RNA (1997), 3:950–951. Cambridge University Press. Printed in the USA.

LETTER TO THE EDITOR

The position of site-directed cleavage of RNA using RNase H and 2′-O-methyl oligonucleotides is dependent on the enzyme source

JON LAPHAM,[1] YI-TAO YU,[2] MEI-DI SHU,[2] JOAN A. STEITZ,[2] and DONALD M. CROTHERS[1,2]

[1]Department of Chemistry, Yale University, New Haven, Connecticut 06511, USA
[2]Department of Molecular Biophysics and Biochemistry, Boyer Center for Molecular Medicine, Howard Hughes Medical Institute, Yale University School of Medicine, 295 Congress Avenue, New Haven, Connecticut 06536, USA

Keywords: chimera; DNA

It has been shown that RNA can be site specifically cleaved by RNase H using 2′-O-methyl RNA/DNA chimeras to direct the cleavage site (Inoue et al., 1987; Hayase et al., 1990; Lapham & Crothers, 1996; Xu et al., 1996). In these studies, the chimeric splint positions four base paired deoxyribonucleotides to the 5′ side of the position of desired cleavage, as shown in Figure 1A. In each of these studies, the cleavage position has been determined to be that shown in the figure with a reaction efficiency of greater then 90%.

Recently, a paper from the Steitz laboratory (Yu et al., 1997) reported the application of chimeric-directed RNA cleavage as a diagnostic tool in determining positions that have been methylated at the 2′ hydroxyl position. It was reported that a chimera containing four deoxyribonucleotides resulted in efficient cleavage of a target RNA at the site 5′ to the ribonucleotide that base pairs to the 5′-most deoxyribonucleotide, as shown in Figure 1B. This cleavage site is different from that described previously (see above). For another target RNA, a chimera containing three deoxyribonucleotides was found to direct specific RNase H cleavage more efficiently than a four deoxyribonucleotide-containing chimera. Again, the cleavage site was 5′ to the ribonucleotide that base pairs to the 5′-most deoxyribonucleotide (Fig. 1B).

We have determined that the apparent discrepancy between these findings can be traced to the source of the RNase H enzyme. In the earlier studies, the RNase H (*Escherichia coli*, cloned) was purchased from either Pharmacia, Sigma, or Takarashuzo. The later work from the Steitz lab utilized RNase H from Boehringer Mannheim (*E. coli*, cloned). An experiment comparing the Pharmacia versus the Boehringer Mannheim enzymes on the same substrate and chimera was performed (data not shown), with the Pharmacia enzyme cleaving as shown in Figure 1A and the Boehringer Mannheim enzyme cleaving as in Figure 1B.

It is unclear why enzymes from different suppliers derived from the same source show different activities. We note that the Boehringer Mannheim RNase H storage buffer does not contain EDTA and is relatively low in salt compared with that from Sigma and Pharmacia. Other differences in the purification protocols may also be contributing to the observed differences. Therefore, when precise cleavage using chimeric oligonucleotides is required (i.e., for ligations or site-specific labeling) we recommend caution in the construction of the oligonucleotides and choice of supplier of enzyme.

Reprint requests to: Donald M. Crothers, Department of Chemistry, Yale University, 350 Edwards Street, New Haven, Connecticut 06511, USA; e-mail: donald.crothers@quickmail.yale.edu.

REFERENCES

Hayase Y, Inoue H, Ohtsuka E. 1990. Secondary structure in formylmethionine tRNA influences the site-directed cleavage of ribonuclease H using chimeric 2′-O-methyl oligonucleotides. *Biochemistry 29*:8793–8797.

Inoue H, Hayase Y, Iwai S, Ohtsuka E. 1987. Sequence-dependent hydrolysis of RNA using modified oligonucleotide splints and RNase H. *FEBS Lett 215*:327–330.

Lapham J, Crothers DM. 1996. RNase H cleavage for processing of in vitro transcribed RNA for NMR studies and RNA ligation. *RNA 2*:289–296.

Xu J, Lapham J, Crothers DM. 1996. Determining RNA solution structure by segmental isotopic labeling and NMR: Applications to *Caenorhabditis elegans* spliced leader RNA 1. *Proc Natl Acad Sci USA 93*:44–48.

Yu Yt, Shu Md, Steitz JA. 1997. A new method for detecting sites of 2′-O-methylation in RNA molecules. *RNA 3*:324–331.

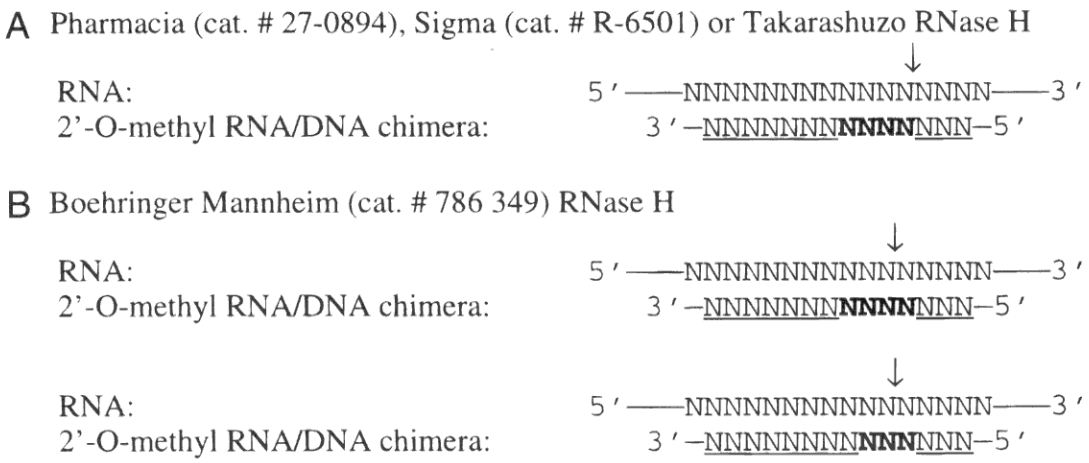

FIGURE 1. Underlined letters represent 2′-O-methyl RNA, bold letters represent the deoxyribonucleotides, and the observed cleavage positions are shown as arrows.

The position of site-directed cleavage of RNA using RNase H and 2'-O-methyl oligonucleotides is dependent on the enzyme source.

J Lapham, Y T Yu, M D Shu, et al.

RNA 1997 3: 950-951