exchange of protons and deuterons between aniline and D_2O. Therefore, no more than 20% of the observed reduction could have occurred via H_2. If all of the reduction has occurred via H_2, there would have been observed 59% deuterium incorporation. We have also observed that there is no appreciable exchange between H_2 and D_2O under similar reaction conditions in the absence of nitrobenzene.

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Stabilizing Effect of Dangling Bases on a Short RNA Double Helix as Determined by Proton Nuclear Magnetic Resonance Spectroscopy

Sir:

The secondary structures of naturally occurring RNA molecules contain short helices which are separated by hairpin or bulge loop regions of noncomplementary bases. Attempts to predict the secondary structure of an RNA from its primary sequence have been based on maximizing the amount of double strandedness in the structure.[1] However, for longer RNA sequences, it is difficult to determine which of a number of secondary structures is most stable, since the effect of looped regions on helix stability is not well quantified.

To further probe the factors which affect helix stability, we have undertaken a series of ^1H NMR investigations of several synthetic oligoribonucleotides. The work reported here shows the effect of a terminal "dangling" base on the stability of a short helix. This study offers two advantages over earlier work in this area.[2,3] Firstly, chemical synthesis of the oligoribonucleotides by the phosphotriester method[4,5] offers more versatility than enzymatic synthesis, both in the number of sequences available for study, and in scale of preparation. Secondly, the chemical shift and coupling constant data give information about the conformational environment of each constituent nucleotide unit of the molecule. The optical methods used in the earlier studies[2,3] are limited in this respect, since they can only follow the overall conformational changes of the oligoribonucleotide.

The self-complementary sequence CAUG[6] was chosen as the reference compound. The procedure of incremental analysis[7] was used to make the chemical shift assignments. The low field proton resonances of CAUG were assigned by comparison with the 90-MHz spectra of CA, CAU, and AUG, all of which were recorded at 70 °C.[8] The chemical shift assignments of CAUGA and CAUGU were made in a similar fashion (Table I).

The effect of temperature variation (over the range of 10–70 °C) on the proton resonances of CAUG, CAUGA, and CAUGU was examined. Figure 1 shows a plot of these data for CAUGU. A general nonlinear variation of chemical shifts with temperature was observed in each case and, at the concentrations used in this work,[9] is associated with the formation of the base paired duplexes. The average melting temperature, T_m, of each duplex was determined from those temperature vs. chemical shift curves which showed upfield, sigmoidal shift changes as temperature decreased. The T_m values were

$$\overset{\text{CAUG}}{\underset{\text{GUAC}}{\cdot\cdot\cdot\cdot}}\ 24 \pm 1^\circ;\ \overset{\text{CAUGU}}{\underset{\text{UGUAC}}{\cdot\cdot\cdot\cdot\cdot}},\ 29.5 \pm 1^\circ;\ \overset{\text{CAUGA}}{\underset{\text{AGUAC}}{\cdot\cdot\cdot\cdot\cdot}},\ 35 \pm 1^\circ\ \text{C}$$

These results clearly indicate that a dangling base stabilizes a double helix, the effect being greater with the dangling A. Preliminary results from work on the duplex formed by the complementary pentaribonucleotides CAAUG and CAUUG (T_m, 30 ± 2 °C) indicate that the increase in T_m resulting from an additional internal A·U base pair is similar to the increase that would result from having a dangling U at each terminus, and *less than* the result from having a dangling A.

