BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **238**, 113–118 (1997)
ARTICLE NO. RC977197

Primer/Template-Independent Synthesis of Poly d(A-T) by Taq Polymerase

Kenichi Hanaki,*‡ Takashi Odawara,* Tomonari Muramatsu,† Yoshiyuki Kuchino,† Michiaki Masuda,* Kenji Yamamoto,‡ Chikateru Nozaki,§ Kyosuke Mizuno,§ and Hiroshi Yoshikura*[1]

**Department of Bacteriology, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113; †Biophysics Division, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104; ‡Laboratory of Clinical Microbiology and Immunology, Bun' in Hospital of University of Tokyo, 7-3-1, Mejirodai, Bunkyo-ku, Tokyo 113; and §The Chemo-Sero-Therapentic Research Institute, Kyokushi, Kikuchi-gun, Kumamoto 869-12, Japan*

Received July 24, 1997

Taq DNA polymerase polymerized dATP and dTTP to poly d(A-T) without requiring added primer/template in the temperature range of 60–70°C. Tth DNA polymerase also catalyzed the reaction, while ΔTth, Vent, Vent(exo-), Pfu, Ultma, BcaBEST, and KOD DNA polymerases did not. The reaction was distinct from the template-nonrequiring terminal deoxynucleotidyl transferase reaction which absolutely required primers. © 1997 Academic Press

Unprimed polymerase reaction using Escherichia coli DNA polymerase I was studied extensively during the early 1960's to 1970's (1-5). Characteristic feature of the polymerization was that (i) the reaction apparently required neither primers nor templates and that (ii) the products were high molecular weight poly dA · poly dT or poly d(A-T) · poly d(A-T). A similar activity was also detected in DNA polymerases isolated from *Bacillus subtillus, Micrococcus luteus* and calf thymus (6-10). However, the description of this activity appears to be omitted from recent literatures (11). This is probably due to the observations that the activity decreased or even disappeared during the purification of the polymerase (3, 5, 12, 13). Therefore, the molecular nature of the enzyme polymerizing dATP and dTTP has remained unclear.

Recently, during attempts to amplify poly d(A-T) sequence using Taq polymerase, we encountered the similar phenomenon which was described more than 35 years ago. The polymerases used in our experiments were highly purified native or recombinant preparations, and the reaction converted near 90% of the substrate dATP and dTTP nucleotides into the high molecular weight poly d(A-T). The reaction took place without added template or primer. In this study, this novel activity associated with Taq polymerase was characterized in detail.

[1] To whom all correspondence should be addressed.

MATERIALS AND METHODS

DNA polymerases. The thermostable DNA polymerases examined were AmpliTaq DNA Polymerase (Perkin–Elmer), AmpliTaq DNA Polymerase, LD (Perkin–Elmer), Taq DNA Polymerase (Gibco BRL), TaKaRa Taq (Takara), rTaq DNA Polymerase (Toyobo), Tth DNA Polymerase (Toyobo), ΔTth DNA Polymerase (Toyobo), Vent DNA Polymerase (New England Biolabs), Vent(exo-) DNA Polymerase (New England Biolabs), Pfu DNA Polymerase (Stratagene), Ultma DNA Polymerase (Perkin–Elmer), BcaBEST DNA Polymerase (Takara), and KOD DNA Polymerase (Toyobo). Except Tth and Pfu polymerases, all of the enzymes were recombinant DNA products. The genes of AmpliTaq, BcaBEST, Tth, KOD and Pfu polymerases were respectively derived from *Thermus aquaticus* (14), *Bacillus cardotenax YT-G* (15), *Thermus thermophilus* (16), *Pyrococcus sp. KOD1* (17), and *Pyrococcus furiosus* (18). Thermus and Bacillus belong to Eubacteria, and Pyrococcus belongs to Archaea.

Reaction condition. Standard reaction mixture contained 5 U of AmpliTaq DNA polymerase, 0.2 mM each of dNTPs (GeneAmp dNTPs [N808-0007], Perkin–Elmer) in 100 μl PCR buffer (1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl [pH 8.3]) (GeneAmp PCR Buffer, [N808-0006], Perkin–Elmer). The mixture was incubated at 65°C (except otherwise stated) using GeneAmp PCR System 2400 (Perkin–Elmer) for an indicated period. Reaction products were precipitated twice with 2 M CH_3COONH_4 and 70% ethanol which removed 99% of unincorporated dNTPs, dissolved in the distilled water, and electrophoresed in a 2% agarose gel (Agarose type 1; LOW EEO [A6013], Sigma). For visualizing DNA, the gel was stained with ethidium bromide (1mg/l).

