Structural Homology of DNA Polymerase β from Various Mammalian Cells*

Kazushi Tanabe, Masamitsu Yamaguchi, Akio Matsukage, and Taijo Takahashi

From the Laboratory of Biochemistry, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464, Japan

DNA polymerase β was purified from various mammalian cells, i.e., mouse myeloma, rat liver, rat ascites hepatoma cells, rabbit liver, pig liver, and calf thymus cells. The apparent molecular weights of the polypeptides composing these enzymes were all about 40,000, as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Moreover, tryptic peptide maps of these enzymes after radiolabelling with [125I]indicated that the molecular structures of the enzymes were basically identical. No difference was detected in the peptide maps of the mouse and rabbit enzymes, and only a few of the 22 spots in the fingerprint of the mouse enzyme were not detected in that of the rat enzyme. Furthermore, the peptide maps of DNA polymerase β's from normal and malignant rat cells differed in only one spot.

Recently, we obtained a nearly homogeneous preparation of DNA polymerase β from chick embryos and demonstrated that the preparation consisted exclusively of a single polypeptide with an apparent molecular weight of 40,000 (11). Tryptic peptide mapping analysis showed partial structural homology between the chick and rat enzymes.

In the present work, we compared the molecular weights and fingerprint patterns of the tryptic peptides of DNA polymerase β purified from several mammalian cells to examine how well the structure of this enzyme was conserved during evolution. The apparent molecular weights of the polypeptides composing these enzyme preparations from different sources were indistinguishable, and autoradiograms of tryptic peptide maps of these polypeptides labeled with [125I]indicated extensive homology of the enzymes.

**MATERIALS AND METHODS**

**Cells**—Mouse myeloma MOPC104E solid tumor (12) and rat ascites hepatoma cells (6) were grown as described previously. Rat and rabbit livers were removed from normal adult animals and stored at -20°C until use. Pig liver and calf thymus were obtained from a slaughter house, frozen in dry ice immediately after removal, and stored at -20°C.

**Chemicals**—Unlabeled deoxynucleoside triphosphates and oligonucleotide (dT)₂₀-₃₀ were purchased from Boehringer Mannheim, West Germany, and poly(rA)-oligo(dT) in addition to activated calf thymus DNA was from Sigma and was activated as a template-primer as described by Schlabach et al. (13). Phosphcellulose was from Sigma, blue agarose (Mätrix Blue A) was from Amicon Corporation, Cambridge, MA, and DEAE-cellulose paper (DB 81) was from Whatman, England. Single-stranded DNA-cellulose was prepared as described by Litman (14), using calf thymus DNA and cellulose powder (Cellex 410) from Bio-Rad, Richmond, CA. Bovine serum albumin, ovalbumin, and a-chymotrypsinogen from Boehringer Mannheim were used as molecular weight markers in SDS-polyacrylamide gel electrophoresis. Acrylamide, N,N'-methylene bisacrylamide, and other chemicals for the preparation of the electrophoresis gel were from Bio-Rad. Silica gel-coated thin layer plates (20 × 20 cm, 0.25-mm thickness) were from E. Merck, West Germany. A 1-cm width of silica gel layer was cut from each edge, and the plates were stored in a desiccator until use.

**Enzymes**—Trypsin and bovine pancreatic DNase were purchased from Boehringer Mannheim. E. coli tRNA polymerase was prepared as described previously (15).

**Assay of DNA Polymerase β**—The standard reaction mixture in 25 µl contained the following: 50 mM Tris-HCl, pH 8.8 at 37°C, 10 mM Mg-acetate, 1 mM dithiothreitol, 100 µM each of dATP, dGTP, and dCTP, 100 µM [3H]dTTP (60 cpm/nmol), 12% (v/v) glycerol, 400 µg/ml of bovine serum albumin, 160 µg/ml of activated calf thymus DNA, 40 mM KCl, and 1-2 µl of enzyme fraction. The mixture was incubated at 37°C for 30 min and then transferred to a DEAE-cellulose paper disc, which was washed as described by Lindell et al. (16). One unit of DNA polymerase activity was defined as the amount catalyzing the incorporation of 1 nmo1 of [3H]dTMP into polymer DNA in 60 min.

