Purification and Characterization of DNA Polymerase from the Archaebacterium *Methanobacterium thermoautotrophicum*†

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ABSTRACT: DNA polymerase from the thermophilic archaebacterium *Methanobacterium thermoautotrophicum* was purified about 16 000-fold by chromatography on heparin–agarose, Blue Sepharose, hydroxylapatite, and phenyl-Sepharose as well as by centrifugation through a glycerol gradient. The enzyme exists in the highly purified preparation as one polypeptide of molecular weight 72K that constitutes its single subunit, as shown by electrophoresis under denaturing conditions, gel filtration, and glycerol gradient sedimentation. With the activity gel technique it is demonstrated that the DNA polymerase is very sensitive to proteolysis and the purified polypeptide could be derived from a larger precursor. The enzyme has a temperature optimum at 65 °C. Both 3′→5′- and 5′→3′-exonuclease activities have been found associated with the DNA polymerase. On the basis of its properties, the enzyme shows certain similarities to prokaryotic DNA polymerases of the type of *Escherichia coli* DNA polymerase I.

DNA polymerases have been purified and characterized in many different organisms from almost all groups of the living world (Kornberg, 1980, 1982). On the basis of their characteristics, it is possible to recognize several similarities between DNA polymerases of both prokaryotic and eukaryotic origin. Hübscher et al. (1981) showed that in both prokaryotes and eukaryotes the polymerizing activity is associated with polypeptides of similar molecular weight as well as of a similar pattern of proteolytic degradation. They suggested that the complex of replicative DNA polymerase contains an evolutionarily conserved polypeptide responsible for chain elongation.

Within prokaryotes and especially eukaryotes, DNA polymerases show a more clearly pronounced relatedness. The multiple DNA-polymerizing activities found in a given organism are not very closely related and can be distinguished from each other by simple biochemical criteria. However, it can be demonstrated that a given activity has its closely related counterparts in a range of other organisms. For instance, DNA polymerase activities found in eukaryotes can be ascribed to one of three distinct classes (Weissbach et al., 1975). Eukaryotes as distinct as fungi, plants, and animals possess DNA polymerase α activities, which show several common biochemical features: inhibition by aphidicolin and *N*-ethylmaleimide, high native molecular weight, and similar subunit composition. No corresponding activity is found in prokaryotes (Huberman, 1981). In animals, DNA polymerases β are also present. They are resistant to both aphidicolin and *N*-ethylmaleimide and are composed of a single small polypeptide with conserved antigenic properties (Chang et al., 1982) and conserved proteolytic cleavage patterns (Tanabe et al., 1981). DNA polymerases γ are characterized by their ability to use ribohomopolymers as templates and are found in mitochondria (Yamaguchi et al., 1980). Whereas eukaroytic DNA polymerases do not possess associated exonuclease activities, this is a property observed in prokaryotic enzymes. Prokaryotic DNA polymerases composed of a single subunit of about 100 kDa are believed to be involved in DNA repair, while multisubunit complexes are engaged in replicative DNA synthesis.

Further studies are required to elucidate the evolution of replication systems, especially the relation of their prokaryotic and eukaryotic counterparts. The investigation of organisms with an intermediate evolutionary position, e.g., lower eukaryotes, should contribute to this aspect.

Another group of organisms well suited for studies of this kind is archaebacteria. They were classified as a third urkingdom of organisms, in addition to "eubacteria" and eukaryotes (Fox et al., 1980), and possess, besides several unique features, also features shared with either eubacteria or eukaryotes (Kandler, 1982).

Isolation and characterization of enzymes involved in DNA replication are the first steps to initiate the comparative analysis of this process in archaebacteria. We have already described a DNA polymerase from a thermoacidophilic archaebacterium *Sulfolobus acidocaldarius* (Klimczak et al., 1985). To extend the basis for these studies, we present here the purification and characterization of DNA polymerase from a separate branch of this urkingdom. This enzyme has been isolated from the methanogenic archaebacterium *Methanobacterium thermoautotrophicum*.