Nearest neighbor frequency analysis. The polymerase reaction was carried out for 3hr with standard method products added with 10 μCi (3.3 pM) of either [α-^{32}P]-dATP or [α-^{32}P]-dTTP (as the primer consisted A and T as shown below). Then, the samples were applied to NICK column (Pharmacia Biotech AB, Uppsalla, Sweden), and the polymer fraction (400 μl) free of the unincorporated dNTPs was collected. For hydrolytic degradation of the sample to deoxynucleo-

0006-291X/97 $25.00
Copyright © 1997 by Academic Press
All rights of reproduction in any form reserved.

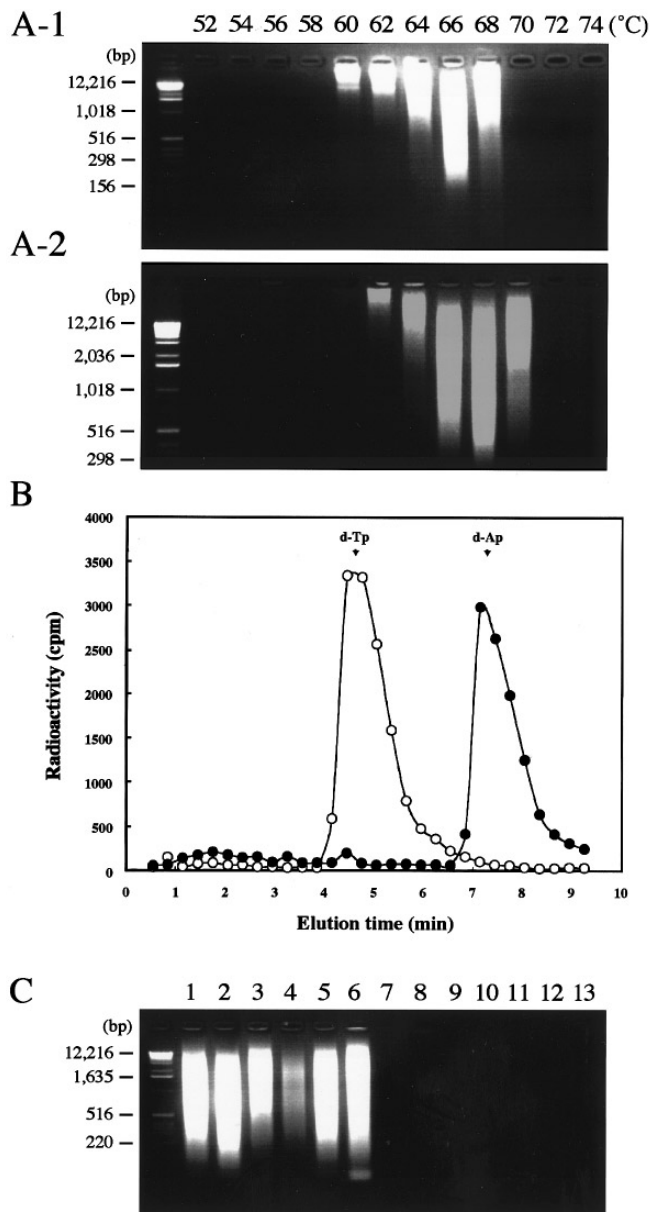

FIG. 1. dAdT polymerization by Taq DNA polymerase at various conditions and that by other thermophile bacteria-derived DNA polymerases. (A) Temperature effect. 5 U AmpliTaq (Perkin–Elmer) and 0.2 mM each of dATP and dTTP (A-1) or all the four dNTPs (A-2) in 100 μl GeneAmp PCR buffer [N808-0006] (Perkin–Elmer) were incubated at different temperatures for 2 hr. The DNA was extracted from 40 μl reaction mixture and dissolved in 40 μl. 15 μl aliquot was applied onto the 2% agarose gel and electrophoresed at 100 V for 20 min. The molecular size marker was the 1 kb DNA Ladder [15615-016] (Gibco BRL). (B) Nearest neighbor base analysis. HPLC of the digests of the product labelled with ^{32}P-ATP (○) or with ^{32}P-TTP (●). The positions of d-Tp and d-Ap are shown. (C) 5 U thermostable DNA polymerase and 0.2 mM each of all the four dNTPs in 100 μl PCR buffer (attached to the each kit) was incubated at 65°C for 2 hr. The DNA was extracted with ethanol from 50 μl reaction mixture and dissolved in 50 μl of the distilled water. 15 μl aliquot was applied onto the agarose gel. According to the suppliers' information, PCR buffer for AmpliTaq (Perkin–Elmer), AmpliTaq, LD (Perkin–Elmer), and rTaq (Toyobo) was 1.