STRUCTURE AND DYNAMICS OF RNA

Edited by
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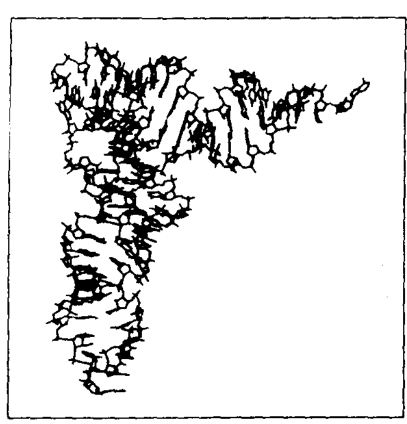

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PREFACE

This volume contains contributions from the speakers at the NATO Advanced Research Workshop on "3D Structure and Dynamics of RNA", which was held in Renesse, The Netherlands, 21 - 24 August, 1985.

Two major developments have determined the progress of nucleic acid research during the last decade. First, manipulation of genetic material by recombinant DNA methodology has enabled detailed studies of the function of nucleic acids in vivo. Second, the use of powerful physical methods, such as X-ray diffraction and nuclear magnetic resonance spectroscopy, in the study of biomacromolecules has provided information regarding the structure and the dynamics of nucleic acids. Both developments were enabled by the advance of synthetic methods that allow preparation of nucleic acid molecules of required sequence and length.

The basic understanding of nucleic acid function will ultimately depend on a close collaboration between molecular biologists and biophysicists. In the case of RNA, the ground rules for the formation of secondary structure have been derived from physical studies of oligoribonucleotides. Powerfull spectroscopic techniques have revealed more details of RNA structure including novel conformations (e.g. left-handed Z-RNA).

A wealth of information has been obtained by studying the relatively small transfer RNA molecules. A few of these RNAs have been crystallized, enabling determination of their three-dimensional structure. It has become apparent that "non-classical" basepairing between distal nucleotides gives rise to tertiary interactions, determining the overall shape of the molecule. Independent evidence for the 3D folding stems from high resolution proton NMR studies of dissolved molecules. Newly started molecular dynamics calculations promise to provide us with a detailed knowledge of the atomic motions in these molecules. Details of the structures and of the interaction with ligands are also derived from data obtained by a variety of spectroscopic techniques. When these are combined with results of (bio)-chemical analysis it is possible to arrive at a clear picture of structure and function of this class of RNA molecules.

In most cells the bulk of RNA is present in ribosomes. The three classes of ribosomal RNA, 5S RNA, 16S RNA and 23S RNA have been studied extensively, although not in such detail as transfer RNA. For all three classes "consensus" secondary structures have been derived These are primarily based on phylogenetic data, but are supported by experiments using (bio)-chemical approaches. Unfortunately, it has as yet not been possible to crystallize a ribosomal RNA (or part of it) and the molecules are too large to be studied by NMR at its current state of the art. However, some progress has been made with fragments of ribosomal RNA. Similarly, through the use of a variety of techniques, including recombinant DNA methods, functional areas in ribosomal RNAs have been mapped.

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Important information has also been obtained regarding the folding of viral RNA. Here, some novel folding principles have been introduced which might also play a role in other RNA molecules.

A recent finding that RNA molecules, like enzymes, have catalytic properties, has attracted much attention. The auto-catalytic selfsplicing of RNA appears to depend on a precise folding pattern of the RNA near the splice junction.

NATO Scientific Affairs Division is gratefully acknowledged for granting an award that made the organization of the workshop possible. Generous financial support was obtained from the Royal Netherlands Academy of Arts and Sciences. Contributions were also made by Amersham Nederland BV, Beckmann Instruments Nederland BV, Boehringer Mannheim BV, Bruker Spectrospin NV, Gibco-BRL, Gist-Brocades BV, Salm & Kipp and Westburg BV (Anglian Biotechnology).

Finally it should be mentioned that the success of the meeting and the high scientific standard of this volume are the result of the enthousiastic co-operation of the participants. There is clearly a need for meetings devoted to RNA at regular intervals in the future.

