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Purification and Characterization of DNA Polymerases from *Bacillus* Species

ECKHARD SELLMANN, KARL-LUDWIG SCHRÖDER, ILSE-MARIA KNOBLICH, AND PETER WESTERMANN†*

Department of Biochemistry, Central Institute of Molecular Biology, O-1115 Berlin-Buch, Germany

Received 4 October 1991/Accepted 1 May 1992

DNA polymerases from *Bacillus stearothermophilus*, *Bacillus caldotenax*, and *Bacillus caldovelox* were purified by chromatography on DEAE-cellulose, phosphocellulose, and heparin-Sepharose and obtained in high yield. The enzyme preparations are free of exo- and endonuclease activities. Additional purification steps, e.g., hydrophobic interaction chromatography and chromatography on a Mono Q column or sucrose density gradient centrifugation, are needed to obtain the enzymes in the form of homogeneous 95-kDa proteins. Each of the three organisms possesses a major DNA polymerase activity comparable to DNA polymerase I. The enzymes require Mg^{2+} (10 to 30 mM) for optimal activity, although 0.4 mM Mn^{2+} could substitute for magnesium. The optimal reaction temperatures were lowest in *B. stearothermophilus* (60 to 65°C) and about equal in *B. caldovelox* and *B. caldotenax* (65 to 70°C). The thermal stabilities of the enzymes increased in the same order. The DNA polymerase from *Thermus thermophilus* was isolated for comparison by using a similar procedure. The enzyme was obtained as a homogeneous 85-kDa protein that was also free of exo- and endonucleolytic activities.

DNA-directed DNA polymerases (DNA deoxynucleotidyltransferase; EC 2.7.7.7) from most thermophilic microorganisms lack the 3′-5′ and 5′-3′ exonuclease activities (3, 4, 8–10, 17) that are intrinsic enzymatic activities characteristic of DNA polymerase I from *Escherichia coli* (16) and *Micrococcus radiodurans* (11). However, contradictory results have been reported concerning the DNA polymerases from *Bacillus* species: enzyme preparations from *Bacillus licheniformis* and *Bacillus stearothermophilus* were obtained apparently free of exonuclease activities (18), whereas minor 5′-3′ and 3′-5′ exonuclease activities have been attributed to *B. stearothermophilus* DNA polymerase (7). In both reports heterogeneous preparations were analyzed, and therefore the effect of contaminating activities could not be excluded.

In this paper we describe an improved procedure for the isolation of DNA polymerases from *B. stearothermophilus*, *Bacillus caldotenax*, *Bacillus caldovelox*, and *Thermus thermophilus*. This method includes chromatography on DEAE-cellulose, phosphocellulose, and heparin-Sepharose and results in DNA polymerase preparations that are essentially free of exo- and endonuclease activities. With the exception of the enzyme obtained from *T. thermophilus*, additional purification steps were required to obtain the DNA polymerases in a homogeneous form.

Because of the apparent absence of exonuclease activities and their thermostability, the DNA polymerases are suitable for DNA sequencing at elevated temperatures.

MATERIALS AND METHODS

Chemicals. Deoxyribonucleoside triphosphates (dNTPs) were purchased from Boehringer Mannheim Corp., [8-^{3}H]dATP (300 MBq/mmol) was from Techsuabexport, DE-52 cellulose and P11 cellulose were from Whatman Inc., and heparin-Sepharose was from Pharmacia (Uppsala, Sweden).

Microorganisms and growth conditions. *B. stearothermophilus* (IMET 10306, = DSM 494) and *B. caldovelox* YT-F (IMET 11433, = DSM 411) were grown by repeated batch fermentation until late log phase. Three liters of nutrient broth (NBI broth; SIFIN, Berlin, Germany) was inoculated with 200 ml of inoculum; the mixture was fermented for 2 h at 60°C. At this time, the pO_2 was ~25% and the cell concentration of *B. stearothermophilus* reached 3×10^8/ml. For *B. caldovelox*, the pO_2 was ~5% and the cell concentration reached 5×10^9/ml. By centrifugation for 0.5 h at 10°C and 40,000 × *g*, 32 and 28 g (wet weight) of cell paste were harvested, respectively.

B. caldotenax YT-G (IMET 11321, = DSM 406) was propagated for 24 h at 70°C, and 2 liters was thereafter inoculated in 20 liters of NBI broth and grown to late log phase (6 h at 70°C; 5 to 3 mg of O_2 per liter; pH <8.0; cell concentration, 3.3×10^8/ml). A total of 10.2 g of cells (wet weight) was recovered by centrifugation for 0.5 h at 10°C and 40,000 × *g*.