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl [pH 8.3], that for Taq (Gibco BRL) was 1.5 mM $MgCl_2$, 50 mM KCl, and 20 mM Tris-HCl [pH 8.4], that for TaKaRa Taq was 3.5 mM $MgCl_2$, 15 mM $(NH_4)_2SO_4$, and 100 mM Tris-HCl [pH 8.9], that for Tth and delta Tth were 1.5 mM $MgCl_2$, 80 mM KCl, and 10 mM Tris-HCl [pH 8.9], that for Vent and Vent(exo-) were 2 mM $MgSO_4$, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, and 20 mM Tris HCl [pH 8.8], that for Pfu was 2 mM $MgCl_2$, 10 mM KCl, 6 mM $(NH_4)_2SO_4$, and 20 mM Tris-HCl [pH 8.0], that for Ultma was 1.5 mM $MgCl_2$, 10 mM KCl, and 10 mM Tris-HCl [pH 8.8], that for BcaBEST was 10 mM $MgCl_2$ and 20 mM Tris-HCl [pH 8.5], and that for KOD was 2 mM $MgCl_2$, 10 mM KCl, 6 mM $(NH_4)_2SO_4$, and 120 mM Tris-HCl [pH 8.0]. Lane 1, AmpliTaq (Perkin–Elmer); lane 2, AmpliTaq, LD (Perkin–Elmer); lane 3, Taq (Gibco BRL); lane 4, TaKaRa Taq (Takara); lane 5, rTaq (Toyobo); lane 6, Tth (Toyobo); lane 7, ΔTth (Toyobo); lane 8, Vent (New England Biolabs); lane 9, Vent(exo-) (New England Biolabs); lane 10, Pfu (Stratagene); lane 11, Ultma (Perkin–Elmer); lane 12, BcaBEST (Takara); lane 13, KOD (Toyobo).

side-3′-monophosphates, an aliquot (30 μl) of this fraction was incubated at 38°C for 3.5 hr in the 50 μl mixture containing 6 U of micrococcal nuclease (Worthington Biochemical Co.), 0.06 U of spleen phosphodiesterase (Worthington Biochemical Co.), 10 mM sodium succinate (pH 6.0) and 5 mM $CaCl_2$. Hydrolyzed samples were mixed with 2.5 μg salmon testis DNA and subjected by high-performance liquid chromatography (HPLC) using a reverse-phase column, J'sphere ODS-H80 (YMC Co. Ltd., Kyoto, Japan) with acetate buffer containing 25 mM sodium acetate, 12.5 mM citric acid, 10 mM acetic acid, 30 mM NaOH and 1% methanol at a flow rate of 1 ml/min. Counts of each fraction (0.3 ml) were measured by the Cerenkov counting method.

