J. Biochem. 119, 186-192 (1996)

DNA Polymerase βs from Liver and Testes of Cherry Salmon, *Oncorhynchus masou*: Purification and Characterization of DNA Polymerase βs with Acidic Isoelectric Points

Toyofumi Yamaguchi,*,[1] Shigeko Nishimura,† Keiko Takahashi,* Michiyasu Yoshikuni,‡ Junya Masaki,* Toshiaki Hirai,* and Mineo Saneyoshi*

*Department of Biological Sciences, The Nishi-Tokyo University, Uenohara, Yamanashi 409-01; †Shizuoka Higashi Hospital, National Epilepsy Center, 886 Urushiyama, Shizuoka 420; and ‡Department of Developmental Biology, National Institute for Basic Biology, Okazaki, Aichi 444

Received for publication, September 26, 1995

DNA polymerase βs from cherry salmon, *Oncorhynchus masou*, liver and testes were purified to near homogeneity, and no substantial differences between the enzymes were observed. The molecular weight of both enzymes, determined by SDS-polyacrylamide gel electrophoresis, was 39,000. The amino acid sequences of the N-terminus of the liver and testes enzymes were determined and compared with that of the rat enzyme. Of the N-terminal 30 amino acid residues of salmon liver DNA polymerase β, 21 (70%) were identical to those of the rat enzyme sequence. However, unlike most eukaryotic DNA polymerase βs, the isoelectric points (pIs) of the DNA polymerase βs from salmon liver and testes were both estimated to be 6.2, which is significantly different from the alkaline isoelectric points (pI=8.5-9.5) established for other highly purified vertebrate DNA polymerase βs. The cherry salmon DNA polymerase βs were still active at below 10°C, compared with the rat enzyme.

Key words: cherry salmon, DNA polymerase β, isoelectric point, N-terminal amino acid sequence, vertebrates.

Higher eukaryotic cells contain five species of DNA polymerases, α, β, γ, δ, and ε (*1*). Of these, DNA polymerase β is thought to play a role in DNA repair synthesis (*2*). This enzyme is found in a wide range of animals (*3*), and has been purified from various eukaryotic cells. The genes of both rat and human DNA polymerase βs have been cloned, and an active recombinant enzyme has been overproduced in *Escherichia coli* (*4-6*). Recently, the crystal structure of rat DNA polymerase β was determined (*7, 8*). However, only a few papers on the properties of DNA polymerase βs from lower vertebrates, particularly from the fish kingdom, have been published (*9, 10*). The purification and characterization of DNA polymerase βs from cherry salmon, *Oncorhynchus masou*, has been undertaken from a comparative biochemical point of view.

In a previous study, we demonstrated the existence of extensive common amino acid sequences in the primary structures of DNA polymerase β molecules from mouse myeloma cells, chick embryos and cherry salmon testes using the tryptic peptide mapping method (*10*).

In this paper, we describe procedures for the purification of DNA polymerase βs from cherry salmon liver and testes, and some of their enzymatic properties and characteristics. The results showed that these DNA polymerase βs from cherry salmon tissue were quite unique, as their isoelectric points (pIs) were lower than those of other highly purified eukaryotic DNA polymerase βs.

[1] To whom correspondence should be addressed.

MATERIALS AND METHODS

Tissues—Mature cherry salmon (2-years-old) were killed, and their livers were removed, frozen promptly with solid carbon dioxide and kept at −80°C until use. Testes were obtained from immature male salmon (2-years-old) and handled identically.

Enzyme—Rat recombinant DNA polymerase β (JMpβ5) (*5*) was kindly provided by Dr. Akio Matsukage, Aichi Cancer Center Research Institute.

Assaying of DNA Polymerase β Activity—The reaction mixture (25 μl) comprised 50 mM Tris-HCl (pH 8.8), 1 mM dithiothreitol, 100 μg/ml bovine serum albumin, 100 mM KCl, 0.5 mM $MnCl_2$, 60 μg/ml poly(A)·oligo(dT)$_{12-18}$ (2 : 1, w/w), 50 μM [^{3}H]dTTP (55 to 220 cpm/pmol), and 1 μl enzyme preparation. When activated DNA was used as the template·primer, 4 mM $MgCl_2$ instead of 0.5 mM $MnCl_2$ was added, poly(A)·oligo(dT) was omitted, and 50 μg/ml activated calf thymus DNA was added. Incubation was carried out for 20 min at 25°C, and the radioactivity in the polynucleotides was measured (*11*). One unit of DNA polymerase was defined as the amount of enzyme that incorporated 1 nmol [^{3}H]dTMP into polymer per 60 min. When the effect of pH on DNA polymerase β activity was examined, the enzyme activity was measured under the standard assay conditions with poly(A)·oligo(dT), except that 50 mM Tris-HCl buffer of pH 7.0, 8.0, 9.0, or 9.5 was used instead of this buffer of pH 8.8.