P.H. van Knippenberg
C.W. Hilbers

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IMPROVED PARAMETERS FOR PREDICTION OF RNA SECONDARY STRUCTURE AND INSIGHTS INTO WHY RNA FORMS DOUBLE HELIXES

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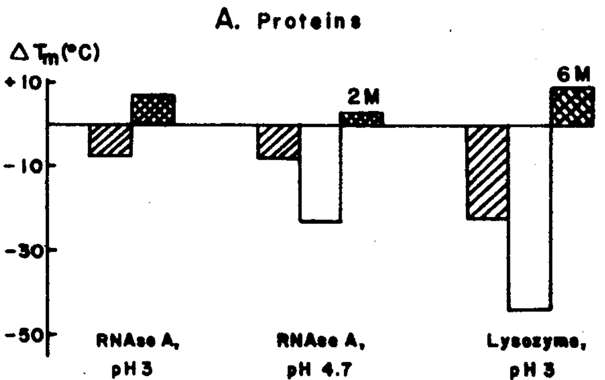
ABSTRACT

Thermodynamic parameters for double helix formation have been measured for a large number of oligoribonucleotides. These data have been analyzed to provide free energy changes associated with base pairs, dangling ends, and base mismatches. The results suggest base stacking and base pairing are important determinants of RNA stability, but that hydrophobic bonding is not. The improved thermodynamic parameters are applied to predict secondary structures for the self splicing intervening sequence from the ribosomal RNA precursor of **Tetrahymena thermophila**.

INTRODUCTION

Knowledge of the forces directing nucleic acid chemistry is important for understanding the structure and dynamics of RNA. There is considerable controversy, however, over the relative contributions of interactions such as hydrophobic bonding, base stacking, and hydrogen bonding. Empirical measures for the magnitudes of these contributions can be obtained from optical studies of double helix



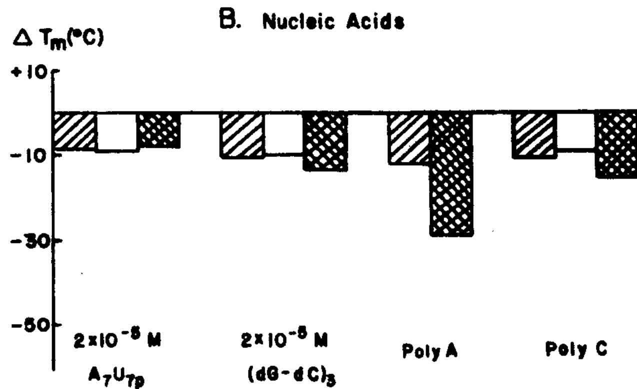


Fig. 1. Cosolvent-induced changes in T_m relative to water. A. Proteins: ribonuclease A (RNAse A) at pH 3 (Brandts and Hunt, 1967; Gekko and Timasheff, 1981b) and pH 4.7 (Schrier and Scheraga, 1962; Schrier et al., 1965; Gerlsma and Stuur, 1974), lysozyme at pH 3 (Parodi et al., 1973; Back et al., 1979). T_m's in H_2O are 45, 61, and 67°C, respectively. B. Nucleic Acids at pH 7: 2 × 10^{-5} M A_7U_7p (Hickey and Turner, 1985a), 2 × 10^{-5} M $(dG\text{-}dC)_3$ (Albergo and Turner, 1981; Freier et al., 1983b), poly A (Dewey and Turner, 1980), poly C (Freier et al., 1981). T_m's in H_2O are 37, 48, 35, and 49°C, respectively. Cosolvents are 10 mol % except where noted and are represented by: [hatched], ethanol; [open], 1-propanol; [cross-hatched], glycerol.

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formation by oligonucleotides. Absorbance versus temperature melting curves are analyzed to provide thermodynamic parameters for the single strand to double helix transition. Such studies also provide parameters for improving predictions of RNA structure from sequence. In this paper, we review the results of several such studies.

