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LETTERS TO NATURE

Improved Estimation of Secondary Structure in Ribonucleic Acids

A SIMPLE method for estimating the most stable secondary structure of an RNA molecule from its sequence was proposed earlier[1]. This method can be used for predicting and assessing possible secondary structures for recently determined RNA sequences[2–4]. New experimental[5–8] and theoretical[9,10] results allow us to improve the method, without making it more complicated.

There are two major changes. First, instead of assigning free energies to each base pair, we will consider the base pairs two at a time. There are now sufficient data on the effect of nearest neighbour sequence on the thermodynamics of oligonucleotide helices to extract a separate free energy for each of the ten different base pair doublets. The necessary thermodynamic data come from study of oligonucleotides of A_nU_n (ref. 11), $(A_nGU_n)\cdot(A_nCU_n)$ (ref. 12), A_nCGU_n (ref. 12) and A_nGCU_n, $(A_3CUG)\cdot(CAGU_3)$, $(A_4G_2)\cdot(C_2U_4)$, $(A_4CG)\cdot(CGU_4)$, U_2CGA_2, A_4UAU_4 (P. N. B., O. C. U., B. D., and I. T., jun., unpublished). The values for the free energies of the helix doublets relative to their corresponding single strands at 25° C in neutral buffer of moderate or high ionic strength are given in the first part of the table. By recognising the effect of helix sequence on free energy, these numbers are a considerable improvement over the previous estimates. For example, two adjacent GC base pairs are found to be significantly more stable than two GC base pairs separated by an AU base pair. The numbers given for the base-paired helical regions are thought to be accurate to ±10%. Different methods of analysing the absorbance "melting" data on the complementary oligonucleotides give values within this uncertainty. Since the variety of model compounds is still rather small, however, future experiments may reveal contributions due to end effects and next neighbour effects not specifically taken into account in the present model.

The second major improvement is a number of changes in the weighting of the types of loops which can occur in RNA. It has been found that interior loops are more stable than hairpin loops[8], presumably because the backbone conformations of the interior loops are more nearly the same as those in the single strands. Also, experiments on $A_6C_mU_6$ (ref. 6) and $A_5GC_mU_5$ (ref. 7) show that hairpin loops with six unbonded bases are the most stable. For less than six unbonded bases steric hindrance and base–base stacking destabilise the loop; for more than six unbonded bases the probability of loop initiation decreases with increasing chain length as expected. These data are converted into positive free energies for the different types of loops in the second part of the Table. The values for loops containing large numbers of unbonded bases were obtained by assuming the loops to be random chains[13]. Experimental information on the thermodynamics of model compounds containing loops is not as extensive as that for helical regions. Thus, although the values for the

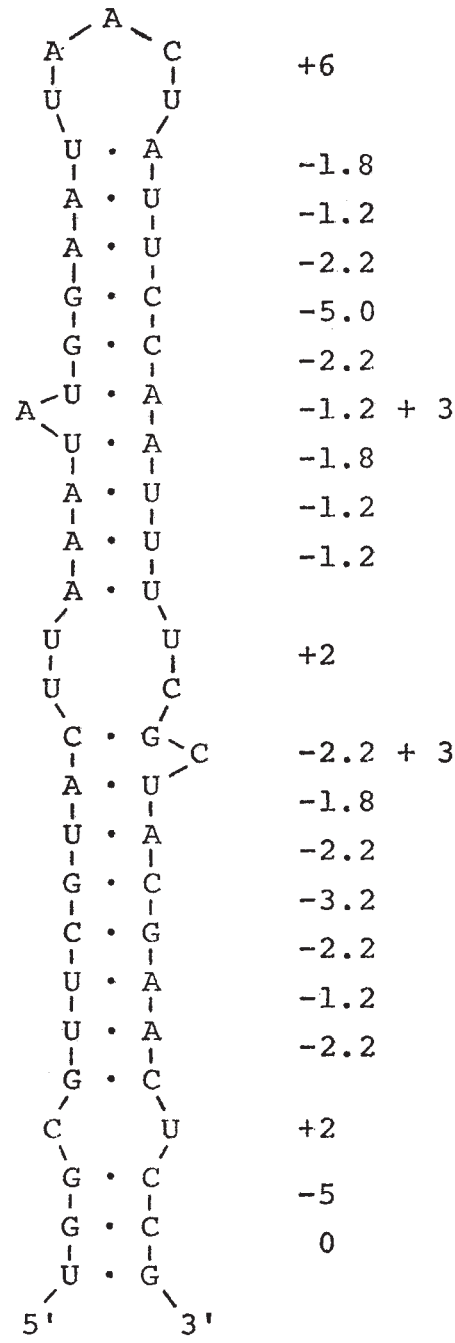
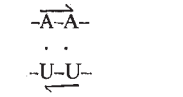
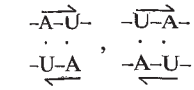
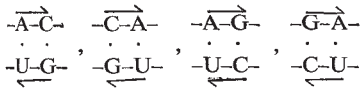
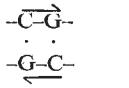
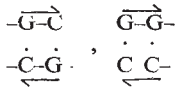
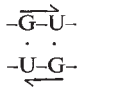
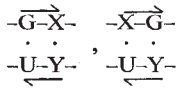