T. thermophilus (IMET 11057, = ATCC 27634) was cultured for 24 h at 70°C in 10 batches of 200 ml of proteose peptone broth (15). These cultures were used to inoculate a 20-liter fermentation batch (69°C; 5 to 3 mg of O_2 per liter; pH <8.0). Cell material was harvested after 9 h by centrifugation for 0.5 h at 10°C and 40,000 × *g*, at which time the culture had reached the early stationary phase, with a cell concentration of 5×10^9/ml. The cell material (140 g [wet weight]) was washed with a buffer containing 50 mM NaCl and 50 mM Tris-HCl, pH 8.0, and stored at −20°C.

Enzyme assays. (i) DNA polymerase activity. DNA polymerase activity was estimated by the method described in reference 8, with the following modifications. The 50-μl mixture contained 25 mM Tris-HCl, pH 8.8; 100 mM KCl; 10 mM $MgCl_2$; 1 mM dithioerythrol (DTE); 0.2 mM (each) dATP, dGTP, dCTP, and dTTP; 150 kBq of [^{3}H]dATP; 12 μg of calf thymus DNA (activated by DNase I treatment by the method described in reference 3); and 0.1 to 1.0 unit of

* Corresponding author.
† Present address: Max-Delbrück-Centre of Molecular Medicine, Robert-Roessle-Strasse 10, O-1115 Berlin-Buch, Germany.

enzyme. After 30 min of incubation at 60°C, 10-μl aliquots were spotted onto GF/C filters. The filters were dried, washed sequentially with 5% trichloroacetic acid containing 0.2% sodium pyrophosphate, 5% trichloroacetic acid alone, and finally ethanol. The filters were dried prior to counting in a toluene-based scintillation mixture. One enzyme unit is defined as the amount which catalyzes the incorporation of 10 nmol of deoxynucleotide monophosphate in 30 min to an acid-precipitable form.

For determining the K_ms, the dNTP concentrations were varied between 0 and 300 μM.

(ii) 3′-5′ Exonuclease activity. 3′-5′ Exonuclease activity was assayed by incubation of enzyme fractions with calf thymus DNA that had been 3′ end labelled with terminal deoxynucleotidyl transferase by the method described in reference 1.

(iii) 5′-3′ Exonuclease activity. 5′-3′ Exonuclease activity was measured by the liberation of ^{32}P-phosphate from 5′-labeled oligonucleotides as described in reference 1.

(iv) Nonspecific endonuclease activity. Nonspecific endonuclease activity was assayed by incubation of 0.5 μg of supercoiled pHMT-22 DNA in 10 μl of reaction buffer (33 mM Tris acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTE, 1 μg of bovine serum albumin) with 1 to 5 μl of enzyme solution for 2 h at 60°C. The reaction was stopped by the addition of 10 μl of 10 mM EDTA, 0.05% bromophenol blue, and 50% glycerol and analyzed by electrophoresis through a 1% agarose gel.

Purification of DNA polymerases free of exo- and endonucleolytic activities. All DNA polymerase purification procedures were carried out at 4°C, and the enzyme fractions were stored at 4°C. Protein concentrations were determined as described in reference 14.

Step I. Preparation of crude extract. Ten grams of frozen cells was thawed, suspended in 60 ml of 50 mM Tris-HCl, pH 7.5–1 mM glutathione–0.3 mM phenylmethylsulfonyl fluoride, and homogenized in a glass-Teflon homogenizer at low speed. The suspension was divided into three 20-ml aliquots, sonicated three times for 30 s each time (Branson sonifier, microtip, 70 W), and centrifuged for 40 min at 4°C and 100,000 × *g*. Potassium phosphate, pH 6.5 (1 M), was added to the supernatant to a final concentration of 0.2 M.

Step II. DEAE-cellulose chromatography I. A 110-ml DE-52 cellulose column was equilibrated with 500 ml of buffer A (0.2 M potassium phosphate, pH 6.5, 10 mM β-mercaptoethanol). The crude extract was pumped onto the column (70 ml/h). Unbound proteins were eluted with buffer A and collected in 11-ml fractions until the A_{280}/A_{260} ratio dropped below 1.0. The pooled protein fractions were diluted with 4 volumes of 10 mM β-mercaptoethanol.