HYDROPHOBIC BONDING

One possible source of free energy driving helix formation by RNA is classical hydrophobic bonding. Many studies indicate hydrophobic bonding is important for protein folding (Brandts and Hunt, 1967; Kauzmann, 1959; Cantor and Schimmel, 1980), and it has been suggested that it is also important for folding of nucleic acids. One indication that hydrophobic bonding stabilizes the folded form of proteins is the decrease in protein melting temperature induced by addition of aliphatic alcohols. Typical results are shown in Figure 1. Presumably, this effect is due to the favorable interactions between the hydrophobic groups of the alcohols and proteins. The observation that propanol is a stronger denaturant than ethanol (see Figure 1) is consistent with this interpretation because the longer aliphatic chain of propanol makes it more hydrophobic. Nucleic acids, however, do not follow this trend. As illustrated in Figure 1, ethanol and propanol have similar effects on the coil to helix transition for double stranded A_7U_7p and $(dG\text{-}dC)_3$, and for single stranded poly (cytidylic acid). Glycerol is a cosolvent that enhances hydrophobic bonding (Gekko and Timasheff, 1981a,b). There is an unfavorable interaction between CHOH and CH_2 groups (Okamoto et al., 1978) that is presumably responsible for this effect. Thus glycerol raises the melting temperatures of proteins, as illustrated in Figure 1. The opposite effect is observed for the coil to helix transition in both single and double strand nucleic acids (see Figure 1). Thus solvent effects on stability differ for proteins and nucleic acids. This should not be surprising since the chemical structures of proteins and nucleic acids are quite different. The interiors of proteins contain many non-polar aliphatic groups, while the buried parts of nucleic acids are largely aromatic and polar. Recent computer simulations of solute-solvent interactions indicate water-water structure increases around non-polar solutes (Geiger et al., 1979; Pangali et al., 1979; Swaminathan et al., 1978) but not around benzene (Linse et al., 1984), and that hydrophobic bonding is not responsible for stacking of purines (Langlet et al., 1980).

STACKING

An empirical measure of the free energy change associated with stacking can be obtained by comparing the stabilities for completely complementary double helixes and double helixes containing terminal unpaired nucleotides (dangling ends) (Petersheim and Turner, 1983a; Freier et al., 1983a, 1985a, 1986a). For example, half the difference of free energy changes for helix formation by CCGG and CCGGA provides the stacking free energy change for a 3' A on a GC base pair. Many such free energy increments have been measured using CCGG, GGCC, and GCGC as core double helixes (Petersheim and Turner, 1983a; Freier et al., 1983, 1985a, 1986a). These are listed in Table 1 for 37°C, and several are shown in Figure 2. Some trends are apparent. Free energy increments for 3' dangling ends are much larger than for 5' dangling ends. In fact, in 1 M NaCl, a 5' dangling end adds essentially the same stability increment as a 5' phosphate. This suggests the base of a 5' dangling nucleotide interacts little with the adjacent base pair.

