# MULTIPLE DNA POLYMERASES IN *ACINETOBACTER CALCOACETICUS*

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## 1. Introduction

The synthesis of DNA is thought to be catalyzed by deoxynucleotide polymerizing enzymes. In the case of procariotic organisms it has recently been shown that *E. coli* and *Bacillus subtilis* possess three different DNA polymerases termed I, II and III [1–5]. DNA polymerase I from *E. coli* was the first enzyme to be characterized and its mechanism of action has been studied extensively [1]. The exact role the various enzymes play in the replication and repair process has not yet been adequately defined. Before any general conclusion can be drawn as to the mechanism of DNA synthesis in bacteria it is necessary to investigate a wide variety of different organisms.

In this laboratory we have for some time been interested in the mechanism of synthesis of nucleic acid in the bacterium *Acinetobacter calcoaceticus* [6]. This is a very widely occurring species which differs from *E. coli* in many respects [7,8]. In the present report we describe the isolation and some properties of the DNA polymerases in this organism.

## 2. Materials and methods

### 2.1. *DNAs and nucleoside triphosphates*

Calf thymus DNA, poly [d(A-T) · d(A-T)] and unlabelled nucleoside triphosphates were products of the Sigma Chemical Company. [$^3$H]- and [$^{14}$C]dATP were obtained from Amersham Radiochemical Corporation. Activated calf thymus DNA was prepared according to a published procedure [9]. $^{14}$C-labelled poly [d(A-T) · d(A-T)] was a gift from Dr. Osland, Bergen.

### 2.2. *Assay system*

The assay system contained 67 mM Tris–HCl ph 8.0, 1.7 mM dithiothreitol, 6.7 mM $MgCl_2$, 33 μM dXTP, 130 μM (P) activated calf thymus DNA or 20 μM (P) poly [d(A-T) · d(A-T)]. The assay period was usually 30 minutes and the temperature 37°C. The radioactively labelled DNA synthesized was precipitated on filter papers as previously described [10]. One unit of activity is defined as the amount of enzyme which catalyzes the incorporation of 10 nmoles nucleotides under the conditions defined above.

The nuclease activity of the various DNA polymerases was assayed using the same buffer system as employed for synthesis except that the nucleoside triphosphates were omitted from the reaction mixture and 50 nmoles [$^{14}$C]poly [d(A-T) · d(A-T)] per ml was added. The degradation was followed by withdrawing aliquots at various time intervals and pipetting these onto DEAE paper strips which were subsequently developed in 0.3 M ammonium formate. The radioactive spots were then cut out and counted in a liquid scintillation system.

### 2.3. *Determination of proteins*

Proteins were determined as described by Lowry et al. [11].

### 2.4. *SDS-gel electrophoresis*

Shandon gel electrophoresis apparatus was employed, the gels being 7.5% polyacrylamide. The proteins were stained with Coomassie Brilliant Blue and

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destaining was carried out in 7% acetic acid. The molecular weight was determined according to a published procedure [12]. The marker proteins were RNA and DNA polymerase I from *E. coli,* bovine serum albumin, hen egg albumin and chymotrypsin.

2.5. *Source of bacteria*

The strain of *A. calcoaceticus* used was originally obtained from the National Type Culture Collection No. 7363.

## 3. Results

3.1. *Purification*

The bacteria were grown in a yeast extract medium and harvested in the late logaritmic phase, then stored at −20°C. All purification steps were carried out at +4°C.