In poly(rA)-oligo(dT) assay, Mg++, dATP, dGTP, and dCTP, and activated DNA were omitted from the standard assay mixture, and 0.5 mM MnCl₂, 16 µg/ml of (rA)₂₀ and 35 µg/ml of (dT)₂₀-₃₀ were added.
Homology of Mammalian DNA Polymerase β

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis—SDS-polyacrylamide slab gel electrophoresis was carried out essentially by the method of Laemmli (17) as described previously (18).

Measurement of Protein Concentration—Protein concentration was determined by the method of Lowry et al. (19). The protein concentration in the final preparation was estimated from the intensity of dye staining after SDS-gel electrophoresis using bovine serum albumin as a standard.

Tryptic Peptide Mapping—Radioiodination, trypsin digestion, and two-dimensional separation of peptides on thin layer plates were carried out as described by Elder et al. (20) with modifications (11, 21). In brief, the stained protein band was cut out of the SDS-polyacrylamide gel electrophoresis slab gel, transferred to a glass tube. Then the peptide was radioiodinated with \(^{125}\)I by the chloramine-T method (22). The gel slice was washed and dried, and then 50 µg of trypsin in 0.5 ml of 50 mM NH\(_4\)HCO\(_3\) (pH 8.4) was added to each tube. After incubation for 20 h at 37°C, the supernatant, which contained most of the trypptic peptide, was lyophilized, and the residue was redissolved in 20 µl of TLE buffer (acetic acid/formic acid: H\(_2\)O = 15:5:80). A 3–6 µl portion of this solution was spotted on a silica gel-coated thin layer chromatography plate, and tryptic peptides were resolved by electrophoresis in the first dimension and ascending chromatography in the second dimension. Electrophoresis was carried out at 900 V for 80 min in TLE buffer using a Pharmacia flat bed apparatus FBE3000 with cooling at 4°C. Chromatography in thin layer chromatography buffer (butanol:pyridine:acetic acid:H\(_2\)O = 32:5:25:6:20) in a thin layer chromatography chamber was continued for about 4 h until the front reached the top. The plate was then dried and autoradiographed using Kodak X-OMAT R film.

Buffers—All pH values given are final values at 4°C. Buffer A is 0.25 M sucrose and 3 mM CaCl\(_2\). Buffer B is 40 mM KPi, pH 7.5, 40 mM NaCl, and 1 mM dithiothreitol. Buffer C is 0.2 M KPi, pH 7.5, 10% glycerol, and 1 mM dithiothreitol. Buffer D is 0.1% Triton X-100, 1 mM dithiothreitol. Buffer F is 0.3 M KPi, pH 7.5, 10% glycerol, and 1 mM dithiothreitol. Buffer G is 50 mM Tris-HCl, pH 7.8, 20% glycerol, 10 mM MgCl\(_2\), 1 mM dithiothreitol, and 0.1 mM EDTA. Buffer H is 20 mM Tris-HCl, pH 7.6, 20% glycerol, 1 mM dithiothreitol, and 0.1 mM EDTA. For I it is 10 mM Tris-HCl, pH 7.6, 0.2 M KCl, 50% glycerol, and 1 mM dithiothreitol.

