Nucleic Acids Research, 1997, Vol. 25, No. 18 **3747–3748**

Fluorescence-, isotope- or biotin-labeling of the 5′-end of single-stranded DNA/RNA using T4 RNA ligase

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Received June 11, 1997; Revised and Accepted August 5, 1997

ABSTRACT

A rapid 5′-labeling method of single-stranded DNA/RNA was developed, which is based on the utilization of an adenylated intermediate in the reaction of T4 RNA ligase. This method is commonly useful for fluorescence-, isotope- or biotin-labeling of the 5′-ends of both oligo- and polynucleotides.

End labeling of DNA/RNA is an essential technique for nucleic acids research. Enzymic methods for end-labeling tend to be easier and more convenient than organic chemical methods due to advantages such as mildness, specificity, controllability and efficiency of enzymic reactions. Enzymic 3′-labeling techniques already exist: TdT methods and T4 RNA ligase methods can add a modified nucleotide to DNA/RNA at its 3′-end (1–4). However, there exists no simple enzymic 5′-end labeling technique which leaves the 3′-end intact for extension. Instead, a rather laborious method was developed for this purpose whereby an oligonucleotide, not a mononucleotide, was organochemically modified and then ligated to a DNA/RNA at its 5′-end by use of T4 RNA ligase (5). Versatile end-labeling such as fluorescence-, RI- and biotin-labeling is also required for highly developed studies.

In this paper, we report that T4 RNA ligase can be exploited for the above purpose. T4 RNA ligase is known to be subject to the reaction scheme shown in Figure 1 (6). Provided with all components, the reaction will proceed to completion, generating the final product. However, if the acceptor is not added (in the reaction mixture), the intermediate product (sufficiently durable; no change observed after one week left at room temperature) will accumulate. Based on this fact, together with our recent finding that 2AP-TP (2-aminopurine riboside triphosphate) and 3′-NH_2ATP (3′-amino, 3′-deoxyadenosine triphosphate) can replace ATP in the ligation reaction of T4 RNA ligase (7), we devised an enzymic 5′-labeling technique.

Since an amino group introduced at the 5′-end can serve as a target for further modifications such as biotination, 3′-NH_2ATP was used to examine whether it can generate the intermediate product. As shown in Figure 2A, the required product was obtained with a yield of $< 50\%$, which was irrelevant to the concentration of 3′-NH_2ATP (0.1–1 mM) when the concentration of oligomer was set at 0.1 μM. This concentration appears to be sufficient for many purposes in direct use. The only by-product was a circularized one that was demonstrated by the insusceptibility to an exonuclease (Klenow fragment) as shown in Figure 2A.

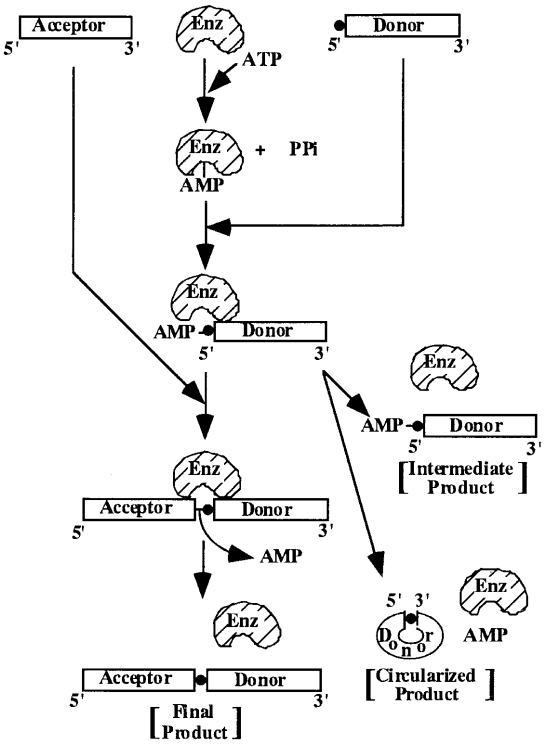

Figure 1. Schematic representation of the widely accepted ligation reaction mechanism of T4 RNA ligase (6). In the absence of acceptors, the intermediate products, i.e., adenylated donors, are accumulated and partly converted to circularized ones. The filled circle represents a phosphate.