RESULT

Polymerization of dATP and dTTP into poly d(A-T) by Taq polymerase. When the mixture of all the four dNTPs and Taq polymerase was incubated in the PCR buffer at 65°C for 3 hr and an aliquot was run in an agarose gel, smeared signals were detected in the high-molecular-weight (HMW) region (Fig. 1-C, lane 1). As the DNA was stained with ethidium bromide, the DNA must have a double-stranded structure at least in part; digestion with S1 nuclease actually failed to digest the DNA under the conditions where M13mp19 plus strand DNA was completely digested (data not shown). The HMW DNA was produced only when the both dATP and dTTP were present in the mixture. In contrast, dGTP and dCTP were dispensable. When the reaction was performed by using ^{32}P-labelled dNTP, radioactive products were generated only when ^{32}P-dATP or ^{32}P-dTTP was in the mixture (data not shown). These results suggested that the polymer contained only A and T. When the reaction was carried out for 2 hr in the presence of dATP and dTTP at different temperatures, the HMW DNA appeared in the temperature range of 60-68°C (Fig. 1-A-1). When the mixture contained all of the four dNTPs, the HMW DNA appeared in the temperature range of 62-70°C (Fig. 1-A-2). In the experiments described below, all of the reactions were performed at 65°C. The reaction required Mg^{++}, and the reaction was observed in Mg^{++} concentration range 1.5-

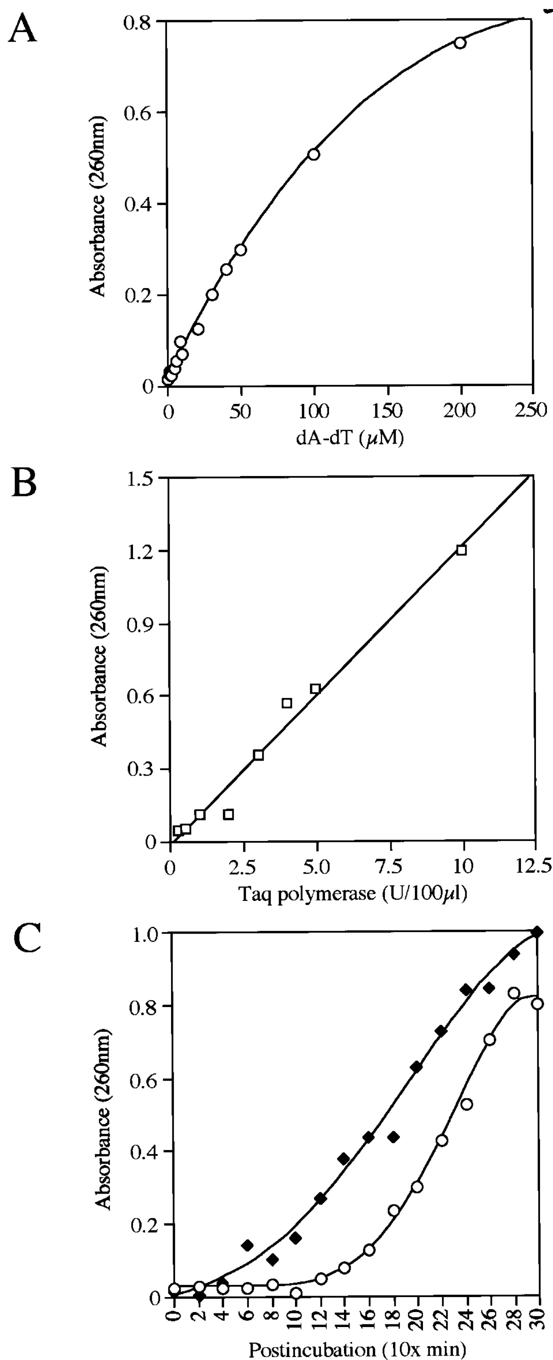

FIG. 2. Dose response of dAdT polymerization and reaction kinetics. (A) 5 U AmpliTaq DNA polymerase [N808-0160] (Perkin–Elmer) and various concentrations of dATP and dTTP (dATP and dTTP were present in equimolar) in 100 μl GeneAmp PCR Buffer (Perkin–Elmer) were incubated at 65°C for 3 hr. DNA was ethanol-extracted from 50 μl of the reaction mixture, dissolved in 100 μl distilled water, and measured for the absorbance at 260 nm. The DNA quantity was estimated from the optic density. (B) Various amount of AmpliTaq and 0.2 mM each of dATP and dTTP in 100 μl GeneAmp PCR Buffer were incubated at 65°C for 3 hr, and the amount of the product DNA was estimated from the optic density at 260 nm. (C) Tubes containing 5 U AmpliTaq and 0.2 mM each of dATP and dTTP (♦) or 5 U AmpliTaq and 0.2 mM each of all the four dNTPs (○) in 100 μl GeneAmp PCR Buffer were incubated at 65°C, and one of the tubes was taken at 20 min interval for analysis of the product. The DNA was ethanol-precipitated and the amount was estimated by the optic density at 260 nm by assuming that the products were double stranded DNA.

