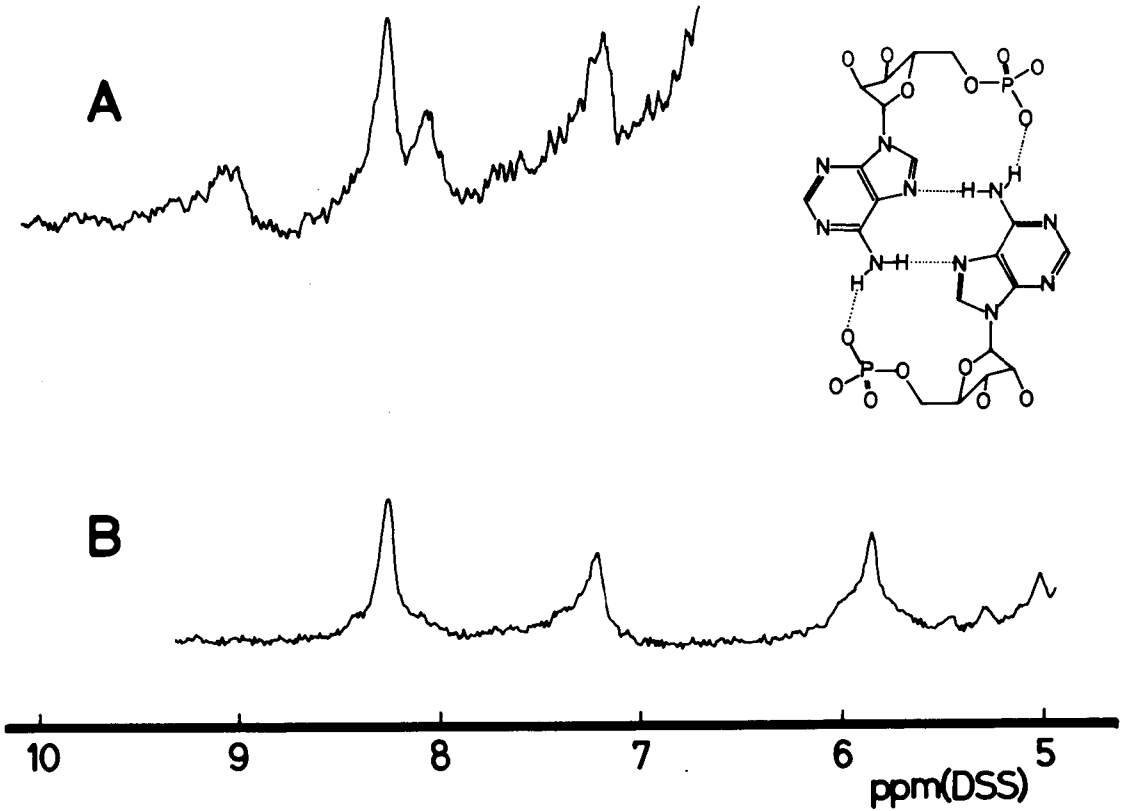


Fig. 2. The 360 MHz spectra of double helical oligo A, approximate chain length 14 nucleotides, recorded in H_2O (A) or D_2O (B) at 25°C, pH 4.5. Concentration oligo A ~ 4 mM. The resonances at 9.1 and 8.1 ppm in (A), not visible in (B), are assigned to the amino protons complexed to AN_7 and the phosphate group, respectively.

TABLE I. Location of the ring N hydrogen-bonded proton resonances of different basepairs, in ppm downfield from DSS[a].

