Nucleic Acids Research, 1993, Vol. 21, No. 9 2275–2276

Reverse transcription using nuclease resistant primers

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Received February 19, 1993; Accepted March 23, 1992

Recent developments in nucleotide chemistry have allowed the synthesis of RNA oligonucleotides modified at the 2′-hydroxyl position on the ribose. The oligo[2′-O-allylribonucleotide], (2′-O-allyl oligo), has been shown to be resistant to a wide array of nucleases (1). Such oligos annealed to RNA form hybrids with a T_m higher than the conventional RNA duplex (2). Furthermore, these hybrids are fully stable in the presence of RNase H. Here we describe the utility of 2′-O-allyl oligos as primers for reverse transcription reactions.

A schematic drawing of primers, template mRNAs as well as the predicted sizes of the reverse transcription products are shown in Figure 1. Figure 2 shows that a 2′-O-allyl oligo, A, (lane 1) functions extremely poorly as a primer for the reverse transcription, whereas a DNA oligo of identical sequence, D, (lane 2) and the internal control DNA oligo (eALAS) are extended efficiently. On the other hand 2′-O-allyl oligos with a single ribonucleotide, rA, or deoxynucleotide, dA, at the 3′ end can readily be extended to yield the expected product (lanes 3 and 4). The minor changes in oligos rA and dA do not significantly alter the T_m of the hybrids with RNA and are too small to induce RNase H cleavage, which requires three to four consecutive DNA/RNA base pairs (3). The results suggest that the relatively bulky allyl group ($-CH_2-CH=CH_2$) at the 2′-hydroxyl position interferes with the addition of the first nucleotide rather than with the recognition of the hybrid between the 2′-O-allyl oligo and RNA by the reverse transcriptase.

We conclude that reverse transcriptase from Avian Myeloblastosis Virus, AMV, (or Moloney Murine Leukemia Virus, MMLV, data not shown) efficiently extends a 2′-O-allyl primer, containing a single RNA- or DNA-nucleotide at its 3′-end, when annealed to RNA. The 2′-O-allyl oligos should therefore provide a solution to performing primer extension assays, such as toe printing analysis (4), even under conditions where RNase H activity is high, e.g. in cell-free translation extracts (5). Presumably these oligos can also be utilised in other normally DNA oligo based assays.

Experimental: Oligos were synthesized as described (1) and labelled at the 5′-end to the same specific activity using T4 polynucleotide kinase and [γ-^{32}P]-ATP followed by removal of non-incorporated material. The CAT and the T7eALAS plasmids were linearised and transcribed in the presence of CAP-analogue (^{m7}GpppG) using T7 RNA polymerase according to the manufacturer's recommendation. Ten fmol of each mRNA were heated to 90°C together with a 50-fold excess of each labelled primer in a 50 μl volume of 0.1 M KCl and allowed to hybridise at 25°C. Reverse transcription was performed at 42°C for 60 minutes with samples adjusted to 10 mM Tris−Cl pH 8.0, 10 mM $MgCl_2$, 0.1% NP40, 3 mM DTT, 0.5 mM of each dNTP and 3 units of AMV reverse transcriptase (Promega) in a volume of 100 μl. The products were precipitated with 2.5 vol. ethanol, re-suspended in formamide dye-mix and analysed on a denaturing 6% polyacrylamide gel followed by autoradiography.

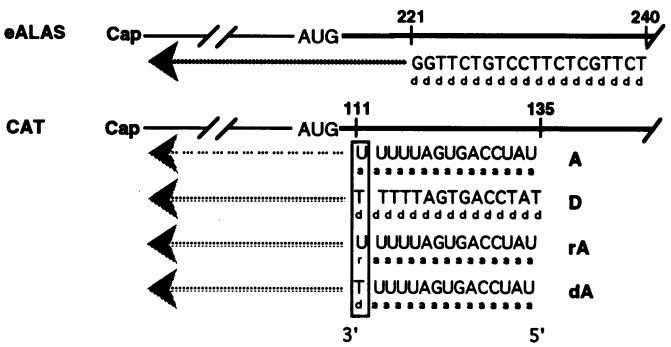

Figure 1. Map of the test (CAT) and internal control (eALAS) template mRNAs. The numbers above the mRNAs indicate the binding sites for the primers in nts from the cap. Letters underneath the sequences of the primers indicate the nucleotide type; a = 2′-O-allyl, d = DNA and R = RNA. The expected products from the reverse transcription are 135 nts for CAT and 240 nts for eALAS.

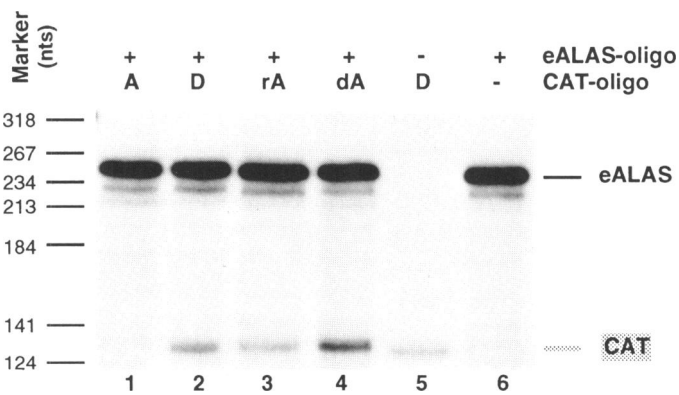

Figure 2. Reverse transcription of eALAS and CAT mRNAs. The indicated primers were used in reactions containing both CAT and eALAS mRNAs. Extension products are marked on the right. The slightly lower mobility of the CAT extension product in lanes 1, 3 and 4 is due to the 2′-O-allyl modification of the primer.

ACKNOWLEDGMENTS

We thank Samantha O'Loughlin and Susan Weston for synthesis of the oligonucleotides and Gábor Lamm for valuable comments on the manuscript. We also appreciate the support from M.W.Hentze in whose laboratory the work was carried out. Ö.M. is a recipient of a postdoctoral fellowship from the EMBO.

REFERENCES

1. Iribarren,A.M., Sproat,B.S, Neuner,P., Sulston,I., Ryder,U. and Lamond,A.I. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7747–7751.
2. Barabino,S.M.L., Sproat,B.S. and Lamond,A.I. (1992) *Nucleic Acids Res.* **20**, 4457–4464.
3. Inoue,H., Hayase,Y., Iwai,S. and Ohtsuka,E. (1988) *Nucleic Acids Res.* Symposium Series No. **19**, 135–138.
4. Anthony,D.D. and Merrick,W.C. (1992) *J. Biol. Chem.* **267**, 1554–1562.
5. Walder,R.Y. and Walder,J.A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5011–5015.