Materials and Methods

Materials. Herring sperm DNA was activated as described by Aposhian and Kornberg (1962). Deoxyribonucleoside triphosphates and polynucleotide templates were obtained from Boehringer, Mannheim, and radiochemicals from Amersham. Heparin–agarose (Ultrogel A_4R) and hydroxylapatite (Ultrogel HA) were purchased from LKB and Blue Sepharose 6B-CL, phenyl-Sepharose 4B-CL, and Sephacryl S-200 from Pharmacia. Specially pure SDS[1] was from British Drug House and acrylamide from Bio-Rad.

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[1] Abbreviations: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; PMSF, phenylmethanesulfonyl fluoride; kDa, kilodalton(s).

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Table I: Purification Scheme of *M. thermoautotrophicum* DNA Polymerase

step	fraction	volume (mL)	total activity (units)	yield (%)	total protein (mg)	specific activity (units/mg)	purification (x-fold)
1	crude extract	80	68	100	950	0.07	1
2	heparin–agarose	40	18	26	18	1.0	14
3	Blue Sepharose	30	28	41	3	9.3	133
4	hydroxylapatite	3	9.1	13	0.09	101	1444
5	phenyl-Sepharose	2	6.2	9	0.01	620	8857
6	glycerol gradient	0.1	5.9	8.7	0.005	1180	16857

Growth of Cells. *M. thermoautotrophicum* (strain Marburg) cells were obtained from Dr. R. K. Thauer, University of Marburg, Marburg, Germany. They were grown at 65 °C under strictly anaerobic conditions according to Schönheit et al. (1980).

DNA Polymerase Assay. The assay was performed in a 40-μL mixture containing 40 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 100 μg/mL bovine serum albumin, 10 μM each of dATP, dGTP, and dCTP, 2.5 μM TTP, 1 μCi of [^{3}H]TTP (specific activity 30 Ci/mmol), and 0.1 mg/mL DNase I activated herring sperm DNA, to which 1 μL of the assayed fraction was added. After incubation at 56 °C for 30 min the reaction was terminated by addition of 10 μL of 0.5 M EDTA containing 10 mg/mL tRNA and the acid-precipitable radioactivity was determined. One unit of DNA-polymerizing activity is defined as the amount of enzyme that catalyzes the incorporation of 10 nmol of TTP under these conditions.

Nuclease Assays. A 20-μL assay mixture contained 40 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 100 μg/mL bovine serum albumin, 2 mM 2-mercaptoethanol, 0.004–0.02 unit of DNA polymerase, and the appropriate substrate. For measurement of 3′→5′-exonuclease, 2×10^4 cpm of activated DNA labeled at the 3′ end with [^{3}H]TTP to a specific activity of 2×10^5 cpm/μg was used, either native or heat denatured. For measurement of 5′→3′-exonuclease, 2×10^4 cpm of [^{32}P]dA–poly(dA) (specific activity 10^6 cpm/μg) was used, either alone or annealed to a 5-fold molar excess of poly(dT). Both assays were incubated for 30 min at 37 °C as well as at 56 °C, and the acid-soluble radioactivity was determined. The analysis of the product of the 5′→3′-exonuclease reaction was performed by thin-layer chromatography of the incubated reaction mixture on poly(ethylenimine) plates with 1 M LiCl as the mobile phase. The endonuclease assay was performed with 0.2 μg of plasmid pBR322 DNA, which was 70% supercoiled and 30% relaxed. After incubation for 30 min at 37 °C for 56 °C the DNA was analyzed on a 1% agarose gel.

Gel Electrophoresis. Electrophoresis under denaturing conditions was performed according to Laemmli (1970), using slab gels containing 4% and 10% polyacrylamide in stacking and separating zones, respectively. Proteins were visualized by a silver staining procedure described by Wray et al. (1981). Detection of active fragments of DNA polymerase in situ (activity gels) followed the protocol of Karawya et al. (1983).

Buffers. Buffer A was 50 mM Tris-HCl, pH 7.0, 5 mM 2-mercaptoethanol, and protease inhibitors; buffer B was 10 mM potassium phosphate, pH 7.0, 250 mM KCl, 5 mM 2-mercaptoethanol, 20% glycerol, and protease inhibitors. The protease inhibitors were 0.5 mM EDTA, 0.1 mM EGTA, 200 mg/L benzamidine hydrochloride, 100 mg/L β-ammoniopropionitrile fumarate, and 10 mg/L soybean trypsin inhibitor. Soybean inhibitor was omitted in the buffer used for glycerol gradient centrifugation.