Purification of DNA Polymerase β from Cherry Salmon

Liver—All the procedures were carried out at 0-4°C. About 90 g of frozen cherry salmon liver was thawed, minced, suspended in 4 volumes of buffer A [20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 10 mM 2-mercaptoethanol, and 10% (v/v) glycerol] containing 0.5 M KCl and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and homogenized for 1 min in a Waring Blender at full speed. After centrifugation of the homogenate (13,000×*g* for 30 min), the supernatant (Fraction I) was filtered though a 200 ml of DEAE-cellulose (DE-52, Whatman) equilibrated with buffer A containing 0.5 M KCl on a Büchner funnel (13 cm diameter), with suction. The flow-through fractions were collected, diluted to 0.1 M KCl with buffer A, and then applied to a column of phosphocellulose (P11, Whatman) (10×5 cm), which was washed with buffer A containing 0.1 M KCl and eluted with buffer A containing 0.8 M KCl. The eluted protein was fractionated with ammonium sulfate. DNA polymerase β was recovered by 80% ammonium sulfate saturation from the 50% ammonium sulfate saturation supernatant (*11*). The precipitate was dissolved in a small amount of buffer B [20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.2 M KCl, and 50% (v/v) glycerol], and then stored at −20°C (Fraction II). Seven preparations of Fraction II were combined (146 ml of preparation was obtained from 620 g of salmon liver), and dialyzed against buffer C [20 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, 10 mM 2-mercaptoethanol, and 20% (v/v) glycerol], and the precipitate formed during the dialysis was removed by centrifugation (13,000×*g* for 30 min). The supernatant was applied to a column of DEAE-cellulose (3×17 cm). After washing the column with buffer C containing 0.05 M KCl, protein was eluted with a linear gradient of KCl, from 0.05 to 0.3 M, in buffer C (800 ml). The eluate (Fraction III, 155 ml) that contained DNA polymerase activity was applied to a column of phosphocellulose (1.5×17 cm). After washing the column with buffer D [20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 10 mM 2-mercaptoethanol, and 20% glycerol] containing 0.15 M KCl, protein was eluted with a linear gradient of KCl, from 0.15 to 0.65 M, in buffer D (160 ml). The fractions containing polymerase activity were pooled (Fraction IV, 48 ml) and diluted with two volumes of buffer D. The resultant solution was applied to a Blue-agarose column (1×6.4 cm, Affi-Gel Blue; Bio-Rad), which was washed with buffer D containing 0.1 M KCl and eluted with a linear gradient of KCl, from 0.1 to 0.6 M, in buffer D (50 ml). The fractions containing polymerase activity were collected (Fraction V, 12.5 ml) and diluted with the same volume of buffer D. The solution was applied to a single-stranded DNA-cellulose column (0.56×6 cm; Pharmacia), which was washed with buffer D containing 0.2 M KCl and eluted with a linear gradient of KCl, from 0.2 to 0.7 M, in buffer D (20 ml). The fractions containing polymerase activity were collected and referred to as Fraction VI (5.6 ml).

Purification of DNA Polymerase β from Cherry Salmon Testes—Cherry salmon testes (150 g) were suspended in 600 ml of buffer A containing 0.5 mM PMSF and then homogenized with a Teflon homogenizer (600 rpm, 4 strokes). The homogenate was centrifuged (13,000×*g* for 40 min), and the residual pellet was rehomogenized in buffer A (600 ml) containing 0.5 mM PMSF and 0.5 M KCl for 1 min in a Waring Blender at full speed. Centrifugation of this homogenate (13,000×*g* for 40 min) yielded a supernatant (Fraction I), which was purified as described above for the liver enzyme.

SDS-Polyacrylamide Gel Electrophoresis—SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli (*12*), with a 10% separation gel.

Isoelectric Point Determination—The isoelectric points (pIs) were determined with a Pharmacia PhastSystem at 15°C, using PhastGel (IEF3-9).

Partial Amino Acid Sequences—After dilution of the purified enzyme preparation (Fraction VI) with the same volume of water, the polypeptide (4 μg) was adsorbed on a poly(vinylidene fluoride) (PVDF) membrane by means of ProSpin™ (Applied Biosystems) and then analyzed by automated Edman degradation with an Applied Biosystems 473A protein sequencer.

RESULTS

Purification of DNA Polymerase βs from Cherry Salmon Liver and Testes—DNA polymerase βs were purified from cherry salmon liver and testes as described by Yamaguchi *et al.* with some modifications (*11, 13*). As shown in Fig. 1, DNA polymerase β activity was eluted as a single peak on each column chromatography. The extent of DNA polymerase β purification is summarized in Table I. Although the recovery of DNA polymerase activity was low (27%) at the Blue-agarose column chromatography step, if this step was omitted, some undesired polypeptides could not be specifically removed from the final preparation (data not shown). Thus Blue-agarose column chromatography was necessary to obtain highly purified salmon DNA polymerase βs, as described for the purification of the chick embryo enzyme by Yamaguchi *et al.* (*13*).

Enzymatic Properties of DNA Polymerase βs from Cherry Salmon Liver and Testes—The optimum pHs of the DNA polymerase βs from liver and testes, like those of other eukaryotic DNA polymerase βs, were in the alkaline range, and the maximum activities were obtained with Tris-HCl buffer of around pH 9 (Fig. 2).

