Strand Ligation in a Double-stranded DNA by T4 RNA Ligase

Yasunori Kinoshita, Koichi Nishigaki,* and Yuzuru Husimi
Department of Functional Materials Science, Saitama University, 255 Shimo-okubo, Urawa 338

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T4 RNA ligase was shown to ligate two DNA strands contained in a double helix with a high efficiency. Experiments on substrate and temperature dependence of this reaction supported that single-stranded ends clamped by a double-stranded region are necessary for this reaction. This finding is widely useful for DNA joining.

T4 RNA ligase ligates both single-stranded RNAs and DNAs (Figure 1a), although DNA ligation is less efficient.[1] Substitution of the 3'-end of an acceptor DNA with a ribonucleotide enhances DNA ligation reaction[2] (Figure 1b). Intramolecular ligation of flaring ss RNAs/DNAs by T4 RNA ligase was also reported[3,4] (Figure 1c). Recently, two flaring ss RNAs clamped by splinting[5] or hybridization[6] were linked(Figure 1d,e). Hitherto, direct ligation of deoxy- to deoxyribonucleotides was shown to be very unfavorable.[4,7] In addition, T4 RNA ligase was regarded to work on ss DNAs but not on ds DNAs. Therefore, strand ligation of ds DNAs without flaring ss regions (Figure 1f) was yet not reported, which evokes interests not only in the mechanism of the interaction between the enzyme and the substrate DNA but also in a general use of this enzyme in DNA technologies.

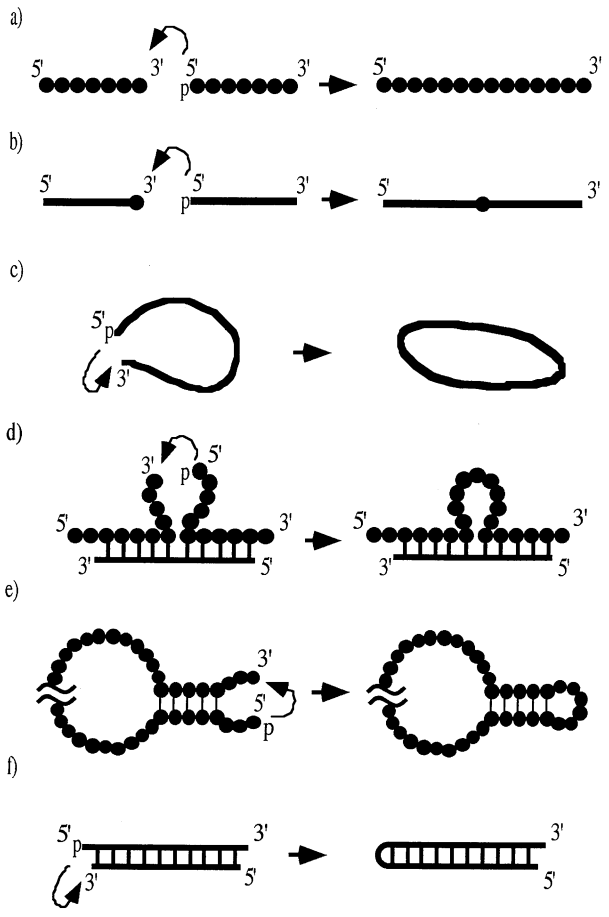

Figure 1. Ligation modes of T4 RNA ligase
The closed circles (●) mean ribonucleotides and bold line segments (▬), deoxyribonucleotides. See text for each reference source.

(p1: 5'p-Oligo-1/Oligo-2)
5' p-ATATGGCTGTTTATTTTGTAACTG-3'
3' -CCGACAAATAAAACA-5'

(p2: 5'p-Oligo-3/Oligo-4)
5' p-GCCAGTTCTTAAAATCGC-3'
3' -GTCAAGAATTTT-5'

(p3: 5'p-Oligo-1/Oligo-5)
5' p-ATATGGCTGTTTATTTTGTAACTG-3'
3' -ATACCGACAAATAAAACATTGAC-5'

(p4: 5'p-Oligo-5/Oligo-1)
5' p-CAGTTACAAAATAAACAGCCATA-3'
3' -GTCAATGTTTTATTTGTCGGTATA-5'

(p5: 5'p-Oligo-4/Oligo-3)
5' p-TTTTAAGAACTG-3'
3' -CGCTAAAATTCTTGACCG-5'

(p6: 5'p-106bp)
5' p-TAAGGCCGA---TCTTTTGCT-3'
3' -TGGTGATTCCGGCT---AGAAAACGA-p5'

Figure 2. Double-stranded DNAs used for this experiment. (p1) A 5'-,3'-extruding double-stranded DNA. Two oligodeoxyribonucleotides, 5'-Oligo-1 (24-mer, 25 pmoles; see the above figure for sequences) and Oligo-2 (15-mer, 25 pmoles), were mixed together with 1 μl of 1 mM ATP, 5 μl of 2x ligation Buffer (0.1 M Tris-HCl (pH 8.0), 20 mM $MgCl_2$, 20 mg/l BSA, 2 mM hexammine cobalt chloride, 50 % PEG 6000)[1] and 3 μl of water, and the mixture was incubated at 65 °C, 5 min and then at 37 °C, 30 min to hybridize two strands. Complete hybridization of two oligonucleotides was confirmed by non-denaturing gel electrophoresis (data not shown). (p2) 2-base-extruding at the 5' end of the donor. 5'p-Oligo-3 (18-mer) and Oligo-4 (12-mer). (p3) 1-base-extruding at the 5' end of the donor. 5'p-Oligo-1 and Oligo-5 (23-mer). (p4) Blunt end at the 5' end of the donor. 5'p-Oligo-5 and Oligo-1. (p5) 4-base-recessing at the 5' end of the donor. 5'p-Oligo-4 and Oligo-3. (p6) 106 bp fragment with one 5-base cohesive end (recessing at the 5' end) and the other blunt end.