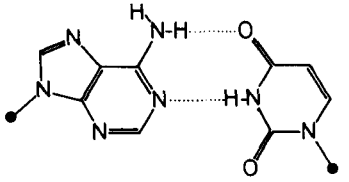
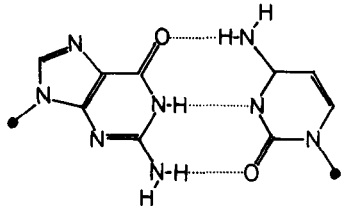
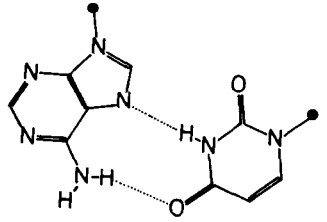
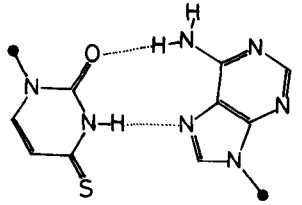
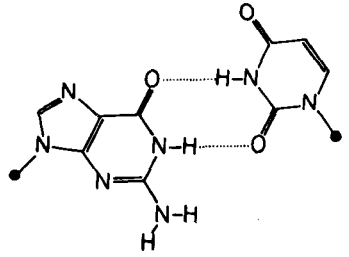
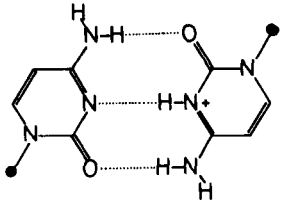
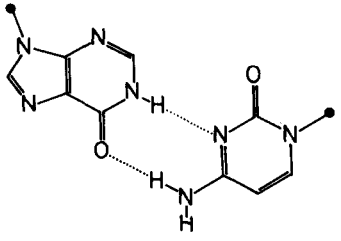
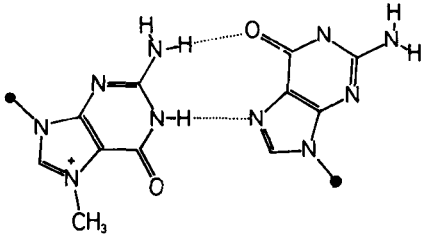
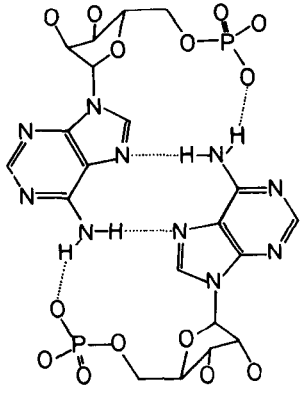
Basepair	*Type*	*Location of resonances*
	*AU* *Watson-Crick*	*14.5-12*
	*GC* *Watson-Crick*	*13.5-11.5*
	*AU* *Hoogsteen*	*14.5-12*
	s^4UA *reversed Hoogsteen*	*15-12*
	*GU*	*12-10.5*

TABLE I (continued)

Basepair	*Type*	*Location of resonances*
	*C-C$^+$* *acid* *oligo C*	 *~16*
	*I-C*	*~15*[b]
	*m^{7}G-G*	*14.5-12.5*
	*A-A'* *acid* *oligo A*	*N-H•••N ~9* *N-H-OP ~8*

[a]*Also included are the positions of the amino proton resonances of acid oligo A.*

[b]*D. J. Patel (private communication, 1977).*

ticodon hairpin studies Kearns (1976) suggests that resonances at ~11.5 ppm common to all tRNA spectra may come from these hydrogen-bonded protons. At present these assignments cannot be considered firmly established, since for yeast $tRNA^{Phe}$ this resonance is not affected after nicking the anticodon loop between U_{33}, which provides a hydrogen bond to phosphate P_{36} and G_{34} (Salemink, P. J. M., unpublished results, 1977).