30 mM; concentrations higher than 35 mM were inhibitory (data not shown).

In order to determine the structure of the polymer, the nearest neighbor base sequence analysis (4, 19) was performed. The phosphate attached to 5′ carbon becomes esterified with 3′ carbon of the deoxyribose at the growing end (next neighbor) of the chain during the polymerization. Therefore, if the phosphate moiety of a oligonucleotide is radio-labeled, the radio activity is transferred to the next neighbor nucleotide after the digestion with the micrococcal nuclease and phosphodiesterase. The analysis using ^{32}P-dATP and ^{32}P-dTTP (note that the polymer consisted of only A and T) revealed that 100% of the labeled phosphate in dATP was recovered as d-Tp and that in dTTP was recovered as d-Ap (Fig. 1-B). Thus, the product was poly d(A-T). The polymerization reaction will hereafter be called dAdT polymerization.

In Fig. 1-C, the dAdT polymerization reaction activities of various thermostable enzymes were compared. Taq polymerases from five different sources and Tth polymerase had the activity. On the other hand, ΔTth, Vent, Vent(exo-), Pfu, Ultma, BcaBEST, and KOD polymerases were devoid of the activity.

Stoichiometry of the dAdT polymerization reaction. Fig. 2-A shows the relationship between the substrate concentration and the relative amount of the reaction products. The reaction mixture contained 5 U of Taq polymerase and the varying concentration of 1:1 mixture of dATP and dTTP in 100 μl PCR buffer. The incubation period was 3 hr. A linear correlation was observed up to 100 μM, and then the curve leveled off. Fig. 2-B shows the relationship between the Taq polymerase concentration and the amount of the products. The reaction mixture contained varying amount of Taq polymerase, 200 μM of dATP and 200 μM of dTTP in 100 μl of PCR buffer. Linear correlation was observed throughout the Taq polymerase concentration range examined.

Fig. 2-C shows the time course of polymerization reaction. When the mixture contained only dATP and dTTP, 88% of dATP and dTTP were converted to the polymer, and if all of the four nucleotides were present, 71% of dATP and dTTP were incorporated to the polymer. The polymer began to appear as early as 1 hr when only dATP and dTTP were present. In the presence of all of the four dNTPs, however, there appeared to be a lag time of 2-3 hr before polymer formation. Thus, it is possible that dCTP and dGTP were inhibitory to dAdT polymerization.