As can be seen in Table II, the preferred template·primer for the salmon DNA polymerase βs was poly(A)·oligo(dT), rather than activated DNA. Both enzyme activities were resistant to 2 mM *N*-ethylmaleimide (NEM). We also examined the inhibitory effect of 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP) on the DNA polymerase βs from liver and testes using poly(A)·oligo(dT) as a template·primer. Both enzyme activities were strongly inhibited by ddTTP, 50% inhibition being achieved with 3 to 5 mM in the presence of 50 μM dTTP as the substrate.

The K_m values for dTTP and dATP for salmon and rat DNA polymerase βs were determined using activated DNA as the template·primer (Fig. 3 and Table III). Similar K_m values for dTTP and dATP were obtained for both the salmon and rat enzymes. The affinities of cherry salmon testes DNA polymerase β and the rat enzyme for three template·primer complexes were estimated (Fig. 4 and Table III). The K_m values for poly(A)·oligo(dT) (10:1), poly(dA)·oligo(dT) (10:1) and activated DNA for the cherry salmon testes enzyme were found to be 8.3, 4.7, and 11 times greater than those for the rat enzyme, respectively. Thus, a remarkable difference in the affinity for template·primers was found between the salmon testes DNA polymerase β and the rat enzyme.

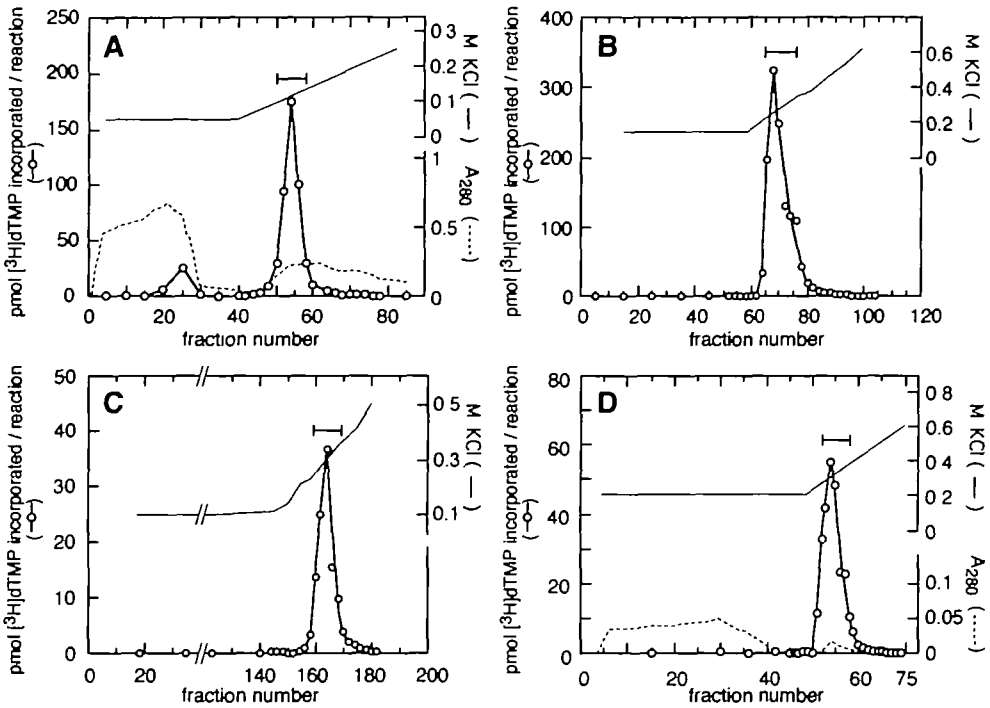

Fig. 1. **Elution profiles of cherry salmon liver DNA polymerase β on DEAE-cellulose (A), second phosphocellulose (B), blue-agarose (C), and single-stranded DNA-cellulose (D) column chromatography.** The DNA polymerase activities in 1 μl (A and B) or 0.1 μl (C and D) aliquots of each fraction in the standard reaction mixture were assayed. The indicated fractions were pooled.

TABLE I. **Purification of DNA polymerase β from cherry salmon liver.**[a]

Fraction	Total protein[b] (mg)	Total activity[c] (units) (%)	Specific activity (units/mg)
I. Crude extract	69,000	126,000(100)	1.8
II. Phosphocellulose and ammonium sulfate	13,900	70,500(56)	5.1
III. DEAE-cellulose	59	49,800(40)	845
IV. Second phosphocellulose	6.2	25,000(20)	4,030
V. Blue-agarose	1.8	6,850(5.4)	3,800
VI. Single-stranded DNA-cellulose	0.033	5,630(4.4)	171,000

[a]The starting material was 620 g of cherry salmon liver. [b]The amount of protein in Fractions I through V was determined by means of the Bio-Rad Protein Assay, and that in Fraction VI was estimated from the staining intensity after SDS-polyacrylamide gel electrophoresis, using bovine serum albumin as a standard. [c]One unit of DNA polymerase activity was defined as the amount of enzyme that incorporated 1 nmol [^{3}H]dTMP into polymer per 60 min.