Protein Determination. Protein was determined according to Sedmak and Grossberg (1977) with bovine serum albumin as the standard.

RESULTS

Purification of DNA Polymerase

All chromatographic steps and dialysis were performed at 4 °C. Intermediate fractions were stored frozen at −20 °C in the corresponding buffers. No substantial loss of activity was observed after several weeks of storage.

The purification procedure, described below, is summarized in Table I.

(*1*) *Preparation of the Cell Extract.* Twenty grams of cells were harvested by centrifugation at 5000*g* for 15 min and washed with 5 volumes of buffer A. The cell pellet was suspended in 3 volumes of fresh buffer A, and the suspension was passed through a French pressure cell at 65 MPa. PMSF was added to the suspension to a final concentration of 50 μg/mL immediately before loading the press. The resulting viscous solution was centrifuged at 32000*g* for 15 min, and the pellet was additionally extracted with 1 volume of buffer A with freshly added PMSF (centrifugation as above). The two supernatants were retained and pooled as fraction I.

(*2*) *Heparin–Agarose Chromatography.* Ten milliliters of heparin–agarose equilibrated with buffer A was added to fraction I, and the suspension was made 10 mM in $MgCl_2$. After 30-min shaking on ice, the suspension was loaded on a half-filled heparin–agarose column (2 × 6 cm) equilibrated with buffer A containing 10 mM $MgCl_2$ and the upper half of the column was formed. After washing with 10 volumes of equilibration buffer, the column was eluted with a linear gradient of 0–600 mM KCl in this buffer (10 volumes). DNA polymerase activity eluted between 400 and 450 mM KCl. The active fractions were pooled as fraction II.

(*3*) *Blue Sepharose Chromatography.* Fraction II was diluted 10 times with buffer A, and $MgCl_2$ and CHAPS were added to final concentrations of 50 mM and 0.1%, respectively. The solution was applied to a Blue Sepharose column (1 × 12 cm) equilibrated with buffer A containing 50 mM $MgCl_2$ and 0.1% CHAPS. The column was washed with 5 volumes of equilibration buffer, and the activity was eluted with a linear gradient of 10 volumes of 0–2 M KCl in this buffer as a single peak emerging at 0.9 M KCl. The peak fractions were pooled as fraction III.

(*4*) *Hydroxylapatite Chromatography.* Fraction III was loaded on a hydroxylapatite column (0.7 × 8 cm) equilibrated with 500 mM KCl in buffer A. The column was then washed with the same buffer (5 volumes) and reequilibrated with buffer B. The column was developed with 8 volumes of a linear gradient of 10–100 mM potassium phosphate, pH 7.0, in buffer B and the activity eluted between 30 and 40 mM potassium phosphate. Fractions containing DNA polymerase were pooled as fraction IV.

(*5*) *Phenyl-Sepharose Chromatography.* Ammonium sulfate was added to fraction IV (20% saturation) and the solution applied to a phenyl-Sepharose column (0.5 × 6 cm) equilibrated with 20% saturated ammonium sulfate in buffer A. The column was washed with 5 volumes of equilibration