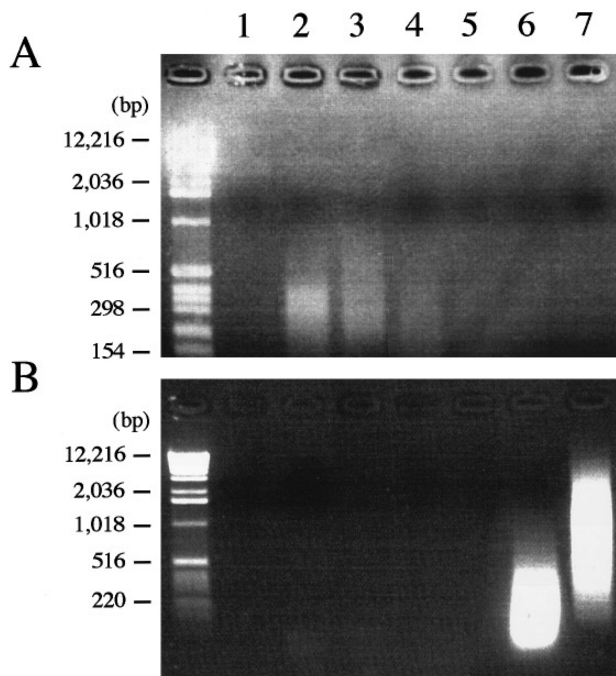

FIG. 3. Comparison of Terminal deoxynucleotidyl transferase (TdT) reaction and dAdT polymerization reaction. Polymerization reaction at 37°C for 16 hr (A) or at 65°C for 3 hr (B). Taq DNA polymerase used was AmpliTaq DNA polymerase (Perkin–Elmer). TdT was Terminal Deoxynucleotidyl Transferase, Recombinant (rTdT [18008-011], Gibco BRL). The PCR buffer was GeneAmp PCR Buffer and the TdT buffer was TdT buffer [16314-015] (Gibco BRL) which contained, 100 mM potassium cacodylate [pH 7.2], 2 mM $CoCl_2$, and 0.2 mM DTT. Lane 1, 5 U / μl Taq, 15 U / μl TdT and 0.2 mM each of dNTPs in TdT buffer; lane 2, 10 pmole oligonucleotide 5′-AGCCATAGTGGTGTGCGGAAC-3′, 15 U / μl TdT, and 0.2 mM dNTPs in TdT buffer; lane 3, 10 pmole oligonucleotide 5′-GCACTCGCAAGCACCCTATCA-3′. 15 U / μl TdT and 0.2 mM dNTPs in TdT buffer; lane 4, 10 pmole 5′-AGCCATAGTGGTGTGCGGAAC-3′, 15 U / μl TdT, 0.2 mM dNTPs and 15 mM $MgCl_2$ in TdT buffer; lane 5, 10 pmole 5′-GCACTCGCAAGCACCCTATCA-3′, 15 U / μl TdT, 0.2 mM dNTPs, and 15 mM $MgCl_2$ in TdT buffer; lane 6, 5 U / μl Taq and 0.2 mM each of dNTPs in TdT buffer; lane 7, 5 U / μl Taq and 0.2 mM each of dNTPs in PCR buffer.

Terminal deoxynucleotidyl transferase activity and dAdT polymerization. Terminal deoxynucleotidyl transferase (TdT) is a group of DNA polymerases which unlike DNA or RNA-dependent polymerases requires neither DNA nor RNA templates but catalyzes linear condensation polymerization reactions (21). It needs a primer at least as large as trinucleotides. The TdT is found almost exclusively in the thymuses of mammalians and birds and in some lymphoid cells (11). The dAdT polymerization and TdT reaction resembled each other in that both reactions required no added template. Calf thymus TdT-mediated template-independent polymerization and Taq-mediated dAdT polymerization were compared. Fig. 3-A shows the reaction at 37°C which was optimal for the activity of TdT and the Fig. 3-B the reaction at 65°C which was optimal for the Taq-mediated dAdT polymerization. The positive TdT reaction was detected as smear bands when the 15 U TdT (Gibco BRL), 200 μM dNTPs, and 10 pmoles of oligonucleotide primers were incubated in TdT buffer (100 mM potassium cacodylate [pH 7.2], 2 mM $CoCl_2$ and 0.2 mM DTT) (lanes 2 and 3 in Fig. 3-A); addition of 15 mM $MgCl_2$ [Mg^{++} was absent in TdT buffer but required for dAdT polymerization by Taq polymerase (see above)] into the TdT buffer was rather inhibitory (lanes 4 and 5 in Fig. 3-A). When Taq polymerase was added instead of the primer to the TdT reaction mixture, TdT product was not produced (lane 1 in Fig. 3-A), indicating that the Taq polymerase preparation did not contain oligonucleotides which could be used as primers in TdT reaction. The dAdT polymerization was detected only in the presence of Taq polymerase and dNTPs in either TdT or PCR buffer (lane 6 and 7 in Fig. 3-B). The positive dAdT polymerization reaction in TdT buffer suggested that Co^{++} could replace Mg^{++} for the dAdT polymerization reaction to occur. TdT had no dAdT polymerizing activity (lanes 2-5 in Fig. 3B). These observations suggested that the dAdT polymerizing activity was distinct from the TdT activity.

DISCUSSION

Our experiments showed that Taq DNA polymerase catalyzed polymerization of dATP and dTTP without added template/primer. The optimal temperature for this reaction was 60-68°C for dATP and dTTP as substrates and 62-70°C for mixture of four dNTPs, about 5-10°C lower than the temperature optimal for PCR elongation reaction which was 72-80°C. The required Mg^{++} concentration was 1.5-30 mM for this reaction.

