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Purificaion and Characteristic Properties of DNA Polymerase α from Sea-Urchin, *Hemicentrotus pulcherrismus*

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From the sea-urchin, *Hemicentrotus pulcherrismus*, we have purified by four column chromatographic steps for DNA polymerase α activity.

The molecular weight of DNA polymerase α was determined to be around 137,000-138,000 by Sephadex G-200 gel filtration and SDS-polyacrylamide gel electrophoresis. The purified enzyme had the optimal activity at pH 7.4. This enzyme showed to be a function of the metal ion K^+, Na^+ and Mg^{2+} employed as activators, the optimum K^+ or Na^+ concentration were 20 *m*M or 25*m*M and the optimum Mg^{2+} concentration was 10 *m*M. The enzyme activity was inhibited by N-ethyl-maleimide, aphidicolin, cytosine β-D-arabinofuranoside 5′-triphoshate (*ara* CTP) and phosphonoacetic acid.

Introduction

Higher eukaryotic cells contain at least three distinct DNA polymerases which have named DNA polymerases(deoxynucleoside triphosphate, DNA deoxynucleotide transferase, E.C. 2.7.7.7).

A nomenclature for the known eukaryotic DNA polymerases was proposal in 1975[1]. This classification which recognized three major classes of cellular DNA polymerase α, β and γ proved to be applicable to a wide range species in the animal kingdom[2].

DNA polymerase α was first isolated from calf thymus and characterized by Bollum[3] and his work was an important starting point in the study of eukaryotic DNA polymerases. DNA polymerase α has been purified from calf thymus[3], sea urchin[4], human cells[5] and yeast[6] among others. Furthermore, the description and properties of these enzymes and initial attempts to understand their metabolic role have been the subject of several reviews [7,8]. Information concerning the function of the different eukaryotic DNA polymerase has only been obtained by studying the relationship of the DNA polymerases. It is generally agreed that DNA polymerase α has a key role in the replication of nuclear DNA and in the synthesis of the DNA in eukaryotic cells[9,10]. Understanding the role of polymerase has been aided by the availability of specific inhibitors such as sulfhydryl group blockers[7], antibiotics[11,12] or the arabinose-cotnaining nucleotides such as *ara*-ATP or *ara*-CTP. The use of these inhibilors supports the concept that DNA polymerase α is the major replicative polymerase in mammalian cells[10,13,14].

Our knowledge of the eukaryotic DNA polymerase is still in the descriptive stage. Most of the investigations have involved animal cells, tissues and virus-infected tissue culture cells. Detailed studies in other organisms and biological systems that offer advantages not seen in eukaryotic systems should be important. In views of these biology of eukaryotic systems, sea urchin embryos could offer powerful genetic and biochemical approaches to complement the DNA polymerase[15,16]. It is worth repeating that finding the role of DNA polymerase in DNA replication will most likely require a knowledge of the role of accessory factors. In this pa-

per, we represent the evidence that DNA polymerase α from the sea urchin embryos, *Hemicentrotus pulcherrismus*, can be highly purified and obtained some results from the purified DNA polymerase α.

Materials and Methods

Chemicals and enzymes

Calf thymus DNA, cytosine β-arabinofuranoside 5′-triphosphate were purchased from Sigma, phosphonoacetic acid was purchased from Aldrich, [^{3}H] dTTP and [^{3}H] dTTP were purchased from New England Nuclear. DEAE-cellulose, hycroxylapatite, Blue Sepharose were purchased from Pharmacia Fine Chemicals. Phosphocellulose was purchased from Whatman. Other chemicals were of reagent grade.

Growing embryos

Sea urchin, *Hemicentrotus pulcherrismus*, mature eggs and sperm were collected from sea urchin by removing the mouth parts with forceps and injecting 5-10 *ml* of 0.5 M KCl into the column. The eggs were collected by placing the sea urchin at the top of a beaker containing sea water. This system was used with artificial sea water because sea urchin sperm are very sensitive to heavy metals which interfere with fertilization. Artificial sea water was prepared from 20 liters of distilled water, with salt as follows, NaCl, 521 *g*;MgCl $6H_2O$, 104 *g* $MgSO_4$ $7H_2O$, 90 *g*;KCl, 13.8 *g*;$NaHCO_3$, 4 *g*;$CaCl_2$ 28.6 *g*. The sperms were collected into small beaker with sea water. The eggs were washed 2-3 times with sea water to remove the gelatinous coat which otherwise prevents fertilization. Washing was accomplished by stirring the eggs with a hand in 100 volumes of sea water, following them to settle and then decanting of the sea water. The process was repeated 2-3 times. The washed eggs were suspended in about 10 volumes of 17°C sea water prior to fertilization. The volume of packed eggs was estimated by putting 10 *ml* of final eggs suspension in a graduated 20 *ml* mess centrifuge tube, centrifuging at 5,000×*g* for 10 *min*, and measuring the volume occupied by the eggs. On the other hand, the sperms were activated by diluting them into sea water to make a slightly turbid suspension. As the eggs were stirred this suspension was added to them in the ratio of 1*ml* of sperm to 100 *ml* of eggs. Fertilization occurred immediately as evidenced by microscopically observing the formation of fertiliation membranes around the eggs. The embryos were stirred gently at 17°C until the hatching stage (17 *h*). After all have been hatched, stirring was stopped. The cultures cooled at 5°C and the embryos are allowed to settle on the bottom. The supernatant was drawn off and the remaining cultures were centrifuged at 2,000×*g* for 10 min to collect the embryos and as a pellet at −20°C. We shed 160 of *Hemicentrotus pulcherrismus* to get 1.300 *ml* packed eggs.