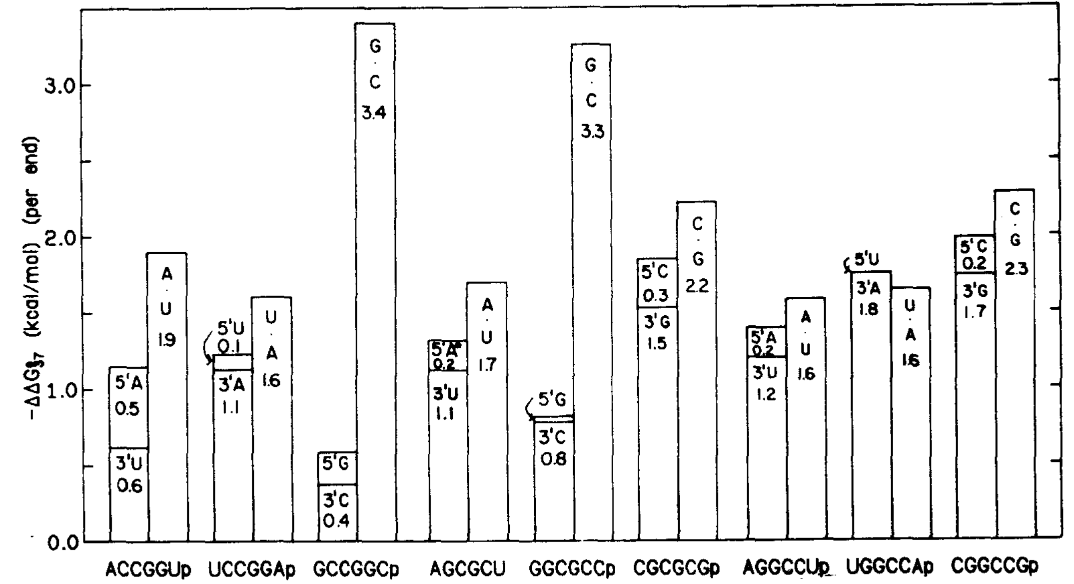

Fig. 2. Free energy increments at 37°C for adding a terminal base pair or dangling end to a CCGG, GCGC, or GGCC core. The left hand bars in each set represent free energy increments for 5' and 3' dangling ends. The right hand bars represent the free energy increments for base pair formation. (*) This free energy increment for a 5' dangling A was measured on AGGCCp.

Table 1. Stability Increments for Adding Terminal Phosphates, Dangling Ends, Terminal Base Pairs and Terminal Mismatches to GGCC, CCGG, and GCGC in 1 M NaCl.[a]

	$-\Delta\Delta G°(37°C)$, kcal/mol		
core helix:	GGCC	CCGG	GCGC
added terminus			
terminal phosphates:			
5' phosphate (5'p)	0.2	0.3	
3' phosphate (3'p)	-0.1	-0.2	
5' dangling ends:			
5'Ap + 3'p	0.2	0.5	
5'Cp + 3'p	0.2		0.3
5'Gp + 3'p		0.2	0.0
5'Up + 3'p	0.0	0.1	
3' dangling ends:			
3'pAp	1.8	1.1	1.7
3'pCp	0.8	0.4	0.8
3'pC	0.9		
3'pGp	1.7	1.3	1.5
3'pUp	1.2	0.6	1.1
Watson-Crick pairs:			
5'Ap + 3'pUp	1.6	1.9	
5'Cp + 3'pGp	2.3		2.2
5'Gp + 3'pCp		3.4	3.3
5'Up + 3'pAp	1.6	1.6	
5'Ap + 3'pU	1.5		1.7
GU pairs:			
5'Up + 3'pGp	1.5	1.4	
5'Gp + 3'pUp		2.3	
5'Gp + 3'pU			1.9
Mismatches			
5'Ap + 3'pAp		1.1	
5'Ap + 3'pCp		1.1	
5'Ap + 3'pGp		1.6	
5'Gp + 3'pAp		1.3	
5'Gp + 3'pGp		1.5	
5'Up + 3'pU			1.2

[a] $\Delta\Delta G°(37°C)$ is half the difference between the $\Delta G°(37°C)$ of helix formation for the molecule containing the core helix plus the added termini and the $\Delta G°(37°C)$ of helix formation for the tetramer core. For example, for the dangling end, 3'pAp on a CCGG core: $\Delta\Delta G°(37°C) = 0.5\ [\Delta G°(CCGGA) - \Delta G°(CCGG)]$. Results are from Petersheim and Turner, 1983a; Freier et al., 1983a; Freier et al., 1985a; Freier et al., 1986a,d.

NMR chemical shifts as a function of temperature for ACCGGp are consistent with this interpretation (Petersheim and Turner, 1983b). This lack of stacking is also consistent with standard A-form RNA geometry (Freier et al., 1985a). For 3' dangling ends, the order of additional stability is A,G>U>C, and they add more stability when adjacent to a C than to a G. The range of free energy increments is -0.4 to -1.8 kcal/mole for 3' dangling ends. For full base pairs, this range is -0.9 to -3.3 kcal/mole at 37°C (see Tables 1 and 3), indicating stacking is an important determinant of nucleic acid stability.

