INVARIANT ADENOSINE RESIDUES STABILIZE tRNA D STEMS

D. ALKEMA, R. A. BELL, P. A. HADER and Thomas NEILSON
Departments of Biochemistry and Chemistry, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

Received 8 October 1981

1. Introduction

Inspection of the sequences of reported tRNAs [1] reveals 2 adenosine residues that nearly always (166 out of 177 cases) occupy positions 14 and 21 at the D-loop-stem junction. Apparently, the nature of these residues has been conserved and perhaps they are involved in some tRNA function, for example, aminoacyl-tRNA synthetase recognition [2,3]. Their presence may also satisfy a structural aspect controlling native conformation, namely an invariant A · U base pair [4,5]. This report resolves the controversy by proposing that the invariant adenosines stabilize D-stem duplexes, features of secondary cloverleaf structure [6]. D-Stems contain only 3 or 4 Watson-Crick base pairs while other stem duplexes have 5 or more. Melting studies have shown D-stems to be the least stable regions in tRNAs [7].

Three-dimensional tertiary structure of yeast tRNA [4] shows adenosine residues 14 and 21 (see fig.1), to be coplanar, and each base-stacked (a vertical electronic interaction between aromatic rings) to the adjacent D-stem duplex [5]. Steric tolerance exists, however, since the adenosine at position 14 is displaced within the helix as a result of its participation in a tertiary Sobell-type A · U base pair [8] with an invariant uridine residue at position 8. This displacement from the normal RNA-A helical geometry at the ends of a duplex can be considered as partial strand unwinding. Extension of base stacking to the invariant adenosine residues, is still possible, and therefore enhances overall D-stem stability.

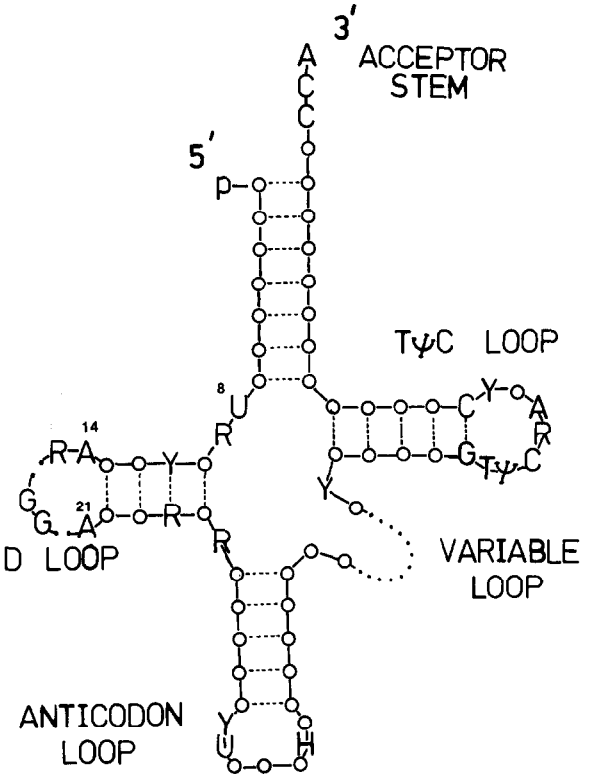

Fig.1. Adapted from a diagram in [5] indicating positions of invariant and semi-invariant bases in tRNA sequences other than initiator tRNAs. Y stands for pyrimidine, R for purine, H for hypermodified purine. Dotted regions represent areas containing a variable number of nucleotides in tRNA sequences. Numbering system corresponds to that of yeast $tRNA^{Phe}$.

2. Materials and methods

The oligoribonucleotides used here were synthesized by the phosphotriester method developed in [9]. Complete details for preparation of these oligomers will appear elsewhere. ^1H NMR spectra were obtained in the Fourier transform mode of Bruker WH-90, WM-250 and WH-400 spectrometers equipped with quadrature detection. Probe temperatures were maintained to within ±1°C by a Bruker variable temperature unit and were calibrated by thermocouple mea-

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.75

surements. The samples were lyophilized twice from D_2O and dissolved in 100% D_2O (Aldrich) containing 0.01 M sodium phosphate buffer (pD 7.0) and 1.0 M NaCl. The sample concentrations were 4–10 mM. *t*-Butanol-d was used as an internal reference and the chemical shifts reported in parts per million (ppm) relative to 2,2-dimethyl-2-silapentan-5-sulphonate (DSS). The field frequency lock was provided by the deuterium signal of D_2O.