DNA polymerase assay

DNA polymerase activities were measured by modification of the methods[17,18]. The reaction mixture for measuring DNA polymerase activities contained the following components. The reaction mixture;25 *m*M KCl, 1 *m*M 2-mercaptoethanol, 4*m*M ATP, 100 *μM* each dATP, 6.7 *μM* [^{3}H] dTTP(specific activity 0.5 Ci/*nmol*), 20 *μg* activated calf thymus DNA, 50 *μl* enzyme fraction. Activated calf thymus DNA was prepared by the method of Aposhian and Kornberg[19]. After incubation at 28°C for 60 *min* an aliquot of the assay mixture was applied on a Whatman (CF/C) filter and washed with 10 *ml* of cold 1% trichloroactic acid and 5 *ml* of ethanol. The filters were dried and incorporation of labeled nucleotide into acid-insoluble material was counted in a liquid scintillation counter[20]. One unit of enzyme activity was defined as the activity sufficient to convert 1 *nmol* of total nucleotide into acid-insoluble material in 60 *min*.

Purification of DNA polymerase α

DNA polymerase α was purified from sea urchin

embryo through step Ⅵ[21]. About 400 g of sea urchin embryos were thawed dna washed twice in 20 ml of cold 1 M dextrose using the low speed centrifugation. The pellet was suspended in 50 *m*M potassium phosphate, pH 7.4, 0.1 *m*M EDTA. 0.2 *m*M 2-mercaptoethanol, 10% glycerol, and 0.5 M KCl. The tissues were disrupted by a glass homogenizer tube with two strokes of a Teflon pestle using Stir R-motor at 1,000 *rpm* with intermittent cooling. The homogenate was then centrifuged at 15,000×*g* for 30 *min* and the pellet was discarded. The supernatant fraction was filtered through four layers of cheese cloth and centrifuged for 30 *min* at 10,000×*g*. The resulting supernatant were pooled and are referred to as fraction Ⅰ.

Ammonium sulfate precipitation: Solid $(NH_4)_2SO_4$ was added to fraction Ⅰ to final concen tration of 60% saturation. The protein preciptate was collected by centrifugation and redissolved by addition of 250 *ml* of 25 *m*M potassium phosphate, pH7.4, 5 *m*M 2-mercaptoethanol and 0.5 *m*M EDTA. The redissolved $(NH_4)_2SO_4$ fraction (800 *ml*) was dialyzed for 18 hr against 10 liters of *m*M 25 potassium phosphate, pH 7.4, 5 *m*M 2-mercaptoethanol and 0.5 *m*M EDTA. The resulting precipitate is removed by centrifugation. The supernatant fraction Ⅱ is immediately loaded to phosphocellulose chromatography (Fraction Ⅱ).

Phosphocellulose column chromatography: Fraction Ⅱ was diluted with 50 *m*M potassium phosphate, pH 7.4, 5 *m*M 2-mercaptoethanol, and was loaded onto a phoshocellulose column (4.5×25 *cm*) previously equilbrated with samebuffer and then were eluted with a 0 to 0.5 M potassium phoshate linear gradient pH 7.2 and 5 *m*M 2-mercapoethanol. The elued DNA polymerase α fraction was precipitated with 50% saturation of ammonium sulfate and the precipitate was dissolved in 50 *m* Mpotassium phosphate, pH 7.4, 5 *m*M 2-mercaptoethanol, 10% glycerol and dialy.ed against two change of 2 liters of lhe same buffer (Fraction Ⅲ).

DEA-cellulose column chromatgoraphy: Fraction Ⅲ was loaded onto DEAE-cellulose colum (2.6×25 *cm*) previously equilibrated with 25 *m*M potassium phosphate, pH 7.4, 5 *m*M 2mercapthanol and 10% glycerol. The column was washed with the same buffer and eluted with linear gradient of 0 to 0.5 M potassium phosphate, pH 7.4, 50 *m*M KCl, 5 *m*M 2-mercaptoethanol and 10% glycerol. Active fractions were pooled as Ⅳ.

Hydroxylapatite column chromatography: Fraction Ⅳ was loaded onto a hydroxylapatite column(1.5×15 *cm*) previously equilibrated with 50 *m*M potassium phosphate and 10% glycerol. The column was washed with same buffer and eluted with 250 *ml* linear gradient of 0 to 0.5 M potassium phosphate, pH 7.4, 5 *m*M 2-mercaptoethanol and 10% glycerol. All enzyme were eluted from the column showing a symmetrical peak at 0.15 M potassium phoshate. The active fraction were pooled (Fraction Ⅴ).

Bleue Sepharose chromatography: Fraction Ⅴ was dialyzed against 50 *m*M potassium phosphate, pH 7.4, 50 *m*M KCl, 5 *m*M 2-mercaptoethanol and 10% glycerol and then loaded to Blue Sepharose column (1.0×10 *cm*) previously equilibrated with 50 *m*M potassium phoshate, pH 7.4, 50 *m*M KCl, 5 *m*M 2-mercaptoethanol and 10% glycerol. The column was washed with