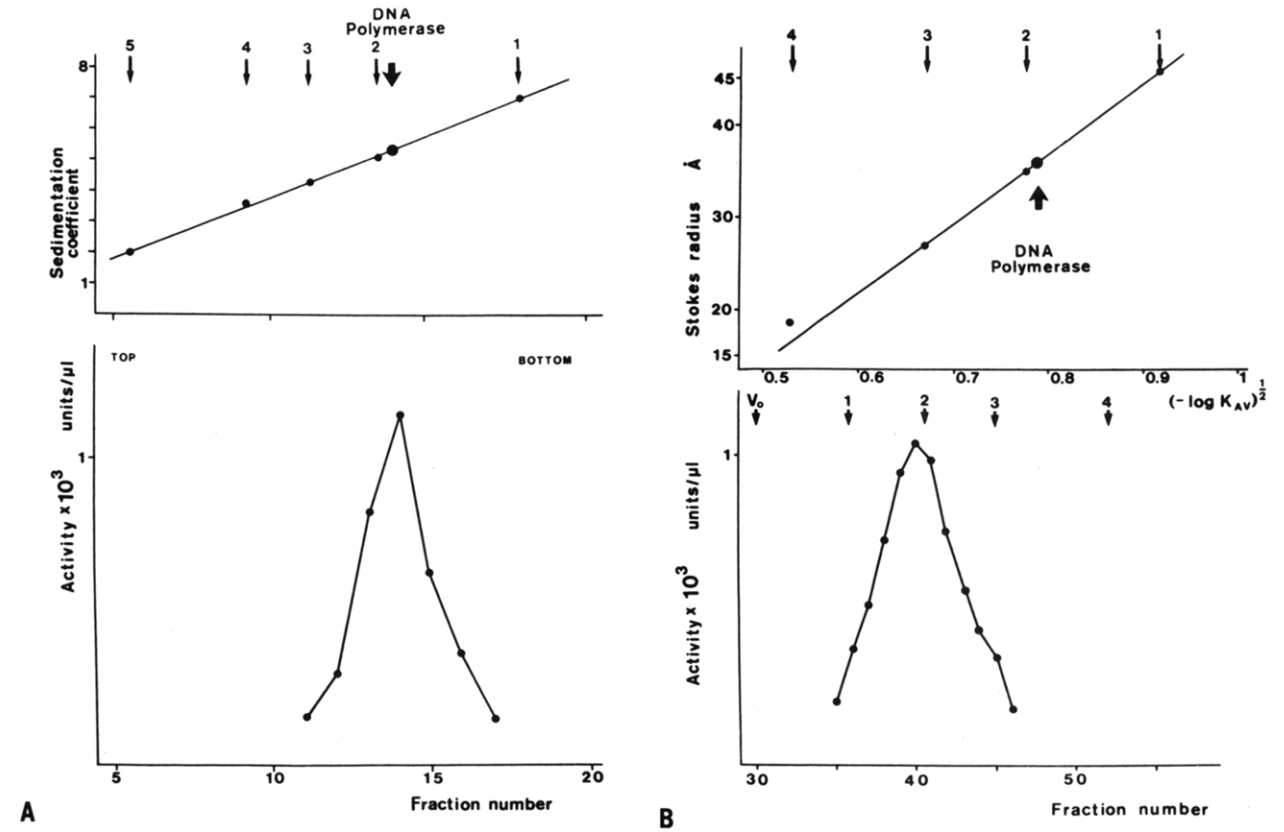

FIGURE 1: Determination of native molecular weight of DNA polymerase. (A) Glycerol gradient sedimentation. A sample of 0.2 unit of DNA polymerase (fraction IV) was loaded on a 10–30% glycerol gradient in buffer A containing 300 mM KCl, and the gradient was centrifuged at 4 °C and 62000 rpm for 16 h in an SW65 rotor. Sedimentation coefficient standards were (1) human γ-globulin ($s_{20,w}$ = 7.0 S), (2) transferrin (5.1), (3) bovine serum albumin (4.3), (4) ovalbumin (3.6), and (5) myoglobin (2.0). (B) Sephacryl S-200 gel filtration. A 0.7 × 50 cm column was equilibrated with 300 mM KCl in buffer A, and a 100-μL sample containing 0.2 unit of DNA polymerase (fraction IV) was eluted overnight. Stokes radius standards were (1) yeast alcohol dehydrogenase (46 Å), (2) bovine serum albumin (35), (3) ovalbumin (27), and (4) myoglobin (18.7).

buffer and was developed with a 8-volume linear gradient of 20–0% ammonium sulfate and 0–50% ethylene glycol in buffer A. The activity eluted as a single peak between 40 and 45% ethylene glycol. Active fractions were pooled and concentrated to 50 μL by using an Amicon microconcentrator (fraction V).

(*6*) *Glycerol Gradient Centrifugation.* Fraction V was loaded on a linear gradient of 10–30% glycerol in buffer A containing 300 mM KCl, prepared in a siliconized tube. The gradient was centrifuged for 16 h at 62000 rpm and 4 °C in an SW65 Beckman rotor. Thirty-one fractions were collected in siliconized tubes, and the active fractions were pooled and concentrated to 50 μL.

