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RNase A Treatment of Taq and Tth DNA Polymerases Eliminates Primer/Template-Independent Poly(dA-dT) Synthesis

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We previously reported Thermus aquaticus DNA polymerase (Taq) and T. thermophilus DNA polymerase (Tth) synthesized poly(dA-dT) at 65°C in the apparent absence of primer/template (2,3). The polymerase preparations were highly purified enzymes provided by various manufacturers such as Invitrogen, Roche Molecular Biochemicals, Toyobo, etc. We examined here the possibility that RNA or DNA, which could serve as a primer/template, was co-purified with Taq and Tth.

Taq treated with 5 U RNase A did not synthesize the poly(dA-dT) (Figure 1A-a) but did so when oligo(dA-dT) was added as a primer/template (Figure 1A-b). Treatment with RNase T1, which specifically cuts RNA on the 3′ side of G, did not affect the polymerase activities (Figure 1B). Tth and other DNA polymerases derived from T. flavus (Promega, Tokyo, Japan), T. icelandicus (ABgene; Epsom, Surrey, UK), and T. ubiquitos (Amersham Pharmacia Biotech, Tokyo, Japan) were examined similarly. RNase A treatment, but not RNase T1, eliminated the poly(dA-dT) synthesis at 65°C (data not shown).

Tth was reported to synthesize repet-
itive high-molecular-weight DNA such as (TACATGTA)_n and (ATACGTAT)_n at 74°C without requiring added primer/template (5). The effect of RNase treatments on high-molecular-weight DNA synthesis at 74°C was checked. Both 5 U RNase A and 10 U RNase T1 treatments eliminated Tth’s capacity of the unprimed high-molecular-weight DNA synthesis (Figure 1, C-a and D-a). The oligo(dA-dT)-primed poly(dA-dT) synthesis run in parallel as a control was not affected by the RNase treatments (Figure 1, C-b and D-b). In these panels, DNA of about 150 bp in the oligo(dA-dT)-primed reaction at 74°C conducted as a control was somehow accumulated. Perhaps the high temperature may have interfered with AT base pairing, resulting in the production of shorter DNA pieces.

∆Tth DNA polymerase (∆Tth) and the Stoffel fragment, both lacking 5’ to 3’ exonuclease activity by the deletion of the respective 250 and 289 N-terminal amino acids of the wild-type DNA polymerases (1,4), were devoid of any activity in the primer-independent DNA synthesis (3). We examined whether these N-terminal-deleted enzymes could synthesize poly(dA-dT) on oligo(rA-rU) primer/template. As shown in Figure 2, Tth and also the Stoffel fragment synthesized poly(dA-dT) when 50 ng or more oligo(rA-rU)_{10} were added in the reaction.

The above experiments were compatible with the hypothesis that high-molecular-weight DNA was synthesized on an RNA primer/template co-purified with the DNA polymerases because (i) RNase treatments eliminated the high-molecular-weight DNA synthesis with expected specificity (i.e., broadly acting RNase A eliminated all the primer/template-independent, high-molecular-weight DNA synthesis), while RNase T1 specifically cleaving RNA on the 3’ side of G eliminated C-containing high-molecular-weight DNA synthesis (5) only, and (ii) the Stoffel fragment or ∆Tth lacking in the activity of primer/template-independent, high-molecular-weight DNA synthesis did so in the presence of added oligo(rA-rU). However, we failed to detect RNA in phenol-chloroform extract of 250 U Taq DNA polymerase (AmpliTaq DNA polymerase, Stoffel Fragment; Applied Biosystems) were mixed with a certain amount of oligo(rA-rU)_{10} and 0.2 mM dNTPs in 50 µL reaction buffer (2.5 mM MgCl2, 25 mM Tris-HCl, pH 8.3, and 10 mM KCl). Five units of ∆Tth (Toyobo, Osaka, Japan) were also mixed with a certain amount of oligo(rA-rU)_{10} and 0.2 mM dNTPs in 50 µL reaction buffer (1.5 mM MgCl2, 10 mM Tris-HCl, pH 8.9, 80 mM KCl, 0.1% Triton® X-100, and 0.05% BSA). The mixtures were incubated at 65°C for 4 h, and 15-µL aliquots were then electrophoresed at 100 V for 18 min on 1.5% agarose gel. Lane M: 1-kb DNA ladder.
smear. Such smear bands obtained with reactions using untreated Taq (Figure 3B, the rightmost two lanes) were eliminated by RNase A treatment (0.01 U/10 µL) of Taq without reduction of the target 3.2-kbp DNA band (Figure 3B, the leftmost two lanes). Therefore, RNase A treatment of Taq DNA polymerase may be useful in eliminating the smear bands in long PCRs.

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