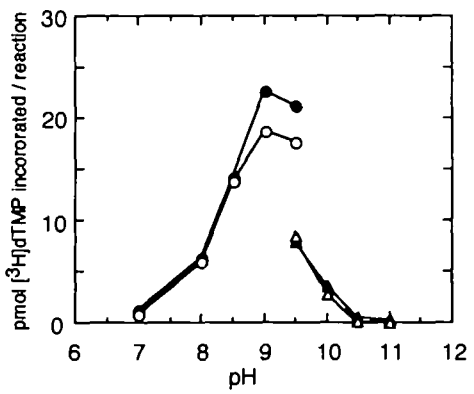

Fig. 2. **Effect of pH on cherry salmon DNA polymerase β activities from liver and testes.** DNA polymerase β activities from liver (●, ▲) and testes (○, △) were measured under the standard assay conditions with poly(A)·oligo(dT), except that 50 mM Tris-HCl (pH 8.8) was replaced by 50 mM Tris-HCl (●, ○) or glycine-KOH (▲, △) buffer of various pHs.

The activity of cherry salmon testes DNA polymerase β was examined at various temperatures and compared with the rat polymerase activity. As shown in Fig. 5, at around 10°C, the rat enzyme exhibited only 20% of the activity at the respective optimal temperature. On the other hand, in the case of the salmon enzyme more than 50% activity remained at the same temperature (10°C), when poly(A)·oligo(dT) was used as the template·primer. Similarly, the salmon enzyme showed 18% activity at 10°C and, in contrast, the rat enzyme showed less than 10% activity at the same temperature in the experiment involving activated DNA as the template·primer (Fig. 5B).

Polypeptide Sizes and Isoelectric Points (pIs) of Cherry Salmon DNA Polymerase βs—When analyzed by SDS-polyacrylamide gel electrophoresis, our enzyme preparations from salmon liver and testes were found to be single polypeptides, and both had a molecular weight (M_r) of approximately 39,000 and were almost homogeneous (Fig. 6). These cherry salmon enzymes were slightly smaller than rat recombinant DNA polymerase β (M_r=41,000). As shown in Fig. 7, the pIs of the salmon DNA polymerase βs were both 6.2, whereas rat recombinant β was characterized as a basic protein (pI=8.7 to 9) under the same conditions. Rat recombinant β yielded two bands, one of which may have been intact recombinant DNA polymerase β and the other, which was acidic with a slightly lower pI, may have been a degraded molecule which had lost the first three N-terminal amino acid residues (fMet-Ser-Lys) (*14*).

Amino Acid Sequence of the N-Terminal Region—Each purified enzyme (Fraction VI) was analyzed directly using

TABLE II. **Template·primer specificity, and effects of *N*-ethylmaleimide (NEM) and 2′,3′-dideoxythymidine 5′-triphosphate (ddTTP) on cherry salmon DNA polymerase β activity.**

Conditions[a]			(%) of control synthesis[b]	
Template·primer	Divalent cation	Inhibitor	Liver enzyme	Testes enzyme
Poly(A)·oligo(dT)	Mn^{2+}	–	100	100
Poly(A)·oligo(dT)	Mg^{2+}	–	19	21
Poly(dA)·oligo(dT)	Mn^{2+}	–	45	44
Poly(dA)·oligo(dT)	Mg^{2+}	–	71	78
Activated DNA	Mg^{2+}	–	15.6[c]	15.6[c]
Poly(A)·oligo(dT)	Mn^{2+}	2 mM NEM	102	93
Poly(A)·oligo(dT)	Mn^{2+}	5 μM ddTTP	45	42
Poly(A)·oligo(dT)	Mn^{2+}	20 μM ddTTP	11	11

[a]The DNA polymerase activity was measured under the standard assay conditions with 60 μg/ml poly(A)·oligo(dT), 60 μg/ml poly(dA)·oligo(dT), or 50 μg/ml activated DNA, as the template·primer, in the presence of 0.5 mM $MnCl_2$ or 4 mM $MgCl_2$, as the divalent cation, and 50 μM [^{3}H]dTTP. About 0.08 unit of enzyme was added to each reaction mixture. Incubation was carried out for 20 min at 25°C. [b]The one hundred percent values of the liver and testes enzymes represent the incorporation of 27 pmol dTMP. [c]The indicated values are based on the incorporation of total dNMP.