Properties of DNA Polymerase

Molecular Weight and Subunit Composition. The native molecular weight of the enzyme was estimated by the method of Siegel and Monty (1966), using gel filtration through a Sephacryl S-200 column and glycerol gradient sedimentation. The sedimentation pattern of DNA polymerase exibited an $s_{20,w}$ value of 5.2 ± 0.2 S (Figure 1A). From the position of DNA polymerase in the gel filtration eluate, a Stokes radius of 36 ● 1 Å was calculated (Figure 1B). These two values were put into the equation of Siegel and Monty (1966), and the native molecular weight of (82 ± 6)K was calculated. Polypeptide composition of fractions obtained at consecutive purification steps was analyzed by SDS–PAGE (Figure 2). An enrichment of a protein with a molecular weight of 72K can be observed in correlation with the enrichment of DNA polymerase activity. This polypeptide comprises at least 80% of the protein in the final fraction of the purification procedure. Since the molecular weight of this protein corresponds to the

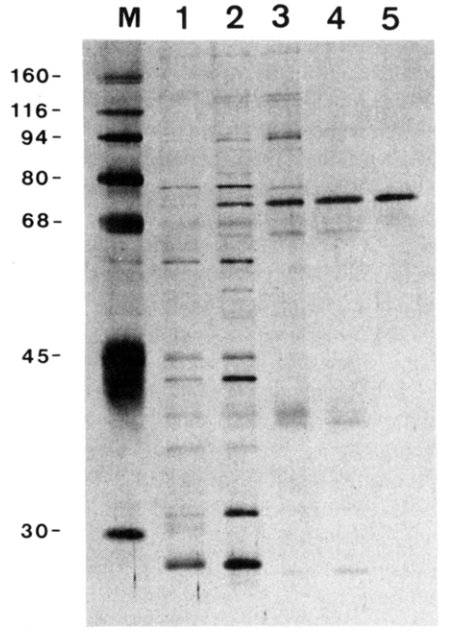

FIGURE 2: SDS–polyacrylamide gel electrophoresis. Lane labels: M, molecular weight standards (given in kilodaltons); 1, heparin–agarose pool (fraction II); 2, Blue Sepharose pool (fraction III); 3, hydroxylapatite pool (fraction IV); 4, phenyl-Sepharose pool (fraction V); 5, glycerol gradient pool (fraction VI).

molecular weight of the native enzyme, it can be concluded that the 72-kDa protein constitutes the single polypeptide of the DNA polymerase.

Activity Gels. To determine directly the molecular weight

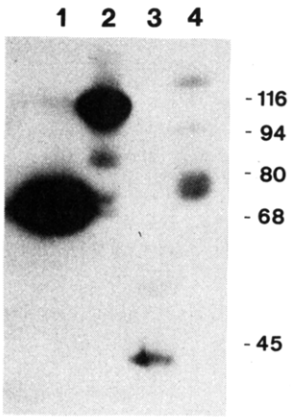

FIGURE 3: Autoradiogram of an activity gel. Lane numbers: 1, *E. coli* polymerase I, large fragment (72 kDa); 2, *E. coli* polymerase I (109 kDa); 3, crude extract (fraction I); 4, Blue Sepharose pool (fraction III). Molecular weight standards (given in kilodaltons) were run in a separate lane, which was then cut off and stained in 10% 2-propanol, 10% acetic acid, and 0.005% Coomassie Blue for 6 h and destained in 10% acetic acid and 5% methanol overnight.

of the active subunit of DNA polymerase, the technique of activity gels was used (Spanos et al., 1981). After separation by SDS–PAGE, the proteins were renatured and DNA polymerase assay was performed in situ. In fraction I (crude extract) one major activity band of 38 kDa was seen upon autoradiography (Figure 3). In fraction II and in other subsequent fractions this band was not visible anymore and bands of higher molecular weight appeared. Most of the activity was contained within a double activity band at 72 and 76 kDa, and weaker bands were observed at 100 and about 130 kDa. The 72-kDa activity band corresponds to the purified polypeptide, while the bands with higher molecular weight could be precursor forms present in amounts not detectable by silver staining. The existence of the 38-kDa form in crude extract indicates that at the initial stages of the purification the DNA polymerase is strongly affected by proteolytic cleavage (see Discussion).

pH, Salt, and Temperature Optima (*Table II*). The enzyme has a broad pH profile with a maximum at pH 8.0 and 60% of the maximal activity at pH 6.0 and pH 9.5.