Step III. DEAE-cellulose chromatography II. A 30-ml DE-52 cellulose column equilibrated with 150 ml of buffer B (20 mM potassium phosphate, pH 6.5, 10 mM β-mercaptoethanol) was loaded with the diluted protein fraction, washed with 15 ml of buffer B, and eluted with a linear gradient of potassium phosphate (0 to 200 mM) in buffer B. Five-milliliter fractions were collected and assayed for polymerase activity as described above. Enzyme fractions were pooled and diluted with 4 volumes of 10 mM β-mercaptoethanol.

Step IV. Phosphocellulose chromatography. An 8-ml P11 cellulose column was equilibrated with 50 ml of buffer B, and the diluted enzyme fraction was loaded onto the column at a rate of 12 ml/h. The column was then washed with buffer B, and the enzyme was eluted by using a gradient of 20 to 300 mM potassium phosphate in buffer B. Five-milliliter fractions were collected and assayed for polymerase activity. The pooled fractions were diluted with 4 volumes of 0.1 mM EDTA, pH 8.0–2 mM DTE–10% glycerol.

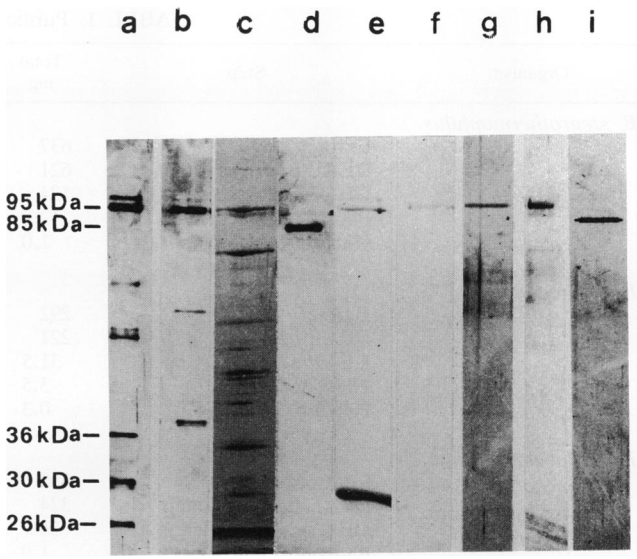

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of DNA polymerase preparations from *B. stearothermophilus* (lanes a, e, and f), *B. caldotenax* (lanes b and g), *B. caldovelox* (lanes c and h), and *T. thermophilus* (lanes d and i). The enzymes were analyzed after heparin-Sepharose chromatography (lanes a to d), sucrose gradient sedimentation (lanes g and i), and Phenyl-Sepharose (lane e) and Mono Q chromatography (lanes f and h). Molecular weights were correlated to those of phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), and chymotrypsinogen (25,000) (data not shown).

Step V. Heparin-Sepharose chromatography. Two milliliters of heparin-Sepharose was equilibrated with buffer C (20 mM potassium phosphate, pH 7.5, 20 mM KCl, 0.1 mM EDTA, 2 mM DTE, 10% glycerol). The diluted enzyme fraction was bound to the column at a rate of 10 ml/h, washed with buffer C, and eluted with a 50-ml linear gradient of 20 to 600 mM KCl in buffer C.

Isolation of DNA polymerases as homogeneous proteins. The enzyme preparation from *T. thermophilus* contained a single 85-kDa component, as shown by polyacrylamide gel electrophoresis (Fig. 1, lane d). The preparation from *B. stearothermophilus* (Fig. 1, lane a), *B. caldotenax* (Fig. 1, lane b), and *B. caldovelox* (Fig. 1, lane c) required further purification (see below).

Sucrose gradient centrifugation. *B. caldotenax* DNA polymerase (0.1-ml portions) was loaded onto 4-ml linear gradients (5 to 20% sucrose in buffer C containing 0.5 M NH_4Cl and 0.05% Triton X-100). Centrifugation was for 24 h at 400,000 × *g* and 4°C in an SW60 Ti rotor (Beckman Inc.). Fractions (0.2 ml) were assayed for enzyme activity as described above.

Hydrophobic interaction chromatography. DNA polymerase preparations from *B. stearothermophilus* and *B. caldovelox* (Fig. 1, lanes a and c) had to be further purified by hydrophobic interaction chromatography, followed by chromatography on a Mono Q column. Step V fractions were dialyzed against 1.5 M ammonium sulfate in 100 mM potassium phosphate (pH 7.5)–2 mM dithiothreitol–0.1 mM EDTA–10% glycerol (buffer D) and loaded onto a Phenyl Superose HR 5/5 column equilibrated with the same buffer.