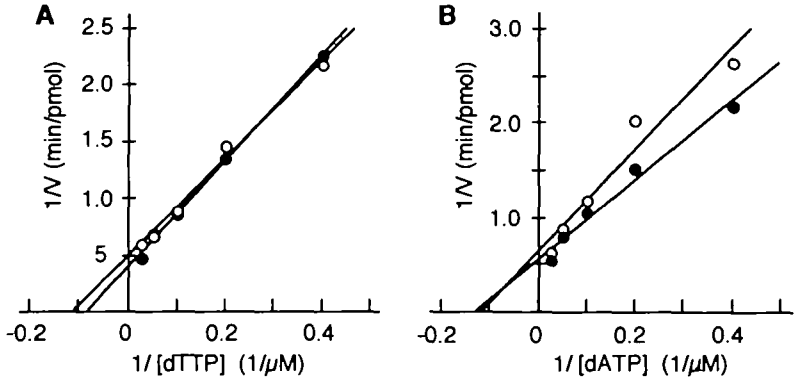

Fig. 3. **Effect of the dTTP (A) or dATP (B) concentration on cherry salmon and rat DNA polymerase βs.** DNA polymerase activity was measured under the conditions described under "MATERIALS AND METHODS" with activated DNA, except that the concentration of [^{3}H]dTTP or [^{3}H]dATP (440 cpm/pmol) was varied as indicated in the figure. About 0.5 unit of cherry salmon DNA polymerase β (○) or 0.33 unit of the rat enzyme (●) was added to each reaction mixture. Incubation was carried out for 15 min at 37°C. The figure presents double-reciprocal plots.

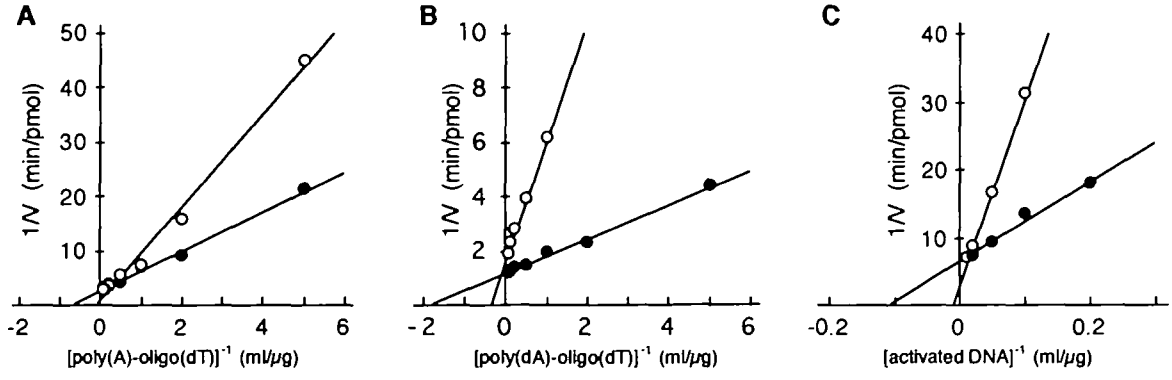

Fig. 4. **Effect of the template·primer concentration on cherry salmon and rat DNA polymerase βs.** DNA polymerase activity was determined under the conditions described under "MATERIALS AND METHODS" with poly(A)·oligo(dT) (A), poly(dA)·oligo(dT) (B), or activated DNA (C), except that concentration of the template·primer was varied as indicated in the figure. When a synthetic homopolymer (base ratio, A to T, was 10) was used as the template·primer, approximately 0.025 unit of cherry salmon DNA polymerase β (○) or the rat enzyme (●) was added to each reaction mixture, and when activated DNA was used, 0.25 or 0.17 unit of cherry salmon DNA polymerase β or the rat enzyme, respectively, was added. Incubation was carried out for 8 min at 25°C (A) or 37°C (B and C).

an automated Edman degradation protein sequencer (Fig. 8). Fortunately, the N-terminal 30 and 20 amino acid residues of the liver and testes enzymes, respectively, could be determined without proteolytic digestion pretreatment. The sequences of the liver and testes enzymes obtained were completely identical.

Alignment of the sequences of the N-terminal regions of the cherry salmon liver DNA polymerase βs with that of the rat enzyme (*8*) revealed that these two polymerases are highly homologous. Of the 30 amino acid residues of the salmon liver enzyme, 21 were identical to those of the rat enzyme sequence. However, 4 amino acids were shown to have been lost from the N-terminal of the cherry salmon enzymes.

TABLE III. **Kinetic constants of the cherry salmon testes and rat DNA polymerase βs for some substrates.**

Substrate	Temperature (°C)	K_m Cherry salmon β-polymerase	K_m Rat β-polymerase
dTTP	37	9.1 μM	11 μM
dATP	37	10	7.7
Poly(A)·oligo(dT)[a]	25	10 μg/ml	1.2 μg/ml
Poly(dA)·oligo(dT)[a]	37	2.5	0.53
Activated DNA	37	100	9.1

[a]Base ratio, A to T, was 10.