The activity is absolutely dependent on Mg^{2+} cations, revealing a plateau of maximal activities between 10 and 20 mM $MgCl_2$. Mn^{2+} cannot replace Mg^{2+} very efficiently, and only 7% of activity found with the latter is observed at the optimal concentration of the former (0.5 mM).

The DNA polymerase was stimulated by KCl with an optimal concentration of 100 mM.

The activity of the enzyme was 10 times higher at the optimum of 65 °C than at 37 °C. At still higher temperatures it decreased rapidly; after 10-min pretreatment at 100 °C no activity was detected even at the optimal temperature.

Template Specificity (*Table II*). Activated DNA was the optimal template; no activity was found in the absence of primer [poly(dA) and poly(dT) as templates, with addition of corresponding priming ribonucleotides] nor with the polyribonucleotide template [poly(rA)·oligo(dT)].

Inhibitors (*Table II*). The DNA polymerase activity was unaffected by aphidicolin even at concentrations 20 times higher than those acting on eukaryotic polymerase α. It was slightly inhibited by dideoxy analogues and more effectively by arabinosine analogues and SH-blocking agents.

Associated Nuclease Activities. Endonuclease activities were removed from the DNA polymerase preparation on Blue Sepharose. A 3′→5′-exonuclease was detected in all fractions, and after chromatography on Blue Sepharose it remained in

Table II: Properties of DNA Polymerase from *M. thermoautotrophicum*

conditions	% activity (100%, standard conditions)	conditions	% activity (100%, standard conditions)
salt optimum		template specificity	
0 mM KCl	100	no DNA	1
100 mM KCl	165	activated DNA	100
		poly(dA)	2
200 mM KCl	140	poly(dA) and 1 mM UTP	1
		poly(dT)[a]	1
300 mM KCl	90	poly(dT) and 1 mM ATP[a]	1
		poly(dA)·oligo(dT)	72
400 mM KCl	20	poly(rA)·oligo(dT)	2
temperature optimum		inhibitors	
		1 mM arabinosyl-CTP	32
		1 mM *N*-ethylmaleimide[b]	74
37 °C	15	25 μM ddTTP[c]	89
56 °C	100	20 μg/mL aphidicolin	100
65 °C	160		
80 °C	25		

[a] [^{3}H]dATP incorporated. [b] 2-Mercaptoethanol omitted. [c] ddTTP:dTTP = 10:1.

a constant ratio to DNA polymerase and solubilized about 40% of the input radioactivity per 0.005 unit of the latter. This exonuclease activity was, similarly to DNA polymerase, strongly stimulated by monovalent cations (170% activity at 100 mM KCl). Its dependence on temperature was not very pronounced (1.6 times stimulation at 56 °C compared to 37 °C). This exonuclease showed a limited preference for single-stranded substrate (activity with denatured template was 1.5 times higher than with the native one). A 5′→3′-exonuclease was also detected up to the final fraction. After heparin–agarose chromatography, it was present at a constant ratio to DNA polymerase (40% of input radioactivity released per 0.01 unit of DNA polymerase). This exonuclease was inhibited by salt (90% inhibition at 100 mM KCl) and was strongly temperature dependent (5 times stimulated at 56 °C in relation to 37 °C). It did not show any preference for the form of the substrate. The products of this exonucleolytic reaction were shown to be monophosphonucleotides.

Discussion

As demonstrated above by SDS–PAGE and gel filtration, the highly purified preparation of *M. thermoautotrophicum* DNA polymerase contains a 72-kDa protein that constitutes the enzyme's single polypeptide. This conclusion is supported by activity gel analysis, which reveals an active polypeptide of 72 kDa.