TABLE 1. Purification of DNA polymerases

Organism	Step	Total mg	Total kU	Sp act (U/mg)	% Recovery	Purification (fold)	Exo- and endonuclease
B. stearothermophilus							
	Extract	637	92	144	100		+, +
	DEAE-cellulose (step II)	621	116	186	126	1.3	+, +
	DEAE-cellulose (step III)	124	73	588	79	4	+, +
	Phosphocellulose	12	54	4,500	59	30	+, −
	Heparin-Sepharose	1.0	80	80,000	87[a]	550	−, −
B. caldotenax							
	Extract	292	130	445	100		+, +
	DEAE-cellulose (step II)	221	130	588	100	1.3	+, +
	DEAE-cellulose (step III)	31.5	68	2,158	52	5	+, +
	Phosphocellulose	3.5	45	12,800	35	28	+, −
	Heparin-Sepharose	0.3	30	100,000	23	225	−, −
B. caldovelox							
	Extract	350	155	443	100		+, +
	DEAE-cellulose (step II)	324	140	432	90		+, +
	DEAE-cellulose (step III)	26	101	3,880	65	9	+, +
	Phosphocellulose	1.9	35	18,200	22	40	+, −
	Heparin-Sepharose	0.4	25	62,500	16	140	−, −
T. thermophilus							
	Extract	254	18.5	73	100		+, +
	DEAE-cellulose (step II)	244	12.7	52	69		+, +
	DEAE-cellulose (step III)	18.8	12	638	65	9	+, +
	Phosphocellulose	0.75	6	8,000	32	73	−, −
	Heparin-Sepharose	0.05	3	60,000	16	820	−, −

[a] After removal of contaminating nucleolytic activity, the activity of the enzyme increases.

The enzymes were eluted by using a decreasing gradient from 1.5 to 0 M ammonium sulfate in buffer D.

Chromatography on Mono Q. Enzyme fractions obtained by hydrophobic interaction chromatography were diluted fivefold with 20 mM Tris-HCl (pH 7.5)–20 mM KCl–2 mM dithiothreitol–0.1 mM EDTA–10% glycerol (buffer E) and loaded onto a Mono Q HR 5/5 column equilibrated with buffer E. DNA polymerases were eluted by using a gradient of 0 to 500 mM potassium chloride in buffer E.

Electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out by the method described in reference 12. The gels were silver stained by the method described in reference 21.

RESULTS

Purification of DNA polymerases from *B. stearothermophilus*, *B. caldotenax*, *B. caldovelox*, and *T. thermophilus*. The DNA polymerases were purified according to the general scheme given in detail in Materials and Methods. Cell extracts were centrifuged at 100,000 × *g*, after which nucleic acids were removed by binding to DE-52 cellulose in the presence of 0.2 M potassium phosphate. The proteins were eluted and separated by three successive chromatographic steps through DE-52 cellulose, P-11 cellulose, and heparin-Sepharose. The application of this separation scheme for the isolation of the four enzymes had several advantages. First, the scheme included four chromatographic steps that could be run sequentially, avoiding intermittent concentration steps. Consequently, the total procedure could be completed within 5 days. Second, the DE-52 cellulose completely removed nucleic acids, making all subsequent steps highly reproducible. Third, the enzymes were purified free of both exo- and endonucleolytic activities and were recovered in high concentrations.

DNA polymerases from *Bacillus* species were indistinguishable by chromatographic steps applied so far: they were eluted from DEAE-cellulose in the range between 120 and 150 mM potassium phosphate, from phosphocellulose between 120 and 180 mM potassium phosphate, and from heparin-Sepharose between 280 and 360 mM potassium chloride. DNA polymerase from *T. thermophilus* exhibited different properties and eluted between 80 and 120 mM potassium phosphate (DEAE-cellulose), between 180 and 240 mM potassium phosphate (phosphocellulose), and between 380 and 420 mM potassium chloride (heparin-Sepharose).

The DNA polymerases from *Bacillus* species were obtained in concentrations between 10 and 20 units/μl (Table 1). The pooled polymerase fractions from *T. thermophilus* had to be concentrated by being bound to a 0.2-ml heparin-Sepharose column and eluted with a small volume of 500 mM KCl in buffer C, resulting in a final concentration of 10 to 20 units/μl (Table 1).