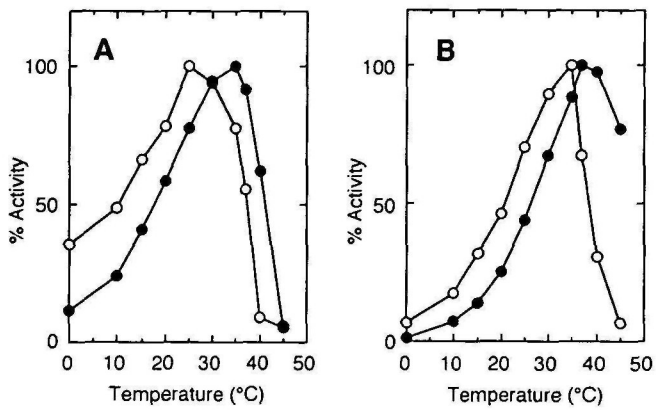

Fig. 5. **Effect of the temperature on DNA polymerase activity.** The activities of DNA polymerase βs were determined under standard assay conditions with poly(A)·oligo(dT) (A) and activated DNA (B). About 0.25 unit of cherry salmon DNA polymerase β (○) or the rat enzyme (●) was added to each reaction mixture, followed by incubation at various temperatures for 10 min. The enzyme activity was compared with those at the optimal temperatures. In panel A, 100% activities of the cherry salmon and rat DNA polymerase βs represent the incorporation of 60 and 94 pmol dTMP, and in panel B, the incorporation of 16 and 22 pmol dNMP, respectively.

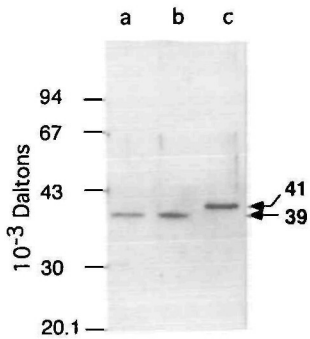

Fig. 6. **Analysis by SDS-polyacrylamide gel electrophoresis of cherry salmon DNA polymerase βs.** About 10 units of the testes enzyme (lane a), 15 units of the liver enzyme (lane b), and 0.18 μg of rat recombinant DNA polymerase β (lane c) were subjected to analysis. The protein bands were detected by staining with Coomassie Brilliant Blue. The marker proteins are phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase B (30,000), and soybean trypsin inhibitor (20,100).

DISCUSSION

Highly purified DNA polymerase βs were obtained from tissue extracts of cherry salmon liver and testes, and no substantial differences between these enzymes were observed. The preferred template·primer for the cherry salmon DNA polymerase βs was poly(A)·oligo(dT), rather than activated DNA. These enzymes were resistant to 2 mM *N*-ethylmaleimide (NEM), and inhibited strongly by ddTTP, and their optimal pH value in Tris-HCl buffer was 9. Therefore, the enzymatic properties of these cherry salmon DNA polymerase βs resemble those of other eukaryotic DNA polymerase βs, but were different from those of DNA polymerase γ. As shown in Fig. 6, the molecular masses of both cherry salmon DNA polymerase βs were 39 kDa, which was slightly smaller than that of the rat enzyme (41 kDa). However, the isoelectric points (pI = 6.2) (Fig. 7) of the cherry salmon enzymes were shown quite unique in comparison with alkaline pIs of 9.5 for calf thymus (*15*), 9.2 for human KB cells (*16*), 8.5 for rat Novikoff hepatoma (*17*), 9.15 for chick embryo (*18*), and 8.1 for sea urchin (*19*). An alkaline isoelectric point is a very typical characteristic for DNA polymerase βs. As the only exception to this, Haraguchi and Nagano reported that the pI value of the DNA polymerase β from starfish ovary was 6.4, however, their enzyme preparation was not purified extensively and the acidic pI value may have been due to the binding with other acidic factor(s) at the low ionic strength (*20*). In contrast to their results, our enzyme preparations (from liver and testes) were homogeneous on SDS-PAGE and their pI values are 6.2, as shown in Fig. 7.

Kumar *et al.* reported that mammalian DNA polymerase β consists of two, N-terminal 8- and 31-kDa, domains connected by a protease-sensitive region (*6*). The 8- and

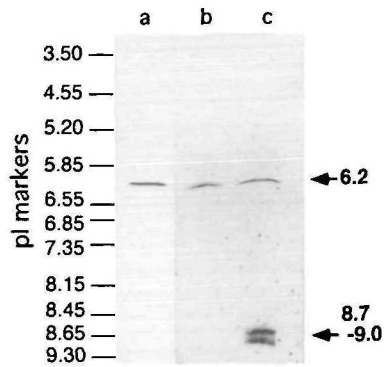

Fig. 7. **Isoelectric focusing of cherry salmon DNA polymerase βs.** About 0.07 μg each of the liver (lane a) and testes (lane b) enzymes and a mixture of the cherry salmon testes enzyme (0.07 μg) and rat recombinant DNA polymerase β (0.1 μg) (lane c) was subjected to isoelectric focusing, as described under "MATERIALS AND METHODS." The protein bands were detected by silver staining.

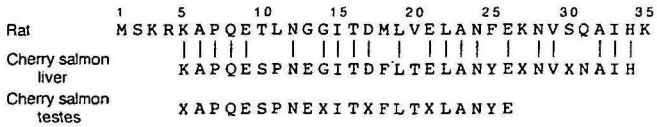

Fig. 8. **Amino acid homology between the N-terminal regions of rat and cherry salmon DNA polymerase βs.** Vertical lines between the residues indicate shared identity. X represents an undetermined amino acid.

31-kDa domains have, respectively, net charges of +9 and −5, and their isoelectric points were calculated to be 9.8 and 5.7, respectively (*8*).

