Volume 8 Number 10 1980 Nucleic Acids Research

DNA-RNA hybrid duplexes containing oligo(dA:rU) sequences are exceptionally unstable and may facilitate termination of transcription

Francis H.Martin and Ignacio Tinoco,Jr.

Department of Chemistry and Chemical Biodynamics Laboratory, University of California, Berkeley, CA 94720, USA

Received 5 March 1980

ABSTRACT

A DNA-RNA hybrid oligonucleotide duplex, $dC(pA)_5pG:rC(pU)_5pG$, which contains a $(dA:rU)_5$ sequence, is at least 200 times less stable at room temperature than the corresponding duplex containing an $(rA:dT)_5$ sequence, $rC(pA)_5pG:dC(pT)_5pG$. This result provides an explanation for the finding that most primary RNA transcripts terminate in several consecutive rU residues, but not rA residues. It strongly supports the idea that instability of the DNA-RNA hybrid at the growing point of transcription plays a role in termination of transcription.

INTRODUCTION

The site of termination of transcription may be determined in part by the instability of the terminal region of the DNA-RNA duplex.[1,2] When relative duplex stabilities are estimated from nearest-neighbor base-pair stacking energies derived from RNA oligomer studies,[3] or simply from G:C content,[1] it is predicted that either A- or T-rich template sequences might equally well serve as terminators. These methods may correctly predict termination sites in some systems.[3] However, most primary transcripts determined so far terminate in a sequence of five or more consecutive U residues.[1-7] Melting studies of synthetic homopolynucleotides have previously indicated that the hybrid complex of polydeoxyadenylic acid and polyribouridylic acid, poly (dA:rU), is much less stable than the poly(rA:dT) duplex.[8] We have found that biologically relevant, short runs of dA:rU base pairs are also exceptionally unstable. This observation that oligomeric (dA:rU) regions are much less stable than (rA:dT) regions provides a strong rationale for biological selection of a string of rU residues at transcriptional termina-

tion sites.

METHODS AND RESULTS

The thermal melting curves of complementary mixtures of the ribo- and deoxyribo-oligonucleotides $dC(pA)_5pG$, $dC(pT)_5pG$, $rC(pA)_5pG$, and $rC(pU)_5pG$ were observed at a series of strand concentrations in the range 10^{-5} -10^{-3} M by measurement of UV absorption at 260 nm. UV spectral changes and Job plots were used to distinguish between double and triple stranded complexes.[9]

Three of the four complementary mixtures studied formed 1:1 complexes (Table 1). The mixture of $dC(pA)_5pG$ and $rC(pU)_5pG$ melted at least 20°C lower than the other complexes and formed only a 1:2 complex, even in 1:1 mixtures.

An estimate for the maximum possible equilibrium constant of the $dC(pA)_5pG$:$rC(pU)_5pG$ 1:1 complex can be made from our measurement that only about 3% of the $rC(pU)_5pG$ strands were in complexes at 25°C at strand concentrations of 1 mM; the value is $K < 3 \times 10^{1}\ M^{-1}$. This is at least a factor of one hundred

TABLE 1

Stabilities of Oligomeric Duplexes in 0.2 M NaCl, 0.01 M Phosphate, 0.1 mM EDTA, pH7

	T_m, [a] °C	$K_{25°C}(M^{-1})$	ΔH°, Kcal/mole [b]
$dC(pA)_5pG$:$dC(pT)_5pG$	27.5	1.8×10^{4}	-41 ± 4
$rC(pA)_5pG$:$dC(pT)_5pG$	22.9	5.5×10^{3}	-48 ± 4
$rC(pA)_5pG$:$rC(pU)_5pG$	19.1	2.2×10^{3}	-44 ± 4
$dC(pA)_5pG$:$rC(pU)_5pG$ [c]	<0	$<3 \times 10^{1}$	--

[a] T_m at strand concentrations = 2×10^{-4} M of each oligomer; estimated from linear plot of $1/T_m$ vs. log c.

[b] ΔH° calculated from slope of plot of $1/T_m$ vs. ln c: $\Delta H° = Rd(\ln c)/d(1/T_m)$.

[c] Double-stranded complex not observed; estimation of maximum possible $K_{25°C}$ as described in text; T_m of 1:2 complex is 0°C, determined as described in note (a), above.

smaller than the equilibrium constant for the other complexes at 25°C. If the complex was mostly 1:2 as indicated by our experiments, the actual equilibrium constant of the 1:1 complex would be even smaller. The order of stabilities of the double-stranded complexes is exactly the same as that of the A and U or T containing homopolymers:[8]

$$dCA_5G:dCT_5G > rCA_5G:dCT_5G > rCA_5G:rCU_5G >> dCA_5G:rCU_5G.$$

The $rCA_5G:dCT_5G$ hybrid complex is about 200 times more stable, as measured by $K_{25°C}$, than the $dCA_5G:rCU_5G$ hybrid, at a minimum, and our data do not exclude the possibility that the difference in stability is considerably larger than this. If the difference in stability is primarily due to the $(dA:rU)_5$ and $(rA:dT)_5$ regions (both complexes contain one dG:rC and one rG:dC pair), we can estimate that each dA:rU pair is about 3 times ($243 = 3^5$) less stable than a rA:dT pair in these complexes.