The DNA polymerases were purified by the general preparation scheme (steps I to V) from 225- to 820-fold (Table 1), and specific activities of 100,000, 80,000, 62,500, and 60,000 enzyme units per mg of protein were obtained for *B. caldotenax*, *B. stearothermophilus*, *B. caldovelox*, and *T. thermophilus*, respectively. Neither endo- nor exonuclease activities were found to be associated with the purified enzymes. Contaminating endo- and exonucleases were separated by phosphocellulose and heparin-Sepharose chromatography, respectively. The pure enzymes were stored for several months at 4°C without reduction in their activity.

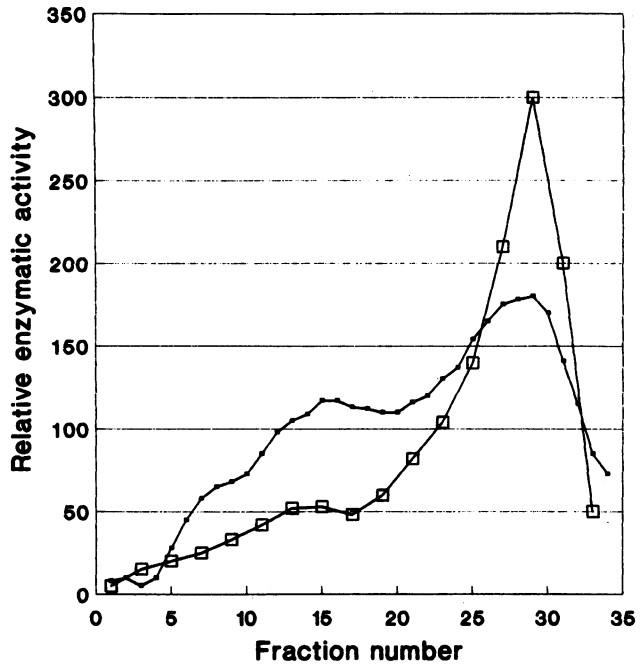

FIG. 2. DNA polymerase preparations from *B. caldotenax* eluted from heparin-Sepharose were centrifuged through a 5 to 20% sucrose gradient in buffer C containing 0.5 M NH_4Cl and 0.05% Triton X-100. ●, A_{280} nm; □, DNA polymerase activity.

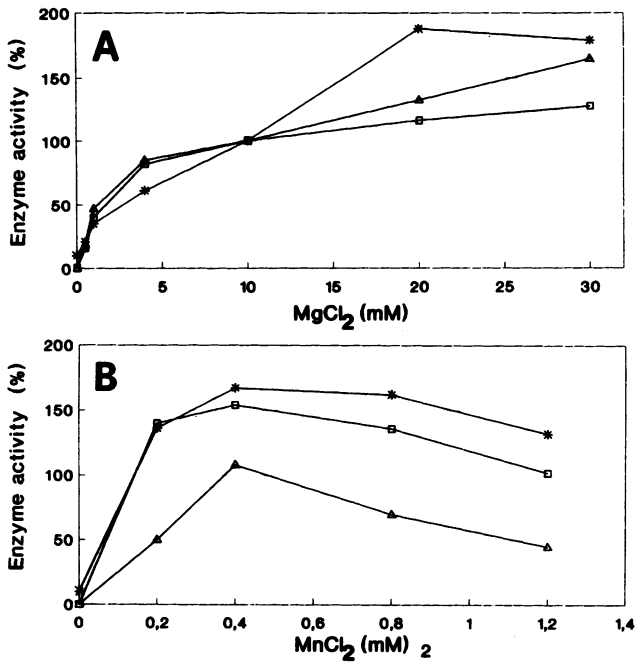

FIG. 3. Effects of the divalent cations Mg^{2+} (A) and Mn^{2+} (B) on DNA polymerase activities. □, *B. stearothermophilus*; △, *B. caldotenax*; *, *T. thermophilus*. Assays were performed as described in Materials and Methods. The values obtained at 10 mM Mg^{2+} were set at 100%.

Isolation of DNA polymerases as homogeneous proteins. The DNA polymerase from *T. thermophilus* was isolated as a single polypeptide of 85 kDa following heparin-Sepharose chromatography (Fig. 1, lane d). The enzymes from *B. stearothermophilus*, *B. caldotenax*, and *B. caldovelox* (Fig. 1, lanes a to c) were heterogeneous, as shown by polyacrylamide gel electrophoresis. The DNA polymerase from *B. caldotenax* could be purified by sucrose gradient centrifugation (Fig. 2), resulting in a homogeneous protein of 95 kDa (Fig. 1, lane g). DNA polymerases from *B. stearothermophilus* and *B. caldovelox* were further purified by hydrophobic interaction chromatography through Phenyl Superose (Fig. 1, lane e), followed by ion-exchange chromatography through Mono Q (Fig. 1, lanes f and h). Both enzymes are 95-kDa proteins.

