

Regioselective ligation of oligoribonucleotides using DNA splints

J.D.Bain and Christopher Switzer[1,*]
Department of Chemistry, University of California, Irvine, CA 92717 and [1]Department of Chemistry, University of California, Riverside, CA 92521, USA

Submitted May 27, 1992

We describe here a novel method for the ligation of oligoribonucleotides with T4 RNA ligase that relies on a DNA splint to approximate the ends of RNA strands, resulting in greatly improved yields of the desired RNA product (1, 2). When sequential ligations are performed, the method may be used to sequence specifically incorporate non-standard nucleotides that may not be compatible with current chemical synthetic technologies. The present method requires that the DNA splint lack elements that base-pair to the immediate vicinity of the splice junction so as to create a *broken interior loop* structure (complex 3, Scheme 1) that is compatible with T4 RNA ligase specificity (3).

Oligoribonucleotide 1 bearing the non-standard nucleotide *iso*-cytidine at its 3'-end was prepared from a 42-mer precursor using T4 RNA ligase and an excess of *iso*-C-3'(2'),5'-diphosphate in 15% yield after denaturing PAGE purification (4). Treatment of RNA/DNA complex **3** first with polynucleotide kinase (5) and then with T4 RNA ligase, furnished ligated product **4** in 53% yield. Oligoribonucleotide **4** was found to be an efficient RNA message for *in vitro* translation, where the new codon (*iso*-C)AG is recognized by a semi-synthetic tRNA bearing its cognate anticodon (1). It is noteworthy that *iso*-C is incorporated into **4** without suffering deamination to U, a side-reaction that is known to occur under the conditions of RNA chemical synthesis (6). This point was confirmed by the failure to observe translation products that would have resulted from a UAG codon had it been present in **4** (1).

The facile ligation in the presence of a DNA splint may be attributed to an increased local concentration of active, single-stranded RNA ends as well as suppressed formation of polymerization and circularization side-products (7).

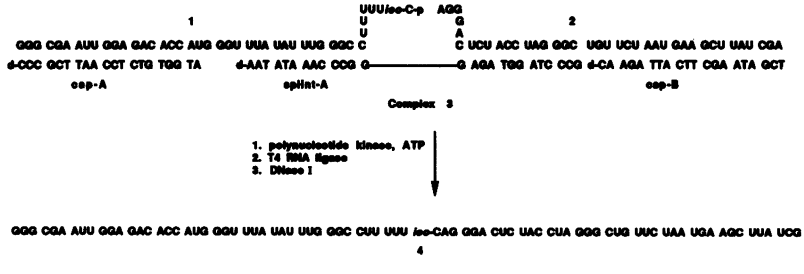
The DNA splint ligation method allows the synthesis of long oligoribonucleotides and even polyribonucleotides containing non-standard bases that are difficult or impossible to make by other methods. The method should find application where there is a need to extend Nature's four ribonucleotide building blocks, such as expanding the genetic code (1).

ACKNOWLEDGEMENTS

We thank Prof. S.A.Benner and Prof. A.R.Chamberlin for helpful discussions, and the National Institutes of Health (GM-42708, J.D.B.; GM-47375, C.S.) for support of this work.

REFERENCES

1. For a recent application, see: Bain,J.D., Switzer,C., Chamberlin,A.R., Benner,S.A. (1992) *Nature* **356**, 537.
2. For a related method, see: Moore,M.J., Sharp,P.A. (1992) *Science* **256**, 992.
3. In the presence of a fully complementary DNA splint the efficiency of ligation is greatly reduced.
4. England,T.E., Bruce,A.G., Uhlenbeck,O.C. (1980) *Meth. in Enzymol.* **65**, 65.
5. Cameron,V., Uhlenbeck,O.C. (1977) *Biochemistry* **16**, 5120.
6. Switzer,C., Moroney,S.E., Benner,S.A. (1989) *J. Am. Chem. Soc.* **111**, 8322.
7. The cap-A and cap-B DNA strands also contribute to the suppression of these side products by protecting the RNA termini during the ligation.
8. Cobianchi,F., Wilson,S.H. (1980) *Meth. in Enzymol.* **65**, 94.



Scheme 1. 1, 2, splint-A, cap-A and cap-B (3 μM, each) were annealed by heating at 90°C for 2 min followed by cooling to 15°C over 40 min. The RNA/DNA complex **3** was kinased using T4 polynucleotide kinase (50 U/mL) in the presence of Hepes (50 mM, pH 6.9), $MgCl_2$ (20 mM), dithiothreitol (3.3 mM), ATP (30 μM), BSA (10 μg/mL), and incubating at 37°C for 1 h. Ligation of oligoribonucleotides **1** and **2** was performed by adding T4 RNA ligase (4 U/mL) to the mixture and incubating at 15°C for 2 h. The oligoribonucleotide product was obtained by (a) adding RNase-free DNase I (8), (b) precipitation of the RNA from EtOH/H_2O, and then (c) HPLC using a Pharmacia ODS 120-T column (triethylammonium acetate, 100 mM, pH 7, (A) and CH_3CN, (B); 100% A to 80% A/20% B, 20 min).

* To whom correspondence should be addressed