Purification of DNA Polymerase β from Rat Liver—DNA polymerase was purified from a crude nuclear fraction of rat liver as described by Chang (1) with modifications. The purification procedure is summarized in Table I. In brief, 200 g of frozen tissue was thawed, minced, passed through stainless steel gauze, and suspended in 4 volumes (800 ml) of buffer A. The preparation was homogenized in a Waring Blender and centrifuged at 4000 rpm for 4 min, and then the pellet fraction was suspended in 400 ml of buffer A and centrifuged at 4000 rpm for 5 min. The pellet was washed twice with buffer A and the supernatant obtained by centrifugation at 9000 rpm for 20 min, was mixed with 300 ml of buffer B, homogenized with a blender, and centrifuged at 9500 rpm for 20 min. The pellet was washed twice with buffer B, and the final pellet (24 g) was suspended in 200 ml of buffer C and homogenized in a blender. The homogenate was then sonicated, stirred at 4°C for 1 h, and centrifuged at 9500 rpm for 20 min. To the supernatant (fraction I, 205 ml), ½ volume of buffer D was added to adjust the KPi concentration to 0.15 M and charged onto a phosphocellulose column (packed volume 120 ml) equilibrated with buffer E. The column was washed with 500 ml of buffer E, and then proteins were eluted with 260 ml of buffer F, and fractions containing high DNA polymerase activity were pooled (fraction II, 54 ml). The KPi concentration of fraction II was reduced to 0.15 M by adding an equal volume of buffer D, and the solution was then charged onto a DEAE-phosphocellulose joint column (5) which had been equilibrated with buffer F. The column was washed with 120 ml of buffer E, and then the phosphocellulose column (22 ml) was separated from the DEAE-cellulose column, and proteins were eluted with a 200-ml linear gradient of 0.15 to 0.3 M KPi containing 10% glycerol and 1 mM dithiothreitol. DNA polymerase activity eluted as a single peak at centered at 0.19 M KPi. The peak fractions were pooled (fraction III, 57 ml), dialyzed overnight against 2 changes of 500 ml of buffer G containing 0.2 M KCl, and then loaded on a Mätrix Blue A column (packed volume 4.8 ml) previously equilibrated with buffer G containing 0.2 M KCl. The column was washed with the same buffer, and then enzyme was eluted with a 60-ml linear gradient of 0.15 to 0.3 M KPi containing 10% glycerol and 1 mM dithiothreitol. Fractions containing the highest DNA polymerase activity were pooled (fraction IV, 22 ml). To fraction IV, 1.1 ml of 0.2 M EDTA was added to chelate Mg\(^{2+}\), and the solution was dialyzed against 2 changes of 300 ml of buffer H containing 0.25 M KCl and then loaded onto a single-stranded DNA-cellulose column (packed volume 1.5 ml) which had been equilibrated with buffer H containing 0.25 M KCl. The column was washed with 10 ml of the same buffer, and enzyme was eluted with 10 ml of buffer H containing 0.6 M KCl. Fractions containing high DNA polymerase activity were pooled and dialyzed against 250 ml of buffer I overnight. This fraction is referred to as fraction V (3.6 ml).

Purification of DNA Polymerase β from Rabbit Liver and Pig Liver—DNA polymerase β's were purified from rabbit liver and pig liver essentially by the method used for the rat liver enzyme.

Purification of DNA Polymerase β from Mouse Myeloma MOPC104E Cells—Preparation of the extract and the first phosphocellulose-cellulose chromatography were carried out as described previously (18) for the purification of chick embryo DNA polymerase α and β (11). The fraction containing DNA polymerase β was precipitated with 80% saturation of ammonium sulfate from the supernatant at 55% saturation of ammonium sulfate. The precipitate was redissolved in a minimal volume of 50 mM Tris-HCl (pH 7.6) containing 1 mM dithiothreitol, 0.1 mM EDTA, and 50% glycerol. This fraction is referred to as fraction II. After extensive dialysis against buffer E, fraction II was loaded onto a DEAE-phosphocelulose joint column. The purification procedure after this step was the same as that for the rat liver enzyme.

Molecular Weight Determination of Polypeptides of DNA Polymerase β's from Various Mammalian Sources by SDS-Polyacrylamide Gel Electrophoresis—The molecular weights of the polypeptides of DNA polymerase β's purified from various mammalian cells were compared by SDS-polyacrylamide gel electrophoresis using bovine serum albumin and ovalbumin as standards.

Activity was measured by a standard assay using calf thymus DNA as a template-primer.

Activity of the final fraction measured by an assay using poly(rA)-oligo(dT) as a template-primer was about 10 times higher than that shown.

Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Chromatin extract</td>
<td>205</td>
<td>779</td>
<td>2,460</td>
<td>3.2</td>
</tr>
<tr>
<td>II. 1st Phosphocellulose</td>
<td>54</td>
<td>27</td>
<td>1,120</td>
<td>60</td>
</tr>
<tr>
<td>III. 2nd Phosphocellulose</td>
<td>57</td>
<td>1.7</td>
<td>1,197</td>
<td>204</td>
</tr>
<tr>
<td>IV. DNA-phosphocellulose</td>
<td>22</td>
<td>0.22</td>
<td>1,188</td>
<td>5,400</td>
</tr>
<tr>
<td>V. DNA-cellulose</td>
<td>3.6</td>
<td>0.0043</td>
<td>684</td>
<td>159,000</td>
</tr>
</tbody>
</table>

* With 200 µg of rat liver as starting material.  
* Protein in fractions I through III was determined by the method of Lowry et al. (11); in fraction IV, protein was estimated from the absorbance at 280 nm, assuming that 1 A\(_{280}\) unit corresponds to 1 mg of protein, in fraction V, protein was estimated from the intensity of dye staining after polyacrylamide gel electrophoresis, using bovine serum albumin and ovalbumin as standards.

RESULTS

Molecular Weight Determination of Polypeptides of DNA Polymerase β's from Various Mammalian Sources by SDS-Polyacrylamide Gel Electrophoresis—The molecular weights of the polypeptides of DNA polymerase β's purified from various mammalian cells were compared by SDS-polyacrylamide gel electrophoresis using bovine serum albumin and ovalbumin as standards.
amidase slab gel electrophoresis. Fig. 1 shows results on the DNA polymerase β's from rat ascites hepatoma AH130 cells (less pure preparation), calf thymus, rat liver, and mouse myeloma MOPC104E cells analyzed on a single slab of gel. All the preparations had major polypeptides with a molecular weight of about 40,000. The stained band of about \( M_r = 68,000 \) seemed to be due to contaminants in the chemicals used for preparation of the gel.

Though the "less pure preparation" of the AH130 enzyme and the calf thymus enzyme contained other polypeptides besides that of \( M_r = 40,000 \), the enzyme preparations from rat liver and mouse myeloma were nearly homogeneous. Extensively purified AH130 enzyme and the enzymes from rabbit and pig liver also contained the major polypeptide of \( M_r = 40,000 \) (data not shown). Thus the sizes of the polypeptides composing the DNA polymerase β's from all the mammalian sources tested were indistinguishable and were estimated as about \( M_r = 40,000 \).

Blue agarose affinity chromatography proved to be very useful for purification of mammalian DNA polymerase β as well as the avian enzyme (11). A combination of phosphocellulose, blue agarose, and single-stranded DNA-cellulose column chromatographies is a simple and effective method for purification of this enzyme.

**Analysis of the Structural Homology of DNA Polymerase β by Tryptic Peptide Mapping**—The \( M_r = 40,000 \) polypeptide of each DNA polymerase sample from various mammalian sources was examined by tryptic peptide mapping after labeling with \(^{125}\)I. The stained bands of \( M_r = 40,000 \) were cut out from the slab gel, the protein in each gel slice was radioiodinated and digested with trypsin, and the tryptic peptides were separated two-dimensionally on a silica gel-coated thin layer plate. The autoradiograms and traced figures are shown in Figs. 2 and 3. Fig. 2A to D, shows the autoradiograms of tryptic peptide maps of DNA polymerase β's from mouse myeloma MOPC104E, rabbit liver, calf thymus, and pig liver, respectively. Fig. 3, A to D, shows the traced diagrams of Fig. 2, A to D. More than twenty spots were detected in each map, and most of the spots (numbers 1 to 22 in Fig. 3A) were common to all these enzymes. No difference was detected between the maps of the mouse and rabbit enzymes, but a
The enzyme may due to differences in the methods used for molecular weight determination and in assumptions of marker size.