Influence of mono- and divalent ions on enzyme activities. The DNA polymerases tested were absolutely dependent on the presence of all four dNTPs and a divalent cation. Both magnesium and manganese supported enzyme activity. The highest activities were attributed to *T. thermophilus* DNA polymerase, reaching plateaus from 20 to 30 mM Mg^{2+} and from 0.4 to 0.8 mM Mn^{2+} (Fig. 3). The activity of *Bacillus* DNA polymerases steadily increased from 5 to 30 mM Mg^{2+}, whereas the highest stimulation with Mn^{2+} was observed at 0.4 mM. The extent of stimulation by Mn^{2+} was different between the *Bacillus* enzymes: the highest activity—150%, compared with the standard assay containing 10 mM Mg^{2+}—was measured for *B. stearothermophilus* DNA polymerase. By contrast, DNA polymerases from *B. caldotenax* (Fig. 3B) and *B. caldovelox* (data not shown) reached only the level of the standard assay.

The use of potassium as a monovalent cation resulted in relatively broad plateaus: between 5 and 100 mM for the *T. thermophilus* enzyme and 100 to 200 mM for the enzymes of the *Bacillus* species (Fig. 4B).

K_ms for dNTPs. The apparent K_ms for the dNTPs were determined by duplicate experiments from two enzyme preparations. The enzymes from *Bacillus* species showed a K_m of 85 ± 10 μM, and for *T. thermophilus* a K_m of 115 ± 12 μM was measured.

pH optima. The pH optima were determined in Tris buffer under conditions of the standard assay. The buffer pH was measured at 20°C, and no corrections are made due to lowering of pH during heating of the Tris buffer to 60°C. The values of maximal activity were estimated for all enzymes at pH 8.8 (Fig. 4A).

Optimal reaction temperatures and thermostabilities. The influence of temperature on incorporation of deoxynucleotide monophosphates into DNA was tested under standard assay conditions (see Materials and Methods). The optimal temperature for *B. stearothermophilus* DNA polymerase was 60 to 65°C, whereas the enzymes from *B. caldotenax* and *B. caldovelox* showed the highest incorporation rate at 65 to 70°C and that of *T. thermophilus* had an optimal reaction temperature between 70 and 75°C (Table 2).

To test the thermostability of each enzyme, they were preincubated for 10 min at the appropriate temperature in a standard reaction mix omitting dNTPs and DNA. The reactions were then started by addition of the missing components, and the extent of incorporation was measured as described in Materials and Methods. The enzyme activities were not affected if the preincubation temperatures did not exceed the optimal temperature by more than 10°C. The activities were reduced significantly at higher temperatures, and the enzymes were inactivated at 80°C (*B. stearothermophilus*), 85°C (*B. caldotenax* and *B. caldovelox*), and 95°C (*T. thermophilus*).