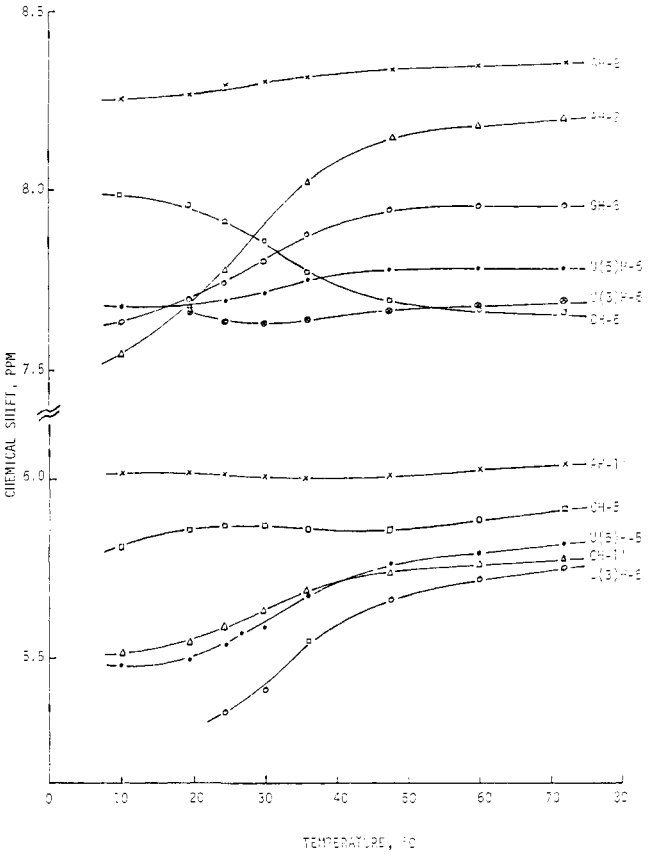

Figure 1. Chemical shift vs. temperature plots for CAUGU.

Table I. Chemical Shifts[a] of the Oligoribonucleotides in D_2O[b] at 70 °C

Proton	CAUG	CAUGU	CAUGA
CH-6	7.662	7.662	7.649
A(2) H-8	8.346	8.352	8.339
A(2) H-2	8.196	8.199	8.167
U(3) H-6	7.692	7.692	7.699
GH-8	7.962	7.958	7.890
U(5) H-6		7.783	
A(5) H-8			8.316
A(5) H-2			8.180
CH-1′	5.765	5.772	5.721
A(2) H-1′	6.039	6.037	6.053
U(3) H-1′	5.845	5.849	5.779
GH-1′	5.813	5.796	5.764
U(5) H-1′		5.853	
A(5) H-1′			6.053
CH-5	5.912	5.912	5.897
U(3) H-5	5.738	5.749	5.741
U(5) H-5		5.813	

[a] Chemical shifts are in parts per million downfield from DSS using *tert*-butyl alcohol-*OD* as an internal reference and are accurate to ±0.005 ppm. [b] pD 7.0 concentrations: CAUG, 9.2×10^{-3} M; CAUGA, 9.2×10^{-3} M; CAUGU, 7.7×10^{-3} M.

For duplexing to occur with short oligoribonucleotides each single strand is probably close to a fully stacked conformation,[12,13] while for long-chain oligoribonucleotides the strands probably have a high percentage of stacked conformers prior to and concommitant with duplexing. Uhlenbeck previously proposed that the dangling base increased favorable stacking interactions between the bases involved in base pairing.[2] We now have evidence of the base-stacking interaction between duplex and dangling base.

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Figure 1 shows that the temperature vs. chemical shift curves of the U(5) H-6 and H-5 of CAUGU are sigmoidal in nature and exhibit an upfield shift as the temperature is decreased. Such behavior is usually attributed to bases which are involved in hydrogen bonding. Although the dangling U does not base pair,[14] it is inherently associated with the duplex through its base-stacking interactions. It is likely, therefore, that it is experiencing the rapid conformational changes associated with both base stacking and duplex formation. Similar results were observed for the H-8 and H-2 resonances of the dangling A in CAUGA. This work clearly shows such base-stacking interactions between duplex and dangling base do exist, while the previous optical studies were only able to suggest the possibility of such an interaction. These results indicate the important role of base stacking in both duplex formation and duplex stability and they are fully consistent with the fine structural details of t-RNA^{Phe} which have recently been elucidated by x-ray crystallographic analysis.[15]

References and Notes

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(6) (a) M. C. Ganoza, A. F. Fraser, and T. Neilson, unpublished work. (b) Oligoribonucleotides are written in the normal 5′–3′ sequence and the bases are numbered from left to right:

CAUG	CAUGU	CAUGA
1234	12345	12345

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(8) The ^{1}H NMR spectra were recorded at 90 MHz on a Bruker WH-90. 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.
(9) Melting temperature versus concentration data for these pentamers are not yet available, but data for AAGCUU,[7] GCUC:GAGC,[10] and CCGG[11] exists over the concentration range 10^{-5} (UV) to 10^{-2} M (^{1}H NMR) and is consistent with the view that base paired duplexing is the only significant process occurring. Interduplex interactions appear to be negligible.
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Reversible Binding of Dioxygen, Nitric Oxide, and Carbon Monoxide by Bis(3,5-di-*tert*-butylcatecholato)vanadium(IV)