The primary structures of our enzymes (Fig. 8) indicate that most of the region corresponding to the 8-kDa domain of the rat enzyme (*21-24*) has been conserved and only 4 amino acids (fMet-Ser-Lys-Arg) were shown to have been lost from the N-terminus. Some proteolytic degradation of the salmon enzymes probably occurred during purification. Moreover, of the 30 amino acid residues of the salmon DNA polymerase βs, 21 (70%) were identical to those of the rat enzyme sequence. Additionally, in our previous study we demonstrated the existence of extensive common amino acid sequences in the primary structures of DNA polymerase β molecules from mouse myeloma cells, chick embryos, and cherry salmon testes, using the tryptic peptide mapping method (*10*). However, the acidic pIs of the cherry salmon enzymes may not only be due to the loss of 4 amino acids from the N-terminus, because only one positive charge would be removed from the DNA polymerase β molecule on this deletion. But, it remains unclear whether or not the C-terminal region of our enzyme proteins was preserved. Removal of the first 10 to 14 amino acid residues (including 3 acidic and 4 basic amino acids) from the C-terminus of rat DNA polymerase β (335 amino acids) can only reduce the positive charge of the molecule by one, and no other deletion from the C-terminus can reduce its positive charge number. Therefore, the acidic pIs of the cherry salmon enzymes may not be due to simple degradation of the polypeptide chain.

As can be seen in Table III, the K_m values for template·primers examined for the cherry salmon DNA polymerase β were somewhat larger than those in the case of the rat enzyme, implying that the salmon DNA polymerase β seems to show lower affinity for template·primers than the rat enzyme. Under the optimum pH (approximately pH 9) for DNA polymerase β activity, many negative charges due to acidic amino acid residues appeared on the cherry salmon DNA polymerase β molecule, but in the case of the rat enzyme, only few negative charges appeared. Presumably the difference in the isoelectric points of these enzymes may influence the ionic interaction between the enzyme protein and nucleic acids as template·primers.

In this context, comparison of the primary structures of the cherry salmon enzyme protein active-site(s) and those of other DNA polymerase β from lower vertebrates, including aquatic animals, would be especially interesting.

Another interesting observation was the effect of temperature on the enzyme activity. Figure 5 shows that the cherry salmon DNA polymerase β was still active at low temperature (more than 50% of the activity remained at 10°C), compared with that of the rat enzyme (only 20% or less of the activity remained) when poly(A)·oligo(dT) was used as the template·primer. Interestingly, our previous studies showed that a similar characteristic as to temperature was observed in the case of RNA polymerases from cherry salmon (*25*) and rainbow trout (*Oncorhynchus myxiss*) (*26*), and also DNA polymerase induced by salmon herpesvirus (OMV) in cultured salmon cells (*27*). Generally, the salmonid fish inhabit relatively cold aquatic environments and their distribution in the world depends on such conditions. Cherry salmon is found only on the Asian side of the north-western Pacific Ocean, and in our country, they are distributed in the northern part or mountain areas of Honshu island (river resident or landlocked form). Our present results may also constitute one of the reasons for the adaptation of salmonids, including cherry salmon, to cold aquatic environments. Furthermore, the roles of DNA polymerase β in DNA replication, repair and chromosome rearrangement in spermatogenesis remain to be elucidated.

We wish to thank Drs. Akio Matsukage and Kazushi Tanabe, Aichi Cancer Center Research Institute, for kindly providing the rat recombinant DNA polymerase β and for their valuable advice. We are also indebted to the Tomakomai Experimental Forest Station, Hokkaido University, and Yamanashi Prefectural Fisheries Technology Center for the fish stocking. This study was carried out under the NIBB Cooperative Research Program.