III. RING CURRENT SHIFT CALCULATIONS

Having located the different types of hydrogen-bonded resonances in particular regions, the first problem one encounters when examining NMR spectra is that of assignment. In exchange and structural studies it is necessary that the resonances be assigned to particular protons in the molecule. In general this is a tedious problem, especially for larger nucleic acid structures like tRNA. Two approaches have been taken so far, namely, selective (chemical) modification of the nucleic acid structure (Wong and Kearns, 1974; Reid *et al.*, 1975; Daniel and Cohn, 1975; Salemink *et al.*, 1977) and the application of ring current shift calculations. The latter are used to explain the secondary shifts, i.e., the shifts from the resonance positions that hydrogen-bonded protons have in an "isolated" basepair, one not surrounded by other basepairs. The resonance position in such a basepair is designated as the intrinsic resonance position. Ring current effects are expected to provide the main shift mechanism, since the bases have aromatic character and are therefore expected to be employable to predict resonance positions. The computations used the ring current shift contours provided by Giessner-Prettre and Pullman (1970) and started from the assumption that the RNA or DNA double helical stretches had A'RNA (Shulman *et al.*, 1973) or B-DNA structure (Patel and Tonelli, 1974) in solution, respectively. Only nearest-neighbor contributions were included in these calculations. By studying a number of model systems (Shulman *et al.*, 1973; Patel and Tonelli, 1974), shifts for the different possible basepair combinations were determined and tabulated and used to derive the resonance position of the ring N protons in "isolated" AU and GC basepairs. Calculated and experimental positions of a number of model systems have been collected in Table II.

In general the calculated positions agree within one- or two-tenths of a ppm with the observed positions except for terminal basepairs, which very often yield experimental resonance positions at higher field from the calculated positions. The latter observations can be rationalized by taking fraying ef-

TABLE II. Observed and calculated positions of the ring N hydrogen bonded proton resonances in different oligonucleotides.[a]

Oligonucleotide	*Basepair*	*Observed position*	*Calculated position*
1234	*A.T. (terminal)*	*13.6*	*14.3*
d AAAGCTTT[b]	*A.T. (2 position)*		*14.1*
•••••••• *TTTCGAAT*	*A.T. (3 position)*	*14.2*	*14.2*
	G.C.	*13.1*	*12.9*
	A.U. (terminal)	*13.2*	*13.4*
r AAGCUU[c]			
•••••• *UUCGAA*	*A.U. (internal)*	*14.25*	*14.0*
	G.C.	*13.5*	*13.3*
	A.T. (terminal)	*13.15*	*13.9*
d ATGCAT[d]			
•••• *TACGTA*	*A.T. (internal)*	*13.8*	*13.85*
	G.C. (central)	*12.75*	*12.74*
d CGCG[e]	*G.C. (terminal)*	*13.4*	*13.3*
•••• *GCGC*	*G.C. (internal)*	*13.25*	*13.0*
d CCGG[f]	*C.G. (terminal)*		*13.4*
•••• *GGCC*	*C.G. (internal)*	*13.3*	*13.1*

TABLE II (Continued)

Oligonucleotide	*Basepair*	*Observed position*	*Calculated position*
d GGCC[f]	G.C. (terminal)		13.3[5]
••••		13.1	
CCGG	C.G. (internal)		13.1[5]
d AGAGAG[g]	G.C. (internal)	12.6-12.7	12.4[5]
••••••			
TCTCTC	A.T. (internal)	13.8-14.0	14.1
d GCGCGC[g]			
••••••	G.C. (internal)	13.1-13.2	13.1
CGCGCG			
d TATATA[g]			
••••••	A.T. (internal)	13.35	13.3
ATATAT			

[a] *All calculations were based on a B-DNA structure, using the ring current shift table by Patel and Tonelli (1974) except for r(AAGCUU) for which an A'RNA double helix structure was used (Kan et al., 1975).*

[b] *Kallenbach et al. (1976).*

[c] *Kan et al. (1975).*

[d] *Patel and Hilbers (1975).*

[e] *Garssen et al. (1977).*

[f] *Patel (1977).*

[g] *Patel and Tonelli (1974).*

fects of helix ends into account (see below) and/or by allowing for interstrand stacking interactions. Recently the shift calculations have been refined by including next nearest-neighbor contributions to the ring current shift (Arter and Schmidt, 1976) as well as contributions due to the anisotropy of the diamagnetic susceptibility other than those from ring currents (Giessner-Prettre and Pullman, 1976). Inclusion of these additional effects requires a readjustment of the intrinsic resonance positions. One may legitimately ask whether addition of