In addition, activity gels reveal in purified fractions several weaker active polypeptides in the range 76–130 kDa. Interestingly, in crude extract only a single polypeptide of 38 kDa is seen. The possibility that the purified fractions contain highly enriched, minority species of the polymerase does not seem probable—a drastic change of the pattern of the active polypeptides takes place in just one step, after chromatography on heparin–agarose. This result could be better explained by proteolytic cleavage of the enzyme—a phenomenon frequently observed with various DNA polymerases (Setlow et al., 1972; Hübscher et al., 1981; Misumi & Weissbach, 1982). DNA polymerase from another archaebacterium, *S. acidocaldarius*, also showed in crude extract an activity polypeptide smaller than that present in purified fractions (Klimczak et al., 1985). The degradation of the *Sulfolobus* DNA polymerase was not very extensive, which allowed a precise correlation of activity and silver-stained bands. As discussed in that work, protease

inhibitors can prevent proteolysis in refrigerated and frozen extracts but are inefficient during the denaturation step of the sample to be loaded on an activity gel. Under the mild denaturing conditions, the proteases present in crude extract can even become activated and generate smaller fragments of corresponding proteins (Pringle, 1975). Such a degradation could explain several examples of smaller active subunits of DNA polymerases observed in activity gels in crude extract and partially purified fractions (Hübscher et al., 1981; Scovassi et al., 1982; Badaracco et al., 1983). In the case of another enzyme from *M. thermoautotrophicum*, methyl coenzyme M reductase, the emergence of lower molecular weight polypeptides has been observed upon storage of the preparations (R. K. Thauer, personal communication), which suggests that significant degradation of proteins can take place in extracts from this organism. After the proteases are removed upon further chromatography, the active subunits can be observed in activity gels in their actual, uncleaved size; therefore, the change of the size of activity polypeptides can occur after a single chromatographic step. In the case of *M. thermoautotrophicum* such a change takes place after chromatography on heparin–agarose and was even observed after the same step in a different purification procedure where heparin–agarose chromatography was performed at a later stage (data not shown). This indicates that the putative proteases are removed on heparin–agarose. Of the activity bands of higher molecular weight observed in purified fractions, the 72-kDa active polypeptide corresponds to the purified polypeptide. Activity polypeptides larger than 72 kDa could be precursors of this form. They are not visible in the final purified fraction, which suggests that they are probably present in low amounts, but renature very efficiently and can be detected in activity gels [see Spanos et al. (1981)]. Since the 72-kDa band represents the smallest activity polypeptide of this group, it could be in fact the most stable species of DNA polymerase, at which the proteolytic cleavage would stop under nondenaturing conditions, allowing its accumulation. Only under mild denaturing conditions could the degradation proceed and the 38-kDa form would be generated. Taking into account the largest active polypeptide detectable, the starting precursor of DNA polymerase could have a molecular weight of about 130K. Whether the 72-kDa polypeptide would be cleaved off from the putative precursors already in vivo or whether it would be artificially generated during purification can be resolved by techniques reducing still more the exposure of DNA polymerase to the action of proteases, e.g., the application of monoclonal antibodies for rapid purification of the enzyme. Such techniques can help to define the precursor–product relationship, as they do in the case of eukaryotic DNA polymerase α (Wahl et al., 1984; Masaki et al., 1984).

The properties of the enzyme are consistent with the physiological characteristics of the organism. The enzyme is typically thermophilic, with its temperature optimum the same as that for *M. thermoautotrophicum* growth (Schönheit et al., 1980). It is also stimulated by KCl, although the optimum is lower than 700 mM, the concentration found intracellularly in this bacterium (Sprott & Jarell, 1981). The DNA polymerase is not sensitive to oxidizing atmosphere, as the methanogenic archaebacteria and some of their biochemical pathways are (Gunsalus & Wolfe, 1978; Jones & Stadtman, 1981). DNA replication probably did not converge with other biochemical adaptations to dependence on a reducing atmosphere.