The product was high molecular weight DNA which consisted of alternating A and T, i.e., poly d(A-T). The stretches of repeated A or T were not detectably present in the nearest neighbor base analysis. Neither dGTP nor dCTP was incorporated into the polymer at a detectable level. The product was not digested with S1 nuclease, suggesting that the product was double-stranded DNA. This, however, does not exclude the possibility that the product was produced as single-stranded poly d(A-T), which folds back onto itself to form a long double-stranded DNA structure. It is also possible that at 65°C the single-stranded poly d(A-T) is denatured and renatured to form the hairpin structure (22), and the elongation of the polymer could proceed by replication and slippage (23) or by self-priming.

Unprimed template-independent DNA synthesis was originally reported for E. coli DNA polymerase I preparations in 1960's (1-5). However, description of this activity has disappeared from recent literatures probably because further purification of the enzyme or modification of the preparation methods gave variable results, such as decrease and disappearance of the activity (3,

5, 12, 13). We ourselves tried to repeat the previously reported polymerization reaction in the same reaction condition (1) using the recombinant E. coli DNA Polymerase I (Toyobo) without success. It absolutely required poly d(A-T) template/primer for polymerization of dATP and dTTP (data not shown).

The critical questions are (i) whether the enzyme catalyzing the dAdT polymerization was actually Taq polymerase itself or a contaminant, and (ii) whether the reaction required any primer/template or not.

As for the first question, probably Taq polymerase itself is catalyzing the polymerization for the following reasons. First, the enzyme preparations are derived from recombinant *E. coli* and highly purified. In addition, all of the Taq preparations from the four different manufacturers were positive for the reaction, i.e., the activity was recovered regardless of the possible different manufacture lines. Secondly, some thermostable DNA polymerases from the recombinant *E. coli,* such as Vent, Ultma, BcaBEST or KOD polymerases was negative for the dAdT polymerization reaction: if the reaction had been due to an *E. coli*-derived contaminant, the activity should be detected in all the samples. Thirdly, though Tth polymerase was positive for the dAdT polymerization, ΔTth polymerase derived from Tth polymerase by deleting the 5′-terminal 751 bp to remove 5′ → 3′ exonuclease activity was negative, i.e., the dAdT polymerization reaction could be genetically manipulated. This also suggests that the 5′-terminal 751 bp region of the Tth gene is required for the dAdT polymerization.

As for the second question, it is very likely that the dAdT polymerization did not require any primer/template, because (i) all the reactions were conducted with the highly purified reagents, and (ii) TdT reaction did not detect any primer activity in Taq polymerase preparation. Nazarenko et al. (11) suggested that dAdT polymerizing activity associated with E. coli polymerase I preparation was due to contamination of dNDP-transferase, an enzyme capable of catalyzing template- and primer-independent polymerization of dNDP (24, 25). But, in view of the purity of the enzyme and reagents and the high dAdT polymerization activity of the enzyme preparation, it is highly unlikely that the dAdT polymerization was carried out by a contaminant.

The dAdT polymerizing activity was detected in Taq and Tth polymerases, but not in ΔTth, Vent, Vent(exo-), Pfu, Ultma, BcaBEST, and KOD polymerases. Taq and Tth polymerase capable of dAdT polymerization were devoid of 3′ → 5′ exonuclease activity and were equipped with both 5′ → 3′ exonuclease activity and a TdT-like activity (26-28) which, unlike the ordinary TdT described above, adds one nucleotide (preferentially dATP) to the 3′ end of the double strand DNA (the suppliers' information) (26). The enzymes devoid of dAdT polymerizing activity, except artificially modified ΔTth and Vent(exo-) polymerases, had the 3′ → 5′ exonuclease activity, little or no 5′ → 3′ exonuclease activity, and no TdT-like activity except BcaBEST polymerase which had the TdT-like activity (the suppliers' information). Whether the dAdT polymerizing activity is functionally associated with these other activities remains to be elucidated.

In conclusion, Taq and Tth DNA polymerases were found to catalyze primer/template-independent polymerization of dATP and dTTP. Though the primer/template-independent polymerization was described more than 35 years ago, its presence has been doubted on account of the crudeness of the reaction preparation. Our present report is probably the first that showed its presence unequivocally.

ACKNOWLEDGMENTS

The work was supported in part by Grant-in-Aid from Ministry of Health. We thank Mari Oyane for assistance in preparing the manuscript.