One-hundred-fifty g cells, wet weight, were mixed with 200 g glass beads and 100 ml of buffer I containing 50 mM $KP_i$ pH 7.0 and 2 mM β-mercaptoethanol was added. After mixing at high speed in a Waring Blendor for 15 min the glass beads were separated and washed twice with an additional 50 ml of buffer. The cell debris were then removed by centrifugation at 10 000 *g* for 30 min and the supernatant made 10 mM with respect to $MgCl_2$. Pancreatic DNase and RNase, 12 mg and 5 mg, were added to the solution which was then subsequently left at room temperature for 60 min. This treatment was necessary in order to break down the nucleic acids to which most of the polymerase activity appeared to be bound. Upon completion of this degradation process the mixture was dialyzed overnight against 5 l of buffer I, after which it was loaded on a DEAE-cellulose column (15 × 7 cm) equilibrated with buffer 1. The column was first washed with 500 ml of buffer I containing 0.1 M KCl, then with 500 ml of buffer I containing 0.4 M KCl. A major peak of protein was eluted off the column with the latter buffer. This peak was pooled (540 ml) then fractionated by ammonium sulfate; first 0–40% saturation, then 40–60%. At least 90% of the polymerase activity was in the latter fraction. This precipitate was dissolved in 20 ml of buffer I. An aliquot of 10 ml of this fraction was subjected to gel filtration on a column of Agarose 1.5 M (95 × 2.6 cm) equilibrated with buffer I. The separation profile is shown in fig. 1. A broad peak of DNA polymerase activity was obtained, which was pooled as shown, the main fraction called I (55 ml) and the leading edge II (58 ml). The remaining part of the ammonium sulfate precipitate was then passed through the column and pooled in the same manner. The pooled fractions I were then loaded on to a column of phosphocellulose (25 × 2 cm) equilibrated with buffer II, 25 mM $KP_i$ pH 7.0 and 2 mM β-mercaptoethanol. After absorption the column was first washed with 200 ml of buffer II then with a gradient of 1400 ml of buffer II containing from 0–0.4 M KCl. The elution profile is shown in fig. 2. The amount of proteins that came off the column was too little to be measured accurately. Two peaks of activity were, however, obtained, termed A and B. The first peak was eluted at approximately 0.15 M KCl and the second at approximately 0.25 M KCl. The pooled fractions II from the Agarose column were also subjected to chromatography on the same phosphocellulose column using the same gradient. In this case a small peak of DNA polymerase activity was found at approximately 0.12 M KCl as well as some traces at 0.15 M and 0.25 M KCl. The enzyme eluted at 0.12 M KCl has been named DNA polymerase C. The three polymerases were concentrated employing a small column of phosphocellulose (2 × 1 cm) and stored at −20°C in 0.1 M $KP_i$ pH 7.0, 1 mM DTT and 50% glycerol.

The yield and specific activity assayed with calf

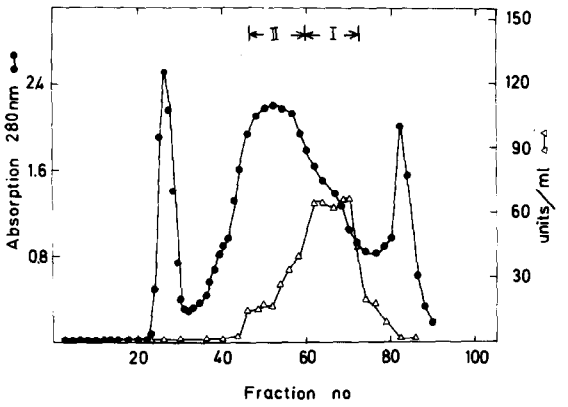


Fig. 1. Purification of DNA polymerases by gel filtration on 1.5 M Agarose. The column (95 × 2.6 cm) was equilibrated with 50 mM $KP_i$ pH 7.0, 2 mM β-mercaptoethanol. Each fraction was 5 ml.

Table 1

Yield and specific activities of the various DNA polymerases

| DNA | DNA pol A specific activity* | Yield** | DNA pol B specific activity | Yield | DNA pol C specific activity | Yield |
|---|---|---|---|---|---|---|
| Calf thymus DNA | 930 | 450 | 260 | 640 | 38>*** | 90 |
| Poly [d (A-T) · d (A-T) ] | 5100 | 2500 | 640 | 1480 | 80> | 210 |

*Specific activity units/mg protein.
**Yield in units.
***The protein concentration was too low to be measured accurately.

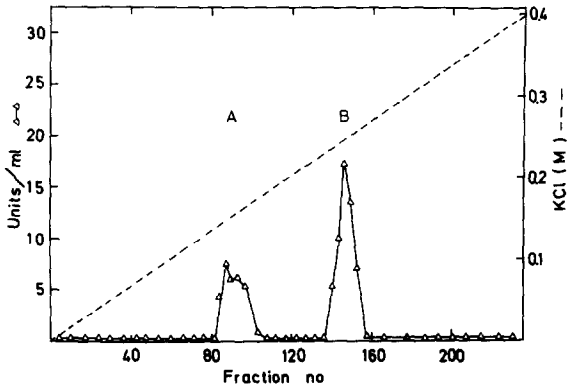


Fig. 2. Purification of DNA polymerases by chromatography on phosphocellulose. The column (25 X 2 cm) was equilibrated with 25 mM $KP_i$ pH 7.0, 2 mM β-mercaptoethanol and the gradient, 1400 ml, was run in the same buffer. Each fraction contained 5 ml.

thymus and poly [d (A-T) · d (A-T)] are shown in table 1. All polymerases gave higher specific activity with poly [d (A-T) · d (A-T)] as a template than with calf thymus DNA. This was most pronounced with DNA polymerase A. The specific activity of this enzyme compares well with that of DNA polymerase I from *E. coli* assayed in the absence of exonuclease III [13]. The overall yield, i.e. the total activity recovered was approximately 7% of that from crude extract.