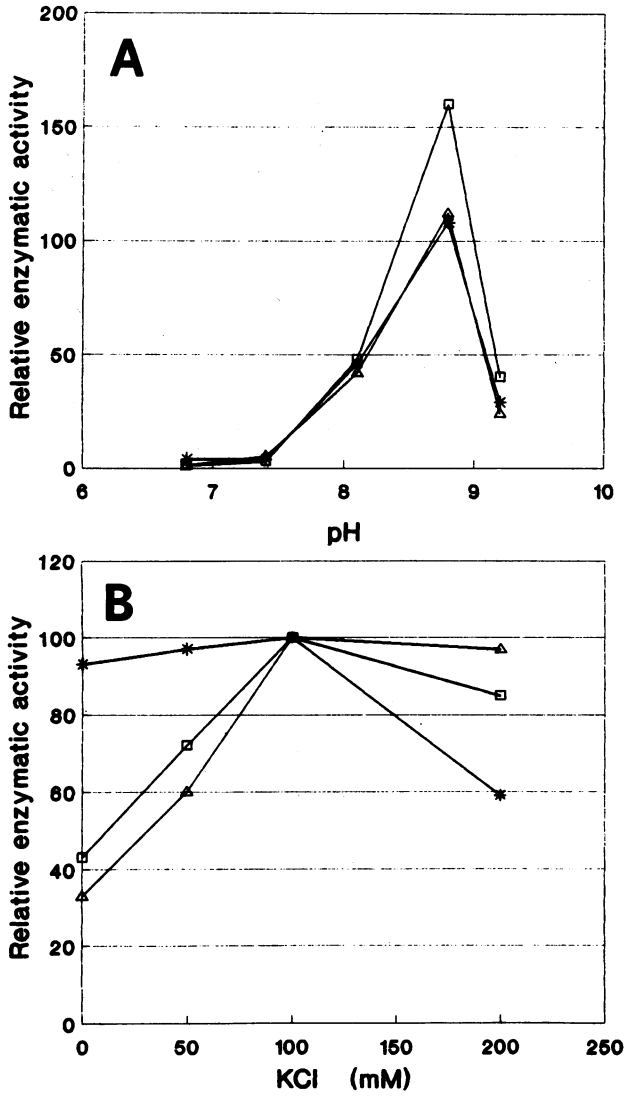

FIG. 4. Dependence of DNA polymerase activities on buffer pH (A) and K^+ concentration (B). The values obtained with 100 mM KCl were set at 100% in panel B. □, *B. stearothermophilus*; △, *B. caldotenax*; *, *T. thermophilus*.

Inhibition by ddNTPs. The DNA polymerases were inhibited by a single dideoxyribonucleoside triphosphate (ddNTP) in the presence of the four dNTPs. By using ratios of ddNTP to dNTP of 1:1 and 5:1, the lowest inhibition was observed with DNA polymerase from *B. caldotenax* of 38 ± 5.1% and 52 ± 9.2%. The enzymes from *B. stearothermophilus* (49 ± 2.8% and 59 ± 4.7%) and *T. thermophilus* (51 ± 2.1% and 61 ± 4.7%) were about equally sensitive.

Each of the latter enzymes was used for two-step DNA sequencing by the method of Innis et al. (6). For this purpose, ratios between dNTPs and ddNTPs of 1:7 (G), 1:40 (A), 1:40 (T), and 1:7 (C) were utilized (data not shown).

DISCUSSION

Molecular masses. DNA polymerases from *Bacillus* species have previously not been purified to homogeneity. Therefore, molecular masses of the enzymes were not known with certainty (7). By using the improved separation scheme described here, DNA polymerases from *B. stearothermophilus*, *B. caldovelox*, and *B. caldotenax* have been purified to homogeneity, with an apparent molecular mass of 95 kDa, according to denaturing polyacrylamide gel electrophoresis.

T. thermophilus DNA polymerase showed an apparent molecular mass of 85 kDa. This value differed from those described previously (17) by using sedimentation analysis (110 and 150 kDa) and polyacrylamide gel electrophoresis (77, 40, and 17 kDa). In contrast to the procedure described in reference 17, we obtained only one major enzymatic activity during all chromatographic steps.

Thermostabilities. Upon comparing DNA polymerases from *Bacillus* and *Thermus* species with DNA polymerase I of *E. coli*, it was evident that the thermostability increased with decreasing molecular mass. In comparison to *E. coli* DNA polymerase I, the DNA polymerase from *T. aquaticus* lacks parts of the domain that is essentially homologous to the exonuclease domain of DNA polymerase I (13). Whether these deletions, which inactivate the exonuclease activity, stabilize the enzyme against heat denaturation is unknown. Interestingly, thermostable DNA polymerases described here and those from *T. aquaticus* (8), *Thermus flavus* (9), *Thermus ruber* (10), and *Sulfolobus acidocaldarius* (4) are all free of exo- and endonucleolytic activities, whereas DNA polymerases from *Thermoplasma acidophilum* (which is stable up to 65°C [5]) and DNA polymerase from *Thermococcus literalis* (stable up to 95°C, according to New England Biolabs product information) both exhibit 3′-5′ exonuclease activity.

However, in addition to the deletions that influence the molecular mass and exonuclease activities, more-subtle sequence modifications might also influence thermostability. Despite the similarities of identical molecular mass and lack of exonuclease activities, *Bacillus* DNA polymerases differ in their optimal reaction temperatures between 65°C and 70°C and are inactivated at temperatures between 70°C and 75°C (Table 2).