Immunological cross-reactivity has been reported not only among mammalian DNA polymerase β’s (28) but also between avian and mammalian enzymes (9). Our previous report (11) also demonstrated the existence of partial structural homology between chick and rat DNA polymerase β by tryptic peptide mapping. Using the same technique, in this work we confirmed the extensive homology in the peptide structure of DNA polymerase β’s from various mammalian sources. The reproducibility and reliability of the peptide mapping technique used here were proved repeatedly in our series of experiments.

The tryptic peptide maps of all the DNA polymerase β’s tested were very similar and indicated extensive amino acid sequence homology among the enzymes. No difference was detected between the mouse and rabbit enzymes, and only a few additional spots were detected in the maps of the calf and pig enzymes. It is interesting that only the rat enzyme lacked some spots that were common to all the other mammalian species, including other rodents. A difference of only one spot was detected between the peptide maps of normal (liver) and neoplastic (ascites hepatoma) rat cell DNA polymerase β. Since the spot not given by the rat liver enzyme is common to all the other enzymes, it is not likely that this spot of the enzyme from neoplastic cells reflects the malignancy of the enzyme source.

Taking the peptide fingerprint pattern of the mouse myeloma enzyme as a standard, the extents of homology of the other enzymes are as follows: rabbit enzyme, 100%; rat enzyme, about 80%; calf thymus enzyme, 85%; pig enzyme, 90%. Previously we calculated the extent of homology between DNA polymerase β from chick embryos and rat ascites hepatoma cells as about 60% (11).

It should be noted that there are some limitations in the method used in this study. The extents of homology estimated here are obtained simply from the numbers of spots of ¹²⁵I-labeled tryptic peptides in the autoradiograms, and they do not correspond to the extent of homology in the amino acid sequences of the proteins. Since ¹²⁵I binds only to susceptible amino acid residues, namely tyrosine, histidine, and phenylalanine (20), and trypsin cuts only the peptide bonds after lysine and arginine, not all the tryptic peptides are labeled with ¹²⁵I. Thus by this method, it is impossible to detect changes of amino acid residues in peptides not labeled with ¹²⁵I.

Homology of Mammalian DNA Polymerase β

Fig. 4. Autoradiograms and traced figures of tryptic peptide maps of rat enzymes. M, 40,000 polypeptide bands of DNA polymerase β’s from rat ascites hepatoma AH130 and rat liver, shown in Fig. 1, are detected in the gels and treated as described under “Materials and Methods.” A, autoradiogram of the tryptic peptide map of DNA polymerase β from rat ascites hepatoma AH130 cells; B, peptide map of rat liver enzyme; C, traced figure of A; D, traced figure of B. Solid spots numbered 1 to 22 are common to all the animals tested. Spots marked e-h are given only by the rat enzymes.

A few unique spots (marked a-d in Fig. 3, C and D) were detected in the maps of the calf and pig enzymes in addition to the common spots.

Fig. 4, A and B, shows the fingerprints of DNA polymerase β’s from rat ascites hepatoma AH130 cells and rat liver, respectively. All except one spot (number 4 in traced diagram Fig. 4C) are common to both maps. Comparison with the peptide maps of the other enzymes shown in Fig. 2 and Fig. 3 indicated that the spots numbered 7 to 17 were common to all the mammalian preparations tested, but spots numbered 18 to 22 were not detected in the maps of the rat enzyme, which had several unique spots instead (marked e-h in Fig. 4, C and D).

**DISCUSSION**

Extensively purified preparations of DNA polymerase β have previously been obtained from various animal cells. The molecular weights reported for the polypeptides composing these DNA polymerase β’s, however, were somewhat different, e.g., rat liver, 29,000 (23); rabbit bone marrow, 40,000–50,000 (24); calf thymus, 44,000 (1); chick embryo, 27,000 (25); human KB cells, 43,000 (2); rat ascites hepatoma, 45,000 (26) and 40,000 (6); Novikoff hepatoma cells, 32,000 (3); *Xenopus laevis* ovaries, 45,000 (27); guinea pig liver, 32,000 (4), and mouse myeloma cells, 40,000 (5). On the other hand, we recently found that the molecular weight of chick embryo DNA polymerase β is the same as that of a mammalian enzyme (40,000) (11). This suggests that the size of this enzyme must be very similar in a wide variety of animals.