PAIRING

We define pairing as the interactions between nucleotides within a base pair. Quantum mechanical calculations indicate hydrogen bonding dominates these interactions (Pullman and Pullman, 1968; 1969). An empirical measure of the free energy increment for pairing is the difference between the free energy change for adding a base pair to a helix and the sum of the free energy changes for adding the corresponding dangling ends (Petersheim and Turner, 1983; Freier et al., 1985a, 1986a). Free energy changes for terminal base pairs and dangling ends are listed in Table 1, and presented as bar graphs in Figure 2. Presumably, stacking of dangling ends provides an upper limit for the stacking contribution to base pair formation since a dangling end has more freedom to adopt an optimum stacking geometry. It is even possible that stacking interactions interfere with base pair formation if they must be disrupted to allow a geometry appropriate for hydrogen bonding. In seven of the nine cases shown in Figure 2, the sums of the free energy increments from 5' and 3' dangling ends are more than half the increments for adding the corresponding base pairs. In two cases, GCCGGCp and GGCGCCp, the sums of the dangling ends are less than one quarter of the increment for the full base pair. This indicates pairing is also an important determinant of nucleic acid stability. In making this comparison, it should be realized that the free energy changes associated with 3' dangling end stacking and base pair formation contain contributions from unfavorable configurational entropy. In principle, this term must be factored out to derive measures of the pure attractive forces driving base pair formation. Unfortunately, there is currently uncertainty about the magnitude of the configurational entropy.

PREDICTING RNA STRUCTURE

Current computer algorithms (Zuker and Stiegler, 1981; Jacobson et al., 1984; Nussinov et al., 1982; Pipas and McMahon, 1975; Salser, 1977, Papanicolaou et al., 1984) for predicting RNA secondary structure from sequence are based on a nearest neighbor model (Tinoco et al., 1971; Gralla and Crothers, 1973). Such a model is reasonable if stacking and hydrogen bonding drive helix formation, since both involve short range forces. The results in Table 1 provide some direct evidence supporting the nearest neighbor model. Specifically, the free energy increments for adding 3' dangling ends, a CG or AU base pair to GGCC are the same, within experimental error, as for adding each to GCGC.

Another test of the nearest neighbor model is shown in Table 2 which lists melting temperatures and free energy changes measured for helix formation by pairs of oligomers that have the same nearest neighbors, but different sequences (Freier et al., 1986b). The

Table 2. Thermodynamic Parameters of Helix Formation for Oligonucleotides with Identical Nearest Neighbors, but Different Sequences[a]

Oligomer	$-\Delta G°$(37°C) (kcal/mol)	T_m(°C) (at 1×10^{-4}M)
AGAUAUCU	6.58	41.4
AUCUAGAU	7.20	45.1
AACUAGUU	7.17	45.7
AGUUAACU	6.36	41.1
ACUUAAGU	6.16	40.2
GAACGUUC	9.30	52.3
GUUCGAAC	8.76	50.4
UCUAUAGA	6.96	43.6
UAGAUCUA	7.25	45.3
GUCGAC	7.09	45.4
GACGUC	7.35	46.2
GCCGGCp[b]	11.24	67.2
GGCGCCp[c]	11.33	65.2
ACUAUAGU	6.98	44.0
AGUAUACU	6.80	43.7

[a] Parameters derived from plots of reciprocal melting temperature (T_m^{-1}) vs. log(concentration). Data from Freier et al., 1986b.
[b] Data from Freier et al., 1985a.
[c] Data from Freier et al., 1986a.

Table 3. Free Energy Parameters for Nearest Neighbor Interactions in 1M NaCl at 37°C.[a,b]

5' Nucleotide	3' Nucleotide A	C	G	U
A	-0.9	-2.2	-1.7	-1.0
C	-1.9	-3.0	-2.2	-1.7
G	-2.2	-3.3	-3.0	-2.2
U	-1.2	-2.2	-1.9	-0.9

[a] Free energy parameters obtained by a multiple linear regression to thermodynamic parameters for single strand to double helix equilibria for 36 oligonucleotides. For each oligonucleotide, thermodynamic parameters were derived from plots of inverse melting temperature vs. log(concentration). Values of $\Delta H°$ and $\Delta S°$ from these plots were within 15% of the $\Delta H°$ and $\Delta S°$ derived by averaging fits of the melting curves to a two-state model.