Double-stranded(ds) oligodeoxyribonucleotides were obtained as shown in Figure 2. ds DNAs thus obtained were subjected to a ligation reaction. In each case, the expected products could be observed, although the blunt end gave a poor product (Figure 3A). A duplex DNA of 106 bp (p6 in Figure 2), which has similar end-types to those of the pairs p4 and p5, could be also ligated by a few percent, which was too low to generate an observable amount of circularized DNAs(Figure 3A). Figure 3B shows a time course of a ligation reaction for p1 which comes to the completion within 15 min.

Through these results, we have attained to a concept that this enzyme has an ability of end-closing of a double-stranded DNA(and,

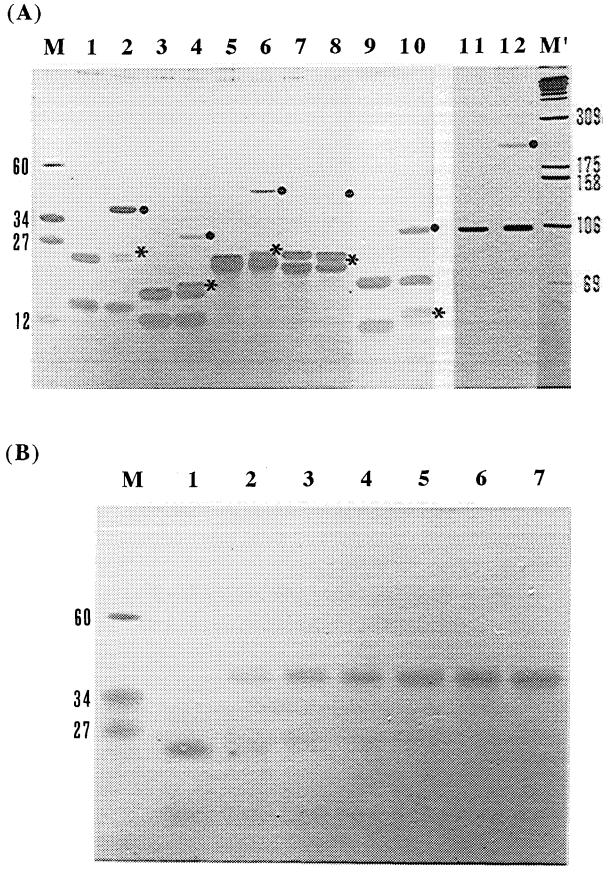

Figure 3. (A) Ligation of double-stranded DNAs. Double-stranded DNAs(from p1 to p6 in Figure 2) were incubated together with T4 RNA ligase(50 units;Takara Shuzo Co.Ltd.) at 37 ℃ for 19 h. Each lane is designated as follows: lane 1,p1; lane2, ligation products of p1; lane 3,p2; lane 4, products of p2; lane 5, p3; lane 6, products of p3; lane 7, p4; lane 8, products of p4; lane 9, p5; lane 10, products of p5; lane 11,p6; and lane 12, products of p6. In each product lane, the dot (•) means the aimed product and the asterisk (*), the intermediate product (*i.e.*, the monophosphoadenylated donor molecule[10]). (B) Time course of ligation reaction of a ds oligodeoxyribonucleotide. A double-stranded DNA (p1 in Figure 2) was incubated with T4 RNA ligase at 37 ℃ as follows : lane 1, 0 min; lane 2, 1 min; lane 3, 2 min; lane 4, 3 min; lane 5, 5 min; lane 6, 10 min ; and lane 7, 20 min. The letters M and M' indicate marker lanes (each number affixed beside shows a degree of polymerization (in nucleotides)). Gel electrophoresis was performed under denaturing conditions.

naturally, RNA). The rate of this reaction seems to depend on the length of the extruding single-stranded region, the length of the double-stranded region and the temperature adopted. Our preliminary experiment on temperature dependence of this reaction supported an idea that, for this reaction, there is a temperature-optimum around the melting temperature of a double-stranded oligo- / polynucleotides (in case of the duplex p1, it was around 37 ℃), which may indicate that the reaction needs a transiently, partially melted structure of a duplex in order to fulfill an equivalent substrate structure to that appearing in ligation of single-stranded RNAs/DNAs but that stable ss structures are not prerequisite. In this vein, the enhanced reaction rate can be explained by the elevation of the local concentration of the substrates, 5'-phosphate of one strand and 3'-OH of the other strand, owing to a clamping effect by the double-stranded region which is consistent with a similar observation by Silber *et al.*[3] The related phenomena to this concentration effect were reported as to this enzyme (by hybridization[6](Figure 1d); by splint[7](Figure 1e)). Irrespective of the knowledge on behaviors of T4 RNA ligase *in vivo*, we can exploit this enzyme to synthesize with high efficiency hairpin or circular structure RNAs/DNAs which must have a wide usage in DNA technologies such as a nuclease-attack-free gene transfer and geometric serial extention of DNAs (which elongates DNAs in a geometric series, using ds DNA templates sealed at one end). Tight, small loop structures thus generated themselves are intriguing objects for structural studies.[8,9]

References and Notes

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