In this study we purified DNA polymerase β from mouse myeloma MOPC104E cells, rat ascites hepatoma AH130 cells, rat liver, rabbit liver, pig liver, and calf thymus cells and demonstrated that all these enzymes had a molecular weight of 40,000 determined by SDS-polyacrylamide gel electrophoresis. The discrepancy in the molecular weights reported for the enzyme may due to differences in the methods used for molecular weight determination and in assumptions of marker size.

The tryptic peptide maps of all the DNA polymerase β’s tested were very similar and indicated extensive amino acid sequence homology among the enzymes. No difference was detected between the mouse and rabbit enzymes, and only a few additional spots were detected in the maps of the calf and pig enzymes. It is interesting that only the rat enzyme lacked some spots that were common to all the other mammalian species, including other rodents. A difference of only one spot was detected between the peptide maps of normal (liver) and neoplastic (ascites hepatoma) rat cell DNA polymerase β. Since the spot not given by the rat liver enzyme is common to all the other enzymes, it is not likely that this spot of the enzyme from neoplastic cells reflects the malignancy of the enzyme source.

Taking the peptide fingerprint pattern of the mouse myeloma enzyme as a standard, the extents of homology of the other enzymes are as follows: rabbit enzyme, 100%; rat enzyme, about 80%; calf thymus enzyme, 85%; pig enzyme, 90%. Previously we calculated the extent of homology between DNA polymerase β from chick embryos and rat ascites hepatoma cells as about 60% (11).

It should be noted that there are some limitations in the method used in this study. The extents of homology estimated here are obtained simply from the numbers of spots of ¹²⁵I-labeled tryptic peptides in the autoradiograms, and they do not correspond to the extent of homology in the amino acid sequences of the proteins. Since ¹²⁵I binds only to susceptible amino acid residues, namely tyrosine, histidine, and phenylalanine (20), and trypsin cuts only the peptide bonds after lysine and arginine, not all the tryptic peptides are labeled with ¹²⁵I. Thus by this method, it is impossible to detect changes of amino acid residues in peptides not labeled with ¹²⁵I.

It is unlikely, however, that different amino acid sequence can give exactly the same maps. On the contrary, only extensive amino acid sequence homology gives extensive similarity in peptide maps. For example, the extent of amino acid sequence homology of the hemoglobin α chains among various mammals is 70–90% (29), but only a few of the ¹²⁵I-labeled tryptic peptides derived from the primary structure are identical; most of the corresponding peptides contain one or more different amino acid residues or are different in size. Thus their peptide maps must be significantly different. In this respect, we conclude that the molecular structures of DNA polymerase β’s from various mammalian cells are nearly identical.

Chang (30) reported that DNA polymerase β, or an enzyme considered to be related to DNA polymerase β, is widely distributed in multicellular animals from sponges to mammals, but is not present in bacteria, plants, or protozoa. The results shown in this and our previous report (11) suggest that the molecular structure of DNA polymerase β is highly conserved in a wide variety of animals. Moreover, it is well known that
this enzyme consists of a single polypeptide. The conservation and simplicity in the molecular structure of this enzyme must be related to the importance of the enzyme in living cells.

In general, most vital functions are thought to be conserved throughout biological evolution. Many studies on the role of DNA polymerase β (31–37) suggest that this enzyme is involved in repair of damaged DNA, which must be an essential cellular function. DNA polymerase β appeared at an early stage of animal evolution and has been conserved for more than half a billion years (30), and its properties have not changed essentially for a long time during evolution. Moreover, the level of enzyme activity remains constant throughout the cell cycle (31). These facts and the structural conservation of DNA polymerase β suggest the essential and vital role of this enzyme.

Acknowledgment—We would like to thank Dr. S. H. Wilson, National Cancer Institute, for advice on tryptic peptide mapping.

REFERENCES