[b] Sources of oligonucleotide data were: Petersheim and Turner, 1983a; Nelson et al., 1981; Freier et al., 1983a, 1985a,b, 1986a,b,c; Groebe, D. R., Uhlenbeck, O. C., Freier, S. M., and Turner, D. H., unpublished experiments.

melting temperatures of the pairs differ from 0.3 to 5.5°C with an average difference of 2.4°C, corresponding to about 1% on the Kelvin scale. The free energy differences range from 0.8 to 15.2% with an average difference of 6%. These results indicate the nearest neighbor model should provide reasonable predictions for helix stabilities.

In order to make the best possible predictions of helix stability with the nearest neighbor model, it is necessary to measure free energy parameters for all ten nearest neighbor interactions (Borer et al., 1974). This has not been previously possible due to limitations in methods for making RNA. Recent advances in synthetic methods have eliminated this constraint (Kierzek et al., 1986; England and Neilson, 1976; Uhlenbeck and Gumport, 1982; Romaniuk and Uhlenbeck, 1983; Beckett and Uhlenbeck, 1984). Table 3 lists free energy changes at 37°C for all ten nearest neighbors as derived from melting studies on 36 oligonucleotides (Freier et al., 1986c).

The largest differences between these parameters and those used most often for prediction of RNA secondary structure (Borer et al., 1974) are for nearest neighbors containing two GC base pairs (Freier et al., 1985b). The values of $\Delta G°(37°C)$ for CG, GG, and GC stacks are -2.2, -3.0, and -3.3 kcal/mole, respectively. If helix stability was determined only by hydrogen or hydrophobic bonding, then $\Delta G°(37°C)$ should be the same for all these stacks. Thus these values also provide additional evidence for the importance of stacking.

In addition to standard AU and GC base pairs, GU pairs are known to occur in RNA. We have measured the thermodynamic parameters for several oligomers containing GU base pairs in order to improve GU parameters. Results from four oligomers containing terminal GU pairs are listed in Table 1. Evidently, a terminal GU pair is essentially equivalent to a terminal AU pair. This confirms previous suggestions based on the binding of the codons AUG and GUG to formylmethionine tRNA (Uhlenbeck et al., 1970; Gralla and Crothers, 1973). Preliminary measurements have also been made on oligonucleotides containing internal GU base pairs. Together with previously published data (Uhlenbeck et al., 1971; Gralla and Crothers, 1973), the results suggest an internal nearest neighbor stack containing GU is roughly 0.1 to 0.4 kcal/mole less stable than the corresponding AU stack (N. Sugimoto, S. M. Freier and D. H. Turner, unpublished experiments).

The thermodynamic parameters for dangling end stacking suggest that terminal mismatches other than GU should also significantly stabilize helixes. Stability increments for six such mismatches are listed in Table 1 (Hickey and Turner, 1985b; Freier et al., 1986d). They range from -1.1 to -1.6 kcal/mole. In the nearest neighbor model, there are a total of 48 different terminal mismatches. Thus it would be useful to have rules for approximating the free energy increments of terminal mismatches without directly measuring each one. One possible approximation is to sum the increments from the corresponding 3' and 5' dangling ends. Figure 3 shows that this is adequate for pyrimidine-pyrimidine and pyrimidine-purine mismatches, but not for A•A mismatches. This might be due to geometrical considerations. Purine-purine mismatches are better approximated by the stability increment of the appropriate 3' dangling end made more favorable by 0.2 kcal/mole for the effect of the 5' phosphate.

To determine the effect of revised nearest neighbor parameters on prediction of RNA structure, the parameters in Table 3 were used with the computer program of Zuker and Stiegler (1981) to predict the structure of the self splicing intervening sequence from the RNA

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