3. Results and discussion

In the quest to evaluate the various factors affecting RNA duplex stability, we have established that base-stacking plays a significant role. The contribution from non-paired 3′-terminal (dangling) adenosines is a major factor influencing overall helical stability [10,11]. Internal A · A non-bonded pairs have been confirmed as centers of instability, for example, CA$\vec{A}$UG:CA$\vec{A}$UG ($T_m > 0°C$) [12] and AG$\vec{A}$CU:AG$\vec{A}$CU ($T_m \sim 25°C$) [13].

To evaluate the effect from opposing adenosine residues a series of synthetic oligoribonucleotides, reference self-complementary tetramer duplex, A$\vec{G}$CU, corresponding duplex with a 3′-dangling adenosine, AG$\vec{C}$UA, and the corresponding duplex with terminal non-bonded A · A pairs, AA$\vec{G}$CUA, were prepared.

Model studies on D-stem melting can be carried out using the duplex formation of AA$\vec{G}$CUA:

$$2\ \mathrm{AA\vec{G}CUA} \rightleftharpoons \begin{matrix} & \overrightarrow{\mathrm{A}} & \mathrm{A} \\ & \mathrm{AGCU} & \\ & \mathrm{UCGA} & \\ \mathrm{A} & \leftarrow & \mathrm{A} \end{matrix}$$

Variable temperature proton NMR was used to determine the stabilities as reflected in the melting temperature (T_m) of the synthetic duplexes (see table 1).

Clearly, terminal non-bonded adenosine residues contribute to duplex stability. The ability of the short duplex to unwind partially at the ends allows the terminal adenosines to exist in opposition to each other; however, the displacement is not of sufficient magnitude to interrupt the extended base stacking which is enhanced by these adenosines.

When these model studies are applied to tRNA secondary structure, D-stems flanked by two adenosines at the neck-loop junction, will be more stable than stems lacking adjacent non-bonded adenosines. Evolution of tRNA conformation [14] has resulted in a delicately balanced steric arrangement where a tertiary Sobell-type A · U base pair displaces an adenosine residue sufficiently to remain opposite another adenosine, but not to interfere with extended base-stacking interactions. The invariance of adenosines at positions 14 and 21 ensures a more stable D-stem as well as distinct loop formation due to strand separation. We note that a pair of purines also exists at the other extremity of the D-stem of most tRNAs.

Table 1
Melting temperature of synthetic duplexes

Oligomer	T_m (°C)
A$\vec{G}$CU	33
AG$\vec{C}$UA	45
AA$\vec{G}$CUA	48

Acknowledgement

The authors thank Phillippe Marlière for helpful discussion.

References

[1] Gauss, D. H. and Sprinzl, M. (1981) Nucleic Acids Res. 9, r1–r23.
[2] Dudock, B., DiPeri, C., Scilippi, K. and Reszelback, R. (1971) Proc. Natl. Acad. Sci. USA 68, 681–684.
[3] Roe, B., Micheal, M. and Dudock, B. (1973) Nature New Biol. 246, 135–138.
[4] Kim, S. H., Suddath, F. L., Quigley, G. J., McPherson, A., Sussman, J. L., Wong, A. H. J., Seeman, N. C. and Rich, A. (1974) Science 185, 435–439.
[5] Rich, A. (1977) Acc. Chem. Res. 10, 388–425.
[6] Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquisee, M., Merrill, S. H., Penswick, J. R. and Zamir, A. (1965) Science 147, 1462.
[7] Hilbers, C. W. and Shulman, R. G. (1974) Proc. Natl. Acad. Sci. USA 71, 3239–3242.
[8] Haschmeyer, A. E. V. and Sobell, H. M. (1963) Proc. Natl. Acad. Sci. USA 50, 872.
[9] Werstiuk, E. S. and Neilson, T. (1976) Can. J. Chem. 54, 2689–2696.
[10] Neilson, T., Romaniuk, P. J., Alkema, D., Hughes, D. W., Everett, J. R. and Bell, R. A. (1980) Nucleic Acids Res. Symp. ser. 7, 293–311.
[11] Alkema, D., Bell, R. A., Hader, P. A. and Neilson, T. (1981) J. Am. Chem. Soc. 103, 2866–2868.
[12] Romaniuk, P. J., Hughes, D. W., Gregoire, R. J., Bell, R. A. and Neilson, T. (1979) Biochemistry 18, 5109–5116.
[13] Alkema, D., Hader, P. A., Bell, R. A. and Neilson, T. (1981) submitted.
[14] Cedergren, R. J., Sankoff, D., LaRue, B. and Grosjean, H. (1981) CRC Crit. Rev. Biochem. 11, 35–104.