RING CURRENT SHIFTS IN GU BASE PAIRS

H. A. M. GEERDES and C. W. HILBERS
Laboratory of Biophysical Chemistry, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

Received 23 August 1979

1. Introduction

NMR spectroscopy has been used extensively during the last years to study the structure and dynamics of RNA and DNA in solution (reviewed [1,2]). Special impetus for these studies has come from the discovery [3], that the resonances of exchangeable hydrogen bonded ring N protons in Watson-Crick base pairs can be resolved in the low-field NMR spectra of double helical RNAs and DNAs in the region between 11 and 15 ppm downfield from the reference signal of 4,4 dimethylsilapentane-1-sulfonate (DSS). Among other approaches the interpretation of these spectra has been attempted by using ring current shift calculations. The first ring current shift tables were derived to predict the hydrogen bonded proton NMR spectra of tRNAs [4]. These tables have been extended and improved and at present ring current shift tables are available for the DNA and RNA double helical structures, which were resolved by means of X-ray fiber diffraction studies [5].

In the helical stems of tRNAs and 5S rRNAs very often GU base pairs are present and a number of experiments have shown that these bases form so called wobble base pairs first indicated by Crick [6] (see fig.1). In the acceptor stem of yeast $tRNA^{Phe}$ a GU pair is present, which in the X-ray crystal model of the molecule is paired according to the scheme given in fig.1 [7–9].

NMR experiments have demonstrated, that also in solution GU base pairs are intact. The most convincing evidence has been provided by double resonance experiments [10]. The resonance positions of the hydrogen bonded ring N protons in GU pairs are found between 12 and 10 ppm downfield from DSS so that the resonances very often are resolved from the other hydrogen bonded proton resonances. Therefore it is worthwhile to make available a table providing the ring current shift contributing from all possible combinations of neighbouring base pairs using a format similar to that in earlier publications. In this way a method is provided to derive the resonance positions of the ring N protons in GU base pairs.

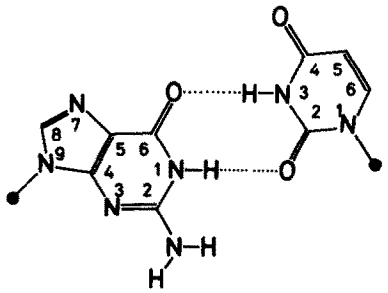

Fig.1. Hydrogen bonding in a GU base pair.

2. Method of calculation

The ring current shift of the GN_1H and UN_3H protons hydrogen bonded in the GU base pair were calculated along the lines indicated [11] using the methods in [12]. Briefly, the ring current shielding effect from a polycyclic aromatic molecule at the position of the proton considered is given by:

$$\Delta\delta = 2.13 \sum_i \frac{I_i}{r_i} G_i \qquad (1)$$

where $\Delta\delta$ represents the calculated ring current

shift. $\sum_i$ represents the summation over the contribution of all aromatic rings in the molecule. In the present calculations only the contributions of rings within a distance of 10 Å from the proton are considered, i.e., only nearest and next-nearest neighbouring base pairs are taken into account. I_i is the ring current intensity of ring i relative to the ring current intensity of benzene; r_i is the radius of the ring i and G_i is a geometrical factor given by:

$$G = \frac{2}{\{(1+\rho)^2 + z_-^2\}^{1/2}} \left\{ K_- + \frac{1-\rho^2-z_-^2}{(1-\rho)^2+z_-^2} \cdot E_- \right\} + \frac{2}{\{(1+\rho)^2 + z_+^2\}} \left\{ K_+ + \frac{1-\rho^2-z_+^2}{(1-\rho)^2+z_+^2} \cdot E_+ \right\} \quad (2)$$

In this equation $K_\pm$ and $E_\pm$ are elliptical integrals of the first and second kind, respectively, with modulus

$$k_\pm^2 = 4\rho \{(1+\rho)^2 + z_\pm^2\}^{-1} \cdot z_\pm = z \pm \langle \bar{z} \rangle$$

where $\langle \bar{z} \rangle$ is the distance of the current loop with respect to a plane through the ring atoms. The loop is located at the average distance of the $2p_z$ orbitals. In the calculations $\langle \bar{z} \rangle$ was taken equal to 0.57 Å [13]. The elliptic integrals were calculated according to [14]. Furthermore ρ and z are the cylindrical coordinates of the GN_1H or the UN_3H proton in the GU base pair defined with respect to the particular aromatic ring of a neighbouring base. The z-axis is chosen through the centre of the ring perpendicular to the plane through the ring atoms; ρ is the distance of the proton from the z-axis.

To calculate the coordinates ρ and z of the hydrogen bonded GN_1H and UN_3H protons in the GU base pair with respect to all possible combinations of nearest and next-nearest neighbour Watson-Crick base pairs use was made of the crystal coordinates of the acceptor stem of yeast $tRNA^{Phe}$, where a GU pair forms the fourth base pair. This yields the ring current shifts for only one possible combination of neighbouring base pairs. The coordinates of other combinations were generated by transferring the neighbouring purine–pyrimidine base pairs in the acceptor stem of yeast $tRNA^{Phe}$ to pyrimidine–purine base pairs. The transformation is effected by making a mirror image of the base pair with respect to the dyad axis, i.e., the line located within the plane of the base pair going through the helix axis and through the middle of the line connecting the purine N_9 with the pyrimidine N_1. In addition, by substituting the coordinates of adenine for guanine and of cytosine for uracil and vice versa the ring current contributions at the hydrogen-bonded protons in the GU base pair have been calculated for all possible combinations of nearest and next-nearest neighbours.

3. Results and discussion

The ring current shifts of the hydrogen bonded protons in GU base pairs were calculated along the lines indicated above using the atomic coordinates from [8,9,15,16]. The average shifts derived from these four sets of coordinates are listed in table 1.

In several RNAs, hydrogen bonded proton resonances have been observed which were attributed to GU base pairs. Upon melting of yeast $tRNA^{Asp}$, which was selected because it contains 3 GU base pairs and 1 Gψ base pair, four resonances were observed in the lowfield NMR spectrum between 10

Table 1
Ring current shifts (ppm) on the hydrogen bonded G(N1) and U(N3) protons in GU base pairs

		AU	GC	UA	CG
G(N1)	1	0	0	0.1	0.1
	2	0.1	0.3	1.2	0.7
	5′ 3′ GU				
	3	1.2	0.5	0.1	0.2
	4	0.1	0.1	0	0
U(N3)	1	0	0	0	0
	2	0	0.1	0.4	0.2
	5′ 3′ GU				
	3	0.5	0.2	0.5	0.2
	4	0.2	0.1	0.1	0.1

1 refers to the second base pair (next nearest neighbour) on the 5′ site of the G residue, 2 refers to the first base pair (nearest neighbour) on the 5′-site of the G residue. See text for details of calculation method. Shifts were taken as the average value in four different sets of crystal coordinates.

and 11.5 ppm at 70°C [17]. They were assumed to come from GU base pairs in the acceptor and anticodon stems of this tRNA. Two resonances were observed [18] between 10 and 12 ppm in the low field NMR spectrum of the 3′-terminal 49 nucleotide fragment of *Escherichia coli* 16 S rRNA, the cloacine fragment. These resonances could not be accounted for by normal Watson-Crick base pairing and were assigned to the resonances of a GU pair in the helical stem of the molecule. In an elegant pulse experiment [10] the resonances at 11.8 and 10.4 ppm in the spectrum of yeast $tRNA^{Phe}$ have been shown by means of saturation transfer of magnetization to come from protons which are magnetically coupled and therefore must be close to one another. The only reasonable candidates are the hydrogen-bonded protons in the GU base pair in the acceptor stem of yeast $tRNA^{Phe}$. Subsequent experiments confirmed these results for GU pairs in *E. coli* $tRNA_1^{fMet}$ and in *E. coli* $tRNA_1^{Val}$ [19].

Using table I and the base sequence around the GU pairs the shifts of the GN_1H and UN_3H protons in the GU pairs in these species have been calculated and are listed in table 2. By combining the ring current shifts with the experimentally observed position for these protons the intrinsic resonance positions could be estimated; a value 12.5 ± 0.1 ppm was found for the U(N3) proton and 12.2 ± 0.1 ppm for the G(N1) proton in the GU base pair. Using these intrinsic positions and the calculated shifts it is possible to predict the resonance positions of the hydrogen bonded protons in GU base pairs. These are included in table 2.

Well resolved resonances of a GU base pair in the so called molecular stalk of the 5 S rRNA of *Bacillus licheniformis* have been observed [20]. The predicted and measured resonance positions are also included in table 2. In general a good agreement between measured and predicted positions can be obtained using this approach. It follows from the present calculations that the original assignment of GU proton resonances of yeast $tRNA^{Asp}$ has to be reassessed [17].

Acknowledgement

We thank Professor A. G. Redfield for making available his results prior to publication.

Table 2
Comparison of predicted and observed resonance positions (ppm) for the hydrogen-bonded protons of GU base pairs in the low field NMR spectra of RNA molecules

		Resonance positions			
		$\Delta\delta_{RC}$	Calc.	Obs.	Ref.
Cloacine fragment	U(N3)	0.7	11.8	11.5	[18]
	G(N1)	1.6	10.6	10.7	
yeast $tRNA^{Phe}$	U(N3)	0.7	11.8	11.8	[10]
	G(N1)	1.6	10.6	10.4	
E. coli $tRNA_1^{fMet}$	U(N3)	0.3	12.2	12.4	[10]
	G(N1)	0.6	11.6	11.6	
E. coli $tRNA_1^{Val}$	U(N3)	0.4	12.1	12.0	[19]
	G(N1)	0.7	11.5	11.4	
B. licheniformis	U(N3)	0.5	12.0	11.8	[20]
5 S rRNA	G(N1)	1.0	11.2	11.0	

The ring current shifts ($\Delta\delta_{RC}$) were obtained from table 1. Intrinsic resonance positions of 12.5 and 12.2 ppm were used for the U(N3) protons and G(N1) protons, respectively

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