### 3.2. *Nuclease activity*

The nuclease activity of the various DNA polymerases was tested with the [$^{14}$C]poly [d (A-T) · d (A-T)] assay and the results are shown in table 2. DNA

Table 2
Nuclease activity of the various DNA polymerases

| | nmoles [$^{14}$C]poly[d(A-T)] d(A-T)] degraded by 1 unit DNA polymerase (poly[d(A-T) · d (A-T)] unit) in 30 min at 37°C | Relative |
|---|---|---|
| DNA polymerase A | 0.04 | 4.5 |
| DNA polymerase B | 0.92 | 100 |
| DNA polymerase C | 0.01 | 1.3 |

polymerase B has a very powerful nuclease activity associated with it whereas the nuclease activity in DNA polymerase A and C was much less.

### 3.3. *Purity and subunit composition*

DNA polymerase A and B were subjected to SDS gel electrophoresis together with marker proteins, fig. 3. In the case of DNA polymerase C the protein concentration was too low for detection on the gels. DNA polymerase A showed one major band with average mol. wt. of approximately 93 000. DNA polymerase B also gave one single band having average mol. wt. of approximately 130 000. The molecular weights of DNA polymerase A and B were also estimated by means of gel filtration using a column of Sephadex G-150 (90 X 1.5 cm). Similar results were obtained as for SDS-gel electrophoresis.

## 4. Discussion

The present work describes purification of three


Fig. 3. SDS gel electrophoresis of DNA polymerase A and B. Approximately 15 μg proteins were applied to the gels.

different DNA polymerases from *A. calcoaceticus*. Two of these enzymes, DNA polymerase A and B, were purified to at least 90% homogeneity and their molecular weights estimated. The question then arises how these enzymes are related to DNA polymerase I, II and III from *E. coli*. In the latter organism it has been estimated that DNA polymerase I accounts for at least 90% of the total deoxynucleotide polymerizing activity [2–4]. In the case of *A. calcoaceticus* DNA polymerase A and B accounted for 38 and 55% of the activity using nicked calf thymus DNA as a substrate. If one in using the affinity for phosphocellulose as a criterion to distinguish between the various DNA polymerases as in the case of *E. coli* then DNA polymerase A might correspond to DNA polymerase I, B to DNA polymerase II and C to DNA polymerase III [2–4]. However, the amount of DNA polymerase II present in *A. calcoaceticus* is then much more than that found in *E. coli*. Furthermore, the substrate specificity for DNA polymerase II and III from *E. coli* is known to be quite different from that of DNA polymerase I [2,4]. Particularly poly [d(A-T)-· d (A-T)] has been found to be a rather inefficient template for these two polymerases. In the case of the polymerases from *A. calcoaceticus* all three showed higher activity with poly [d (A-T) · d-(A-T) ] than with calf thymus DNA. This was particularly the case with DNA polymerase A. Based on these facts we think it is unlikely that DNA polymerase B is a DNA polymerase II type enzyme. Instead we favour the following hypothesis: In *E. coli* it is known that DNA polymerase I can be cleaved into two fragments [14,15]. One of these contains the polymerizing activity and $3'$-$5'$ exonuclease activity and possesses a mol. wt. of approximately 70 000. The other fragment has a mol. wt. of approximately 30 000 and contains the $5'$-$3'$ exonucleolytic activity. In the case of *A. calcoaceticus* DNA polymerase A might be a fragment of DNA polymerase B containing the polymerizing activity. DNA polymerase B would then correspond to DNA polymerase I. The molecular weights, affinity for phosphocellulose and exonucleolytic activity of the two enzymes are in complete agreement with this view. The fact that the amount of DNA polymerase A varied considerably from one preparation to another also supports this hypothesis.

Regarding DNA polymerase C it is possible that this is a DNA polymerase III type enzyme. Both the elution volume from Agarose [4] and affinity for phosphocellulose [3] favour this hypothesis. Further studies are now in progress dealing with physical and enzymatic properties of the various polymerases.

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