Naturally, modification of 3′-OH of a donor oligonucleotide could eliminate this by-product. Complete biotination of an amino group thus introduced was ascertained by a conventional method as shown in Figure 2B (8). Restriction fragments (69–6407 bases) of M13 ssDNA with *Hae*III were also competent for this labeling reaction (data not shown).

Labeling of an oligonucleotide at its 5′-end with a fluorescent nucleotide, 2AP-TP, was performed in T4 RNA ligase buffer containing 10 pmol of an oligonucleotide, 2 mM 2AP-TP and 50 U T4 RNA ligase for 3 h at 25°C (Fig. 2C), resulting in an almost complete reaction when 2AP-TP ≥2 mM was applied. The fluorescence of the adduct was monitored using HPLC with a fluorescence detection unit.

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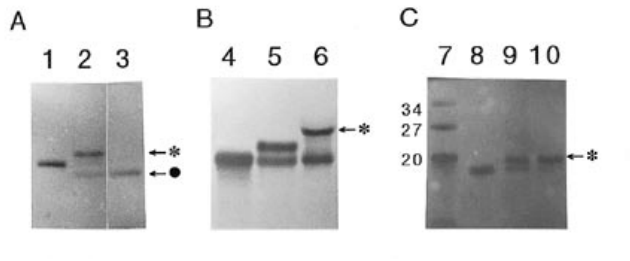

Figure 2. 5′-labeling with an amino-containing or a fluorescent nucleotide. Reaction mixtures were analyzed with denaturing 20% polyacrylamide gel electrophoresis and silver staining. (**A**) Labeling of an oligodeoxynucleotide with 3′-NH_2ATP. 1 pmol of 5′-phosphorylated oligo-1 (5′-CCTTGGGGAAT-CCCTTATAT-3′) was reacted with 3′-NH_2ATP by T4 RNA ligase. Lane 1, oligo-1 (1 pmol); lane 2, reaction products (1 pmol); lane 3, the reaction products (3 pmol) processed with Klenow fragment. (**B**) Biotination of an amino group introduced at the 5′-end of an oligomer. 5′-phosphorylated and 3′-dideoxyadenylated oligo-2 (5′-CGTCGCAAAAAAAAAAAA-3′) was labeled with 3′-NH_2ATP as described above. After purification with oligo(dT)cellulose, the reactant was further processed with 50 mM biotinamidecaproate *N*-hydroxysuccinimide ester in 0.2 M carbonate buffer (pH 9.0)/dimethylformamide (1:1) at room temperature for 2 h. Lane 4, 5′-phosphorylated and 3′-dideoxy-adenylated oligo-2 (10 pmol); lane 5, 3′-NH_2ATP labeled oligo-2 with the excess unreacted mixture; lane 6, further biotinated product. (**C**) Labeling of an oligonucleotide with fluorescent 2AP-TP. The reaction mixtures containing 5′-phosphorylated and 3′-dideoxyadenylated oligo-2, 2AP-TP and T4 RNA ligase as described in the text were incubated for 1 h (lane 9) or 3 h (lane 10); lanes 7 and 8, marker oligomers (the sizes are shown in nucleotides) and unreacted mixtures. *, Labeled oligonucleotides; ●, circularized product.

Since ATP and dATP react more efficiently than 3′-NH_2ATP and 2AP-TP (7), this method can also be used for radio-isotopical 5′-labeling of oligonucleotides by use of [α-^{32}P]ATP/dATP (at a concentration of 0.1 mM when 1 μM of oligonucleotide is used), which means that the same RI species (α-^{32}P) as is used for incorporation experiments can also be applied for end-labeling. Therefore, fluorescence-, RI- and biotin-labelings at the 5′-end of DNA/RNA are possible by the same method utilizing T4 RNA ligase, which can be used in small amounts, under mild conditions and in a substrate-specific mode.

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