REFERENCES

1. Wang, T.S.-F. (1991) Eukaryotic DNA polymerases. *Annu. Rev. Biochem.* **60**, 513-552
2. Fry, M. and Loeb, L.A. (1986) *Animal Cell DNA Polymerases*, CRC Press, Boca Raton, FL
3. Chang, L.M.S. (1976) Phylogeny of DNA polymerase-β. *Science* **191**, 1183-1185
4. Abbotts, J., SenGupta, D.N., Zmudzka, B., Widen, S.G., Notario, V., and Wilson, S.H. (1988) Expression of human DNA polymerase β in *Escherichia coli* and characterization of the recombinant enzyme. *Biochemistry* **27**, 901-909
5. Date, T., Yamaguchi, M., Hirose, F., Nishimoto, Y., Tanihara, K., and Matsukage, A. (1988) Expression of active rat DNA polymerase β in *Escherichia coli*. *Biochemistry* **27**, 2983-2990
6. Kumar, A., Widen, S.G., Williams, K.R., Keder, P., Karpel, R.L., and Wilson, S.H. (1990) Studies of the domain structure of mammalian DNA polymerase β. Identification of a discrete template binding domain. *J. Biol. Chem.* **265**, 2124-2131
7. Pelletier, H., Sawaya, M.R., Kumar, A., Wilson, S.H., and Kraut, J. (1994) Structures of ternary complexes of rat DNA polymerase β, a DNA template-primer, and ddCTP. *Science* **264**, 1891-1903
8. Davies, J.F., II, Almassy, R.J., Hostomska, Z., Ferre, R.A., and Hostomsky, Z. (1994) 2.3 Å crystal structure of the catalytic domain of DNA polymerase β. *Cell* **76**, 1123-1133
9. Philippe, M. and Chevaillier, P. (1980) Study of dogfish (*Scyliorhinus caniculus*) deoxyribonucleic acid polymerases α and β. Extraction, separation, characterization and changes during spermatogenesis. *Biochem. J.* **189**, 635-639
10. Tanabe, K., Yamaguchi, T., Saneyoshi, M., Yamaguchi, M., Matsukage, A., and Takahashi, T. (1984) Conservation of molecular structure of DNA polymerase β in vertebrates probed by tryptic peptide mapping. *J. Biochem.* **96**, 365-370
11. Yamaguchi, M., Matsukage, A., and Takahashi, T. (1980) Chick embryo DNA polymerase γ. Purification and structural analysis of nearly homogeneous enzyme. *J. Biol. Chem.* **255**, 7002-7009
12. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685
13. Yamaguchi, M., Tanabe, K., Taguchi, Y.N., Nishizawa, M., and Takahashi, T. (1980) Chick embryo DNA polymerase β. Purified enzyme consists of a single M_r=40,000 polypeptide. *J. Biol. Chem.* **255**, 9942-9948
14. Shirakihara, Y., Matsukage, A., Nishimoto, Y., and Date, T. (1994) Crystallization of 31 kDa C-terminal fragment of rat DNA polymerase β. *J. Mol. Biol.* **235**, 1342-1344
15. Bollum, F.J. (1975) Mammalian DNA polymerases in *Progress in Nucleic Acid Research and Molecular Biology* (Cohn, W.E., ed.) Vol. 15, pp. 109-144, Academic Press, New York
16. Sedwick, W.D., Wang, T.S.-F., and Korn, D. (1972) Purification and properties of nuclear and cytoplasmic deoxyribonucleic acid polymerases from human KB cells. *J. Biol. Chem.* **247**, 5026-5033
17. Stalker, D.M., Mosbaugh, D.W., and Meyer, R.R. (1976)

Novikoff hepatoma deoxyribonucleic acid polymerase. Purification and properties of a homogeneous β polymerase. *Biochemistry* **15**, 3114-3121
18. Stavrianopoulos, J.G., Karkas, J.D., and Chargaff, E. (1972) DNA polymerase of chicken embryo: Purification and properties. *Proc. Natl. Acad. Sci. USA* **69**, 1781-1785
19. Suzuki-Hori, C., Nagano, H., and Mano, Y. (1977) DNA polymerase-β from the nuclear fraction of sea urchin embryos: Characterization of the purified enzyme. *J. Biochem.* **82**, 1613-1621
20. Haraguchi, T. and Nagano, H. (1983) Isolation and characterization of DNA polymerases from mature oocytes of starfish, *Asterina pectinifera*. *J. Biochem.* **93**, 687-697
21. Zmudzka, B.Z., SenGupta, D., Matsukage, A., Cobianchi, F., Kumar, P., and Wilson, S.H. (1986) Structure of rat DNA polymerase β revealed by partial amino acid sequencing and cDNA cloning. *Proc. Natl. Acad. Sci. USA* **83**, 5106-5110
22. Matsukage, A., Nishikawa, K., Ooi, T., Seto, Y., and Yamaguchi, M. (1987) Homology between mammalian DNA polymerase β and terminal deoxynucleotidyltransferase. *J. Biol. Chem.* **262**, 8960-8962
23. Yamaguchi, M., Hirose, F., Hayashi, Y., Nishimoto, Y., and Matsukage, M. (1987) Murine DNA polymerase β gene: Mapping of transcription initiation sites and the nucleotide sequence of the putative promoter region. *Mol. Cell. Biol.* **7**, 2012-2018
24. SenGupta, D.N., Zmudzka, B.Z., Kumar, P., Cobianchi, F., Skowronski, J., and Wilson, S.H. (1986) Sequence of human DNA polymerase β mRNA obtained through cDNA cloning. *Biochem. Biophys. Res. Commun.* **136**, 341-347
25. Nakayama, C., Saneyoshi, M., Takiya, S., and Iwabuchi, M. (1983) Partial purification and characterization of DNA-dependent RNA polymerases I and II from cherry salmon (*Oncorhynchus masou*). *Comp. Biochem. Physiol.* **74B**, 719-723
26. Gillam, S., Aline, R., Jr., Wylie, V., and Smith, M. (1979) RNA synthesis and RNA polymerase activities in germ cells of developing rainbow trout testis. *Biochim. Biophys. Acta* **565**, 275-292
27. Suzuki, S., Kimura, T., and Saneyoshi, M. (1986) Characterization of DNA polymerase induced by salmon herpesvirus, *Oncorhynchus masou* virus. *J. Gen. Virol.* **67**, 405-408