The properties of the purified DNA polymerase from *M. thermoautotrophicum* clearly do not resemble those of the eukaryotic DNA polymerase α, especially in its insensitivity to aphidicolin. The enzyme differs also from eukaryotic polymerases β and γ as well as from the prokaryotic replicative complex, like *Escherichia coli* polymerase III. Being a single polypeptide of about 100 kDa with associated exonuclease activities, it bears a gross resemblance to prokaryotic DNA polymerases involved in DNA repair, like *E. coli* polymerase I, although those enzymes do not constitute a homogeneous group with relations established by simple biochemical criteria. In this regard, the described enzyme is similar also to DNA polymerase isolated from *S. acidocaldarius* (Klimczak et al., 1985). However, there are clear differences between these two enzymes. The *Sulfolobus* enzyme does not possess a 5′→3′-exonuclease. This could be still a minor difference, since the absence of this activity is also observed, e.g., in *Bacillus subtilis* DNA polymerase I, an enzyme believed nevertheless to be related to *E. coli* polymerase I, in which this activity is present. DNA polymerases from *S. acidocaldarius* and *M. thermoautotrophicum* show significant differences in their Mg^{2+} optima, which could indicate structural differences in the active centers. The enzymes differ also in optima for monovalent cations and binding properties to several chromatographic materials. These pronounced differences are in agreement with the fact that the two arachaebacteria belong to two distinct and probably little related branches of the archaebacterial urkingdom. Their precise relationship can be evaluated only on the basis of structural data.

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Nucleotide Sequence of the cDNA Coding for Human Complement C1r†

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ABSTRACT: C1r is a zymogen of a serine protease that is involved in the activation of the first component of the classical pathway of the complement system. cDNAs coding for human C1r have been isolated from libraries prepared from poly(A) RNA from human liver and Hep G2 cells. From DNA sequence analysis, the overlapping cDNA inserts were shown to span 2493 nucleotides of the C1r mRNA, not including the poly(A) tail. The cDNA sequence coding for C1r contained a 5′ noncoding region, 2115 nucleotides coding for a polypeptide precursor of 705 amino acids, and a 3′ noncoding region. Some variability in the length of the 3′ noncoding sequence was observed with the cDNA inserts, although most contained a polyadenylation signal followed by a poly(A) tail. The A or noncatalytic chain of $C\bar{1}r$, which originates from the amino-terminal end of the precursor molecule, contains a potential growth factor domain and two different pairs of internal repeats. One pair of these internal repeats is closely related to the amino-terminal sequence of C1s, while the other pair of repeats is homologous to the tandem repeats present in β_2-glycoprotein I, complement factor B, the b subunit of factor XIII, and a single region present in the α^1 chain of haptoglobin. The B chain of $C\bar{1}r$ contains the catalytic portion of the enzyme and is homologous to the trypsin family of serine proteases.

Plasma serine proteases participate in a variety of physiological processes, such as blood coagulation (Davie et al., 1979), fibrinolysis (Christman et al., 1977; Collen, 1980), and complement activation (Muller-Eberhard, 1975; Porter & Reid, 1979; Reid & Porter, 1981). They exist in plasma as single- or two-chain zymogens that are activated by specific and very limited proteolytic cleavage (Neurath & Walsh, 1976). These serine proteases also show considerable structural similarities in their catalytic chains.

Complement C1r is one of three distinct glycoproteins that comprise the first component of the classical pathway of complement (Porter & Reid, 1979; Reid & Porter, 1981; Sim, 1981). C1r along with C1q and C1s forms a calcium-dependent complex referred to as component C1. C1q recognizes and binds to antibody–antigen complexes, whereas C1r and C1s are zymogens of serine proteases that participate as enzymes in the early phase of complement activation. The binding of C1q to immune complexes is thought to induce conformational changes within the C1 complex, resulting in an autocatalytic activation and conversion of C1r to the serine protease $C\bar{1}r$. $C\bar{1}r$ then activates C1s, converting it to the active serine protease $C\bar{1}s$. The latter, in turn, activates complement components C2 and C4.

C1r is a single-chain glycoprotein with a molecular weight of about 83 000 (Sim et al., 1977). Upon activation, it is cleaved into an A chain (M_r 56 000) and a B chain (M_r 27 000), and these two chains are held together by a disulfide bond. The A chain, which includes the amino-terminal portion of the precursor protein, is thought to participate in the initial reactions leading to the activation of component C1. It has been partially sequenced by Gagnon and Arlaud (1985). The complete amino acid sequence of the B chain of human $C\bar{1}r$ has been determined and shown to contain the catalytic region of the enzyme (Arlaud et al., 1982; Arlaud & Gagnon, 1983).

As a general approach to isolating cDNAs coding for serine proteases synthesized in the liver, a strategy was chosen that involved the screening of a human liver cDNA library with a short synthetic oligodeoxynucleotide probe coding for a highly conserved region near the active site in a variety of different serine proteases. In this manner, clones were isolated

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