Use of DNA polymerases in DNA sequencing. The purified DNA polymerases from *Bacillus* species were tested in DNA

TABLE 2. Effect of temperature on the activities of DNA polymerases

Organism	DNA polymerase activity[a] (%) at a reaction temp of:						
	37°C	50°C	60°C	65°C	70°C	75°C	80°C
B. stearothermophilus	15	50	95	100	35	0	0
B. caldotenax	10	50	85	100	100	0	0
B. caldovelox	ND	35	80	100	100	5	0
T. thermophilus	15	45	70	80	100	95	55

[a] Assays were performed as described in Material and Methods, and incorporation of deoxyribonucleoside triphosphate is given for the individual enzyme as a percentage of the maximal activity. ND, not determined.

sequencing reactions. By use of a system modified from Innis et al. (6) and a reaction temperature of 60°C, clear sequencing patterns were obtained, even in the case of C-rich templates (data not shown).

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REFERENCES

1. **Bernad, A., L. Bianco, J. M. Lazaron, and G. Martin.** 1989. A conserved 3′-5′ exonuclease active site in prokaryotic and eukaryotic DNA polymerases. Cell **59:**219–228.
2. **Chaudhuri, T. R., and T. J. Green.** 1987. A sensitive urea-silver stain method for detecting trace quantities of separated proteins in polyacrylamide gels. Prep. Biochem. **7:**93–99.
3. **Chien, A., D. B. Elgar, and J. M. Trela.** 1976. Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus aquaticus*. J. Bacteriol. **127:**1550–1557.
4. **Elie, C., A. M. de Recondo, and P. Forterre.** 1989. Thermostable DNA polymerase from the archaebacterium *Sulfolobus acidocaldarius*. Purification, characterization and immunological properties. Eur. J. Biochem. **178:**619–626.
5. **Hamal, A., P. Forterre, and C. Elie.** 1990. Purification and characterization of a DNA polymerase from the archaebacterium *Thermoplasma acidophilum*. Eur. J. Biochem. **190:**517–521.
6. **Innis, M. A., K. B. Myambo, D. H. Gelfand, and M. A. D. Brow.** 1988. DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. Proc. Natl. Acad. Sci. USA **85:**9436–9440.
7. **Kaboev, O. K., L. A. Luchkina, A. T. Akhmedov, and M. L. Bekker.** 1981. Purification and properties of deoxyribonucleic acid polymerase from *Bacillus stearothermophilus*. J. Bacteriol. **145:**21–26.
8. **Kaledin, A. S., A. G. Slyusarenko, and S. J. Gorodetskij.** 1980. Isolation and properties of DNA polymerase from the extremal thermophilic bacteria *Thermus aquaticus*. Biokhimiya **45:**644–651.
9. **Kaledin, A. S., A. G. Slyusarenko, and S. J. Gorodetskij.** 1981. Isolation and properties of DNA polymerase from the extremal thermophilic bacteria *Thermus flavus*. Biokhimiya **46:**1576–1584.
10. **Kaledin, A. S., A. G. Slyusarenko, and S. J. Gorodetskij.** 1982. Isolation and properties of DNA polymerase from the extremal thermophilic bacteria *Thermus ruber*. Biokhimiya **47:**1783–1791.
11. **Kityama, S., Y. Ishizaka, and S. Miyai.** 1978. An exonuclease activity associated with DNA polymerase I of *Micrococcus radiodurans*. Biochim. Biophys. Acta **520:**122–130.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
13. **Lawyer, F. C., S. Stoffel, R. K. Saiki, K. Myambo, R. Drummond, and D. H. Gelfand.** 1989. Isolation, characterization, and expression in *Escherichia coli* of the DNA polymerase gene from *Thermus aquaticus*. J. Biol. Chem. **264:**6427–6437.
14. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
15. **Oshima, T., and K. Imahori.** 1974. Description of *Thermus thermophilus* (Yoshida and Oshima) comb. nov., a nonsporulating thermophilic bacterium from a Japanese thermal spa. Int. J. Syst. Bacteriol. **24:**102–112.
16. **Richardson, C. C., C. L. Schildkraut, H. V. Aposhian, and A. Kornberg.** 1964. Enzymatic synthesis of deoxyribonucleic acid. XIV. Further purification and properties of deoxyribonucleic acid polymerase of Escherichia coli. J. Biol. Chem. **239:**222–232.
17. **Rüttiman, C., M. Cotoras, J. Zaldivar, and R. Vicuna.** 1985. DNA polymerases from the extremely thermophilic bacterium *Thermus thermophilus* HB-8. Eur. J. Biochem. **149:**41–46.
18. **Stenesh, J., and B. A. Roe.** 1972. DNA polymerase from mesophilic and thermophilic bacteria. I. Purification and properties of DNA polymerase from *Bacillus licheniformis* and *Bacillus stearothermophilus*. Biochim. Biophys. Acta **272:**156–166.