Fig. 1 The contribution of sequence dependent base paired regions and various loops to the free energy of a possible secondary structure for a fifty-five base fragment from R17 virus[12]. ΔG (25° C) = −21.8 kcalorie.

Table 1 Free Energies at 25° C for RNA Structures

Base paired regions	ΔG(kcalorie) ± 10%
	−1.2
	−1.8
	−2.2
	−3.2
	−5.0
	−0.3
	0

Unbonded regions	ΔG(kcalorie) ± 1 kcalorie	
Number of bases unbonded	Interior loops	
2–6	+2	
7–20	+3	
m (>20)	+1+2 log m	
	Bulge loops	
1	+3	
2–3	+4	
4–7	+5	
8–20	+6	
m (>20)	+4+2 log m	
	Hairpin loops	
	Closed by G·C	Closed by A·U
3	+8	>8
4–5	+5	+7
6–7	+4	+6
8–9	+5	+7
10–30	+6	+8
m (>30)	3.5+2 log m	5.5+2 log m

The free energies for the base paired regions refer to the free energy of adding a base pair to a pre-existing helix; the magnitude thus depends on the sequence of two base pairs. Each helical region is assumed to be bounded by either an interior loop, a bulge loop, a hairpin loop, or a single stranded region. Other possible contributions to the free energy of an RNA structure, which may be labelled tertiary structure, are not considered.

loops are stated with an accuracy of ±1 kcalorie, ±2 kcalorie may be more appropriate for some situations. The major source of inaccuracy is that the hairpin loops and interior loops studied contained only unbonded cytosine residues. We do not know enough about the base sequence and base composition dependence of loop free energies and there is some evidence that it is quite significant. For example, hairpin loops with unbonded AUG in the loop are 1 to 2 kcalorie more stable than loops containing C (ref. 14). Thus, it may be appropriate to subtract 1 kcalorie from the values given in the table if the hairpin loop contains a U residue. For an interior loop of two unbonded cytosines surrounded by G·C base pairs, the free energy was found to be zero[8]. The data for bulge loops were obtained from data for unbonded adenine N^1 oxides in the bulge loops[5] and from analysis[1] of melting behaviour of random polymers with U or I in the bulge loops. Fresco (personal communication) has found from further studies on random polymers that a single U in a bulge loop is more stable than any other single base in a bulge loop. This sequence and base composition effect on loops is most important for loops containing a small number of unbonded bases (three or less), and therefore at present we prefer to emphasise the data for the larger (four or more unbonded bases) loops.

Figure 1 shows the free energy of the most stable pairing scheme of a fifty-five base fragment from R17 viral RNA[15] calculated according to the numbers in the table.

Further improvements can be made in this method for predicting RNA structure. Additional model compounds should be studied to increase confidence in the numbers in the table and to resolve the uncertainties mentioned above. The temperature dependence of all the free energies should be obtained, so that the "melting" behaviour of RNA molecules can be predicted more accurately than with existing parameters[7].

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Structural Changes in Sarcoplasmic Reticulum Membrane induced by SH Reagents

USING sarcoplasmic reticulum (SR) membranes, stacked and orientated by centrifugation, Dupont, Harrison and Hasselbach[1] demonstrated that it was possible to record Bragg reflections to 15 $Å^{-1}$ resolution. The phases of the reflections and the electron density profiles were obtained to a resolution of 30 Å. In an attempt to label the calcium