REFERENCES

1. Schachman, H. K., Adler, J., Radding, C. M., Lehman, I. R., and Kornberg, A. (1960) *J. Biol. Chem.* **235,** 3242–3249.
2. Radding, C. M., and Kornberg, A. (1962) *J. Biol. Chem.* **235,** 2877–2882.
3. Radding, C. M., Josse, J., and Kornberg, A. (1962) *J. Biol. Chem.* **235,** 2869–2876.
4. Josse, J., Kaiser, A. D., and Kornberg, A. (1961) *J. Biol. Chem.* **236,** 864–875.
5. Burd, J. F., and Wells, R. D. (1970) *J. Mol. Biol.* **53,** 435–459.
6. Okazaki, T., and Kornberg, A. (1964) *J. Biol. Chem.* **239,** 252–268.
7. Zimmerman, B. K. (1966) *J. Biol. Chem.* **241,** 2035–2041.
8. Harwood, S. J., Schendel, P. F., and Wells, R. D. (1970) *J. Biol. Chem.* **245,** 5614–5624.
9. Henner, D., and Furth, J. J. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3944–3946.
10. Henner, D., and Furth, J. J. (1977) *J. Biol. Chem.* **252,** 1932–1938.
11. Kornberg, A. (1992) *DNA Replication,* 2nd ed., pp. 223–225, Freeman, San Francisco.
12. Richardson, C. C., Schildkraut, C. L., Aposhian, H. V., and Kornberg, A. (1964) *J. Biol. Chem.* **239,** 222–232.
13. Lezius, A. G., Hennig, S. B., Menzel, C., and Metz, E. (1967) *Eur. J. Biochem.* **2,** 90–97.
14. Chien, A., Edgar, D. B., and Trela, J. M. (1976) *J. Bacteriol.* **127,** 1550–1557.
15. Uemori, T., Ishino, Y., Fujita, K., Asada, K., and Kato, I. (1993) *J. Biochem.* **113,** 401–410.
16. Carballcira, N., Nazabal, M., Brito, J., and Garcia, O. (1990) *BioTechniques* **9,** 276–281.
17. Morikawa, M., Izawa, Y., Rashid, N., Hoaki, T., and Imanaka, T. (1994) *Apple. Environ. Microbiol.* **60,** 4559–4566.
18. Lundberg, K. S., Schemaker, D. D., Adams, M. M. W., Short, J. M., Sorge, J. A., and Mathur, E. J. (1991) *Gene* **108,** 1–6.
19. Swartz, M. N., Trautner, T. A., and Kornberg, A. (1962) *J. Biol. Chem.* **237,** 1961–1967.
20. Arakawa, T., Jongsareejit, B., Tatsumi, Y., Tanaka, K., Ikeda,

K., Komatsubara, H., Inoue, H., Kawakami, B., Oka, M., Emi, S., Yomo, T., Shima, Y., Negoro, S., and Urabe, I. (1996) *DNA Research* **3,** 87–92.

21. Bollum, F. J. (1974) *in* The Enzymes (Boyer, P. D., Ed.), Vol. 10, pp. 145–171, Academic Press, New York.
22. Vilpo, J. A., and Ridell, J. (1983) *Nucl. Acids Res.* **11,** 3753–3765.
23. Kornberg, A., Bertsch, L. L., Jackson, J. F., and Khorana, H. G. (1964) *Biochemistry* **51,** 315–323.
24. Nazarenko, I. A., Potapov, V. A., Romashchenko, A. G., and Salganik, R. I. (1978) *FEBS Lett.* **86,** 201–204.
25. Nazarenko, I. A., Bobko, L., Romashchenko, A. G., Khripin, Yu. L., and Salganik, R. I. (1979) *Nucl. Acids Res.* **6,** 2545–2560.
26. Clark, J. M., Joyce, C. M., and Beardsley, G. P. (1987) *J. Mol. Biol.* **198,** 123–127.
27. Niedenthal, R. K., and Hegemann, J. H. (1993) *Nucl. Acids Res.* **21,** 4413.
28. Nakayama, H., and Nishikata, T. (1996) Bio-Jikken Illustrated, Vol. 2, Shunjusha, Tokyo. [In Japanese]