Sir:

To gain insight to the properties and function of vanadocytes (cells found in the blood of Ascidians that contain high concentrations of vanadium(III)[1-5]), a study of model complexes has been undertaken. Although complexes of various catechols with vanadium have been prepared and studied previously,[6-12] few of the complexes have been isolated from solution and characterized. During the course of the preparation of the vanadium complexes that are formed by 3,5 di-*tert*-butylcatechol, the bis(catecholato)vanadium(IV) complex (**1**) has been isolated. We wish to report the reversible formation of O_2, NO, and CO adducts with **1** in methanol and dimethyl sulfoxide solutions.

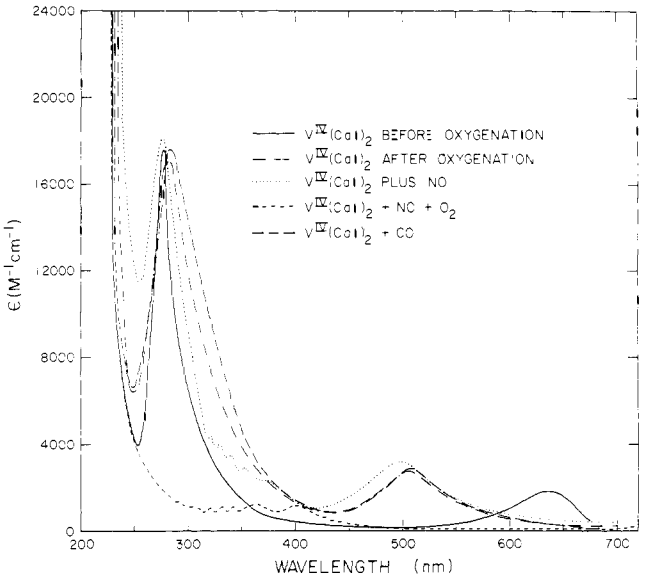

Figure 1. Absorption spectra for 2 mM bis(3,5-di-*tert*-butylcatecholato)-vanadium(IV) in methanol and for the adducts formed by O_2, NO, and CO when present at 1 atm.

When vanadyl acetylacetonate (0.26 g, 0.001 mol) in methanol is combined with a solution of 3,5 di-*tert*-butylcatechol (0.44 g, 0.002 mol) in methanol under an inert atmosphere (argon), a green solution forms initially, which becomes blue on standing. Exposure of this blue solution to oxygen yields a deep violet colored species. The violet solution is reconverted to the blue form by deaeration with argon for 20 min. This cycle between the two species can be repeated by alternately bubbling argon and oxygen through the solution. Kinetic and equilibrium measurements are in progress. However, the initial results indicate that the formation of the O_2 adduct at 25 °C in Me_2SO has a $P_{1/2}$ value of 33 Torr.

Further evidence for the formation of an O_2 adduct is provided by the IR spectrum for the purple species, both in $CHCl_3$ solution and in the solid state (KBr disk). Bands are observed at 1135 and 890 cm^{-1} which are consistent with vibrations that are attributable to a superoxo and a peroxo formulation, respectively.[13] These bands are not observed for **1** or for the NO and CO adducts. The presence of both bands for the O_2 adduct indicates that the purple species probably is a peroxo-bridged dimer which results from an initially formed 1:1 vanadium–dioxygen adduct. Alternately, there may be an equilibrium between the two forms. Kinetic studies that are in progress support the postulate of a two-step adduct formation process.

The unoxygenated (blue) species has been isolated by evaporation of the solution to a small volume, whereupon a dark blue-black precipitate is formed.[14] The complex also can be prepared in Me_2SO, and vanadyl chloride or vanadyl sulfate can be used in place of vanadyl acetylacetonate. On the basis of the elemental analysis for the complex, the two catechol ligands apparently form a four-coordinate array about the vanadium(IV) center. Evidence for a symmetric tetrahedral coordination geometry around the vanadium is provided by EPR spectroscopy, which yields an isotropic eight-line spectrum, centered at a g value of 1.98, 805 G wide and with a peak-to-peak separation of 107.8 G (frozen Me_2SO solution at liquid nitrogen temperature). An EPR spectrum is not ob-

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