The circular dichroism spectrum of the DNA-DNA oligomer duplex resembles that of B-form DNA; whereas the circular dichroism spectrum of the RNA-RNA oligomer duplex resembles that of A-form DNA, as expected. The circular dichroism spectra of both hybrid complexes are similar to the RNA-RNA complex implying A-type geometry, although the results for the unstable hybrid are more uncertain. Arnott and Selsing[10] found that poly(dA:dT) existed in a B-type structure and that it could not be changed into an A-type structure under dehumidifying conditions. However, poly(dA:dT:dT) and poly (dA:rU:rU) do occur in A-type geometry. These results for the polynucleotides and oligonucleotides show that dA residues in a duplex A-type conformation (3'-*endo* sugar) are very unstable. They will not form double strands with rU residues, which are stable only in A-type conformation. In triple strands the dA and rU residues can barely form a stable structure (Table 1).

DISCUSSION

The experimental finding that dA sequences as short as five base pairs in length strongly prefer a DNA B-form conformation and thus will not form stable DNA-RNA hybrids can have important

biological implications. We have already mentioned the explanation of a dA sequence as a termination signal in transcription. According to the Gilbert model[1] the lability of the terminal portion of the DNA:RNA hybrid duplex at sites where RNA polymerase pauses (just after transcription of G:C rich or possibly hairpin-forming[2,5] regions) may aid in dissociation of the transcriptional complex. Our results indicate that an oligo (dA:rU) sequence would be much more effective than any other sequence in this process, providing a physical basis for the observation of oligo rU sequences at 3' termini of transcripts in procaryotes and eucaryotes.

Poly(rA:rBrU) has a T_m about 30°C higher than poly (rA:rU);[11] substitution of BrU for U might also be expected to stabilize DNA-RNA hybrids. Farnham and Platt[12] have observed that transcription of the *E. coli* tryptophan operon leader region *in vitro* with BrUTP results in greater readthrough than normal at the attenuator site. Other factors may operate in some classes of termination, such as termination in the presence of rho-factor.[4] There has also been speculation about the importance of a B-to-A transition in the initiation of transcription.[13,14]

Reverse transcription is another mechanism where hybrid stability is important. During reverse transcription of Rous sarcoma virus, lability of a $(dA:rU)_4$ region at the growing end may aid in dissociation of the DNA strand from the 5'-terminus of 35S RNA and reassociation with the redundant sequence at the 3'-terminus.[15] Hybrid instability may play a role in other processes such as the apparent anomalous slippage of reverse transcriptase on an oligo rU template next to a palindrome near the 3' end of strain C997 of foot and mouth disease virus.[16]

The exceptional instability of (dA:rU) duplexes should be taken into account in the choice of DNA oligomers to be hybridized to RNA and vice versa, and in interpretation of R-looping[17] results. It is indeed fortunate for DNA-sequencers that eucaryotic messengers terminate in poly rA and not poly rU; these terminal sequences, of course, may imply other biological results of hybrid stabilities.

ACKNOWLEDGMENTS

This work was supported in part by NIH grant GM 10840 and by the Division of Biomedical and Environmental Research of the U.S. Department of Energy under contract No. W-7405-Eng-48. We are grateful to Prof. Marvin H. Caruthers and members of his research group for instruction in the chemical synthesis of DNA. We thank Mr. David Koh and Ms. Barbara Dengler for excellent work in synthesis and data acquisition, and Prof. John E. Hearst and Dr. Richard Reisbig for useful discussions of transcriptional control.

REFERENCES

1. Gilbert, W. (1976) in RNA Polymerase, Losick, R. and Chamberlin, M. J. Eds., pp. 193-205 Cold Spring Harbor Laboratory, New York.
2. Rosenberg, M. and Court, D. (1979) Ann. Rev. Genetics 13, 319-353.
3. McMahon, J.E. and Tinoco, I., Jr. (1978) Nature 271, 275-277.
4. Adhya, S. and Gottesmann, M. (1978) Ann. Rev. Biochem. 47, 967-996.
5. Schaller, H., Beck, E. and Takanami, M. (1978) in The Single Stranded DNA Phages, Denhardt, D.T., Dressler, D. and Ray, D.S. Eds., pp. 139-161 Cold Spring Harbor Laboratory, New York.
6. Erdmann, V.A. (1979) Nucleic Acids Res. 6, r29-r44.
7. Majumder, H.K., Maitra, U. and Rosenberg, M. (1979) Proc. Natl. Acad. Sci. USA 76, 5110-5113.
8. Riley, M., Maling, B. and Chamberlin, M.J. (1966) J. Mol. Biol. 20, 359-389.
9. Martin, F.H., Uhlenbeck, O.C. and Doty, P. (1971) J. Mol. Biol. 57, 201-215.
10. Arnott, S. and Selsing, E. (1974) J. Mol. Biol. 88, 509-521.
11. Felsenfeld, G. and Miles, H.T. (1967) Ann. Rev. Biochem. 36, 407-448.
12. Farnham, P.J. and Platt,T. (1980) Cell 20, in press.
13. Arnott, S. and Selsing, E. (1974) J. Mol. Biol. 88, 551-552.
14. Arnott, S., Fuller, W., Hodgson, A. and Prutton, I. (1968) Nature 220, 561-564.
15. Haseltine, W.A., Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 989-993.
16. Porter, A.G. and Fellner, P. (1978) Nature 276, 298-301.
17. White, R.L. and Hogness, D.S. (1977) Cell 10, 177-192.