Contribution of a G·U Base Pair to the Stability of a Short RNA Helix

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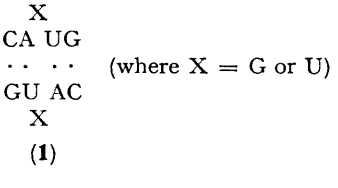
Summary Variable temperature ^{1}H n.m.r. spectroscopic studies have shown that the contribution of an internal G·U base pair approximates that of an A·U base pair in the stability of a short RNA helix.

THE existence of G·U base pairs was first proposed in the wobble hypothesis of Crick[1] to explain the codon–anticodon interactions between mRNA and tRNA. This base pair has since been included in the proposed secondary structures of several native RNAs[2,3,4] despite the fact that several physical studies on the existence of G·U base pairs were inconclusive.[5,6] Substantial evidence for the presence of a G·U interaction has recently been provided by the *X*-ray crystal structure of yeast $tRNA^{Phe}$ [2] and the n.m.r. spectrum of polyd(GpT).[7] In order to study both the formation and relative stability of a G·U base pair in a short RNA helix flanked by normal Watson–Crick base-pairs, we have synthesized the pentaribonucleotides, CAGUG and CAUUG,† by the phosphotriester method.[8] We have examined the duplex formation of these complementary sequences by ^{1}H n.m.r. spectroscopy.

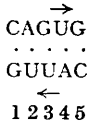
These pentaribonucleotides were chosen because their sequence permits the use of the self-complementary tetraribonucleotide, CAUG,[9] as a reference compound. The low field aromatic protons in the 90 MHz spectra of CAGUG and CAUUG at 70 °C‡ were assigned by incremental analysis[10] and by comparison with the CAUG spectrum recorded at the same temperature.[9] Spectra were then obtained for each of the control pentaribonucleotides over the temperature range 10–70 °C and the plots of chemical shifts *vs.* temperature did not exhibit any sigmoidal behaviour. We interpret these results to mean that self-association of each pentanucleotide to form structures of the type (**1**) is not a significantly favoured process.



(where X = G or U)

(**1**)

† Oligoribonucleotides are written in the normal 5′—3′ sequence and the base pairs are numbered from left to right:



‡ The ^{1}H n.m.r. spectra were recorded at 90 MHz on a Bruker WH-90 spectrometer. Each sample was lyophilized once from D_2O and then dissolved in 100% D_2O which contained 0·01 M sodium phosphate buffer (pD 7·0) and 1·0 M sodium chloride.

When CAGUG and CAUUG were mixed and the spectrum recorded at 70 °C the purine aromatic protons displayed chemical shifts which were essentially identical to those in the single strands. Assignment of the pyrimidine H-6 doublets was more complicated owing to the overlap of these signals. As the temperature of this sample was reduced, non-linear chemical shift changes were observed (Figure). This is interpreted as the formation of a base paired duplex.[9,10] The average melting temperature of the duplex, $T_m = 23{\cdot}4 \pm 2{\cdot}0$ °C, was determined from those temperature *vs.* chemical shift curves which showed upfield, sigmoidal changes as the temperature decreased. This result demonstrates that an internal G·U base pair has relatively little effect on the helix stability when compared to CAUG (Table). As shown in the Figure the T_m of the G(3)H-8 resonance of the G·U base pair is significantly lower than the T_ms of the non-wobble base pairs, implying that the internal G·U base pair is a region of local instability within the duplex.

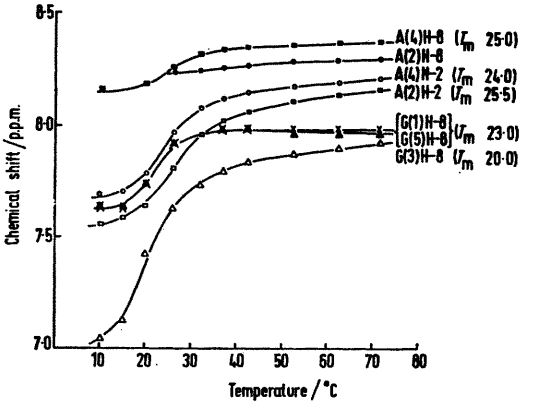


FIGURE. Chemical shift *vs.* temperature plot for the duplex CAGUG:CAUUG.

In the model system used for this study, the addition of an internal A·U or G·C base pair significantly raises the T_m of the duplex relative to the reference CAUG duplex and a corresponding duplex containing a non-hydrogen bonded U·U pair (Table). It has previously been observed by optical methods that internal G·U base pairs do not significantly increase duplex stability,[11] while a terminal G·U base pair in a codon–anticodon interaction was found to be as stable as an A·U base pair.[5] It was proposed that a G·U wobble pair would disrupt the regular stacking within a helix, but have little effect at the terminus of a helix.[11] This explanation received support from ^{1}H n.m.r. observations on yeast tRNA^{Leu}, which indicated that a G·U base pair slightly perturbed the nearest neighbour A·U base pair.[12]

TABLE. Melting temperatures and concentrations of base paired duplexes.

Duplex	T_m/°C	Concentration/M
CAUG · · · · GUAC	24·0 ± 1·0	$9{\cdot}2 \times 10^{-3}$
CAGUG[a] · · · · · GUUAC	23·4 ± 2·0	$1{\cdot}8 \times 10^{-2}$
CAAUG[a] · · · · · GUUAC	28·5 ± 2·1	$1{\cdot}1 \times 10^{-2}$
CAGUG[a] · · · · · GUCAC	38·4 ± 0·6	$3{\cdot}2 \times 10^{-3}$
CAUUG · · · · · GUUAC	<0 °C	$1{\cdot}1 \times 10^{-2}$

[a] Each single strand of the duplex, when examined on its own, showed no sigmoidal behaviour over the temperature range 10—70 °C.

These n.m.r. studies have allowed us to follow the conformational environment of each base pair in the duplex. The results summarised in the Figure show unequivocally that an internal G·U base pair is formed in this duplex, as well as indicating its stability relative to the surrounding A·U and G·C base pairs.

There are two possible explanations for our observations. If the integrity of regular stacking is maintained, then the Gibbs energy gain from the hydrogen bonding of an internal G·U base pair is less than that for a terminal G·U base pair in the codon–anticodon wobble position.[5] Alternatively, to accommodate a full wobble G·U base pair, a base stacking perturbation takes place which affects even the terminal base pairs of the duplex. It is entirely plausible that in solution both situations are contributing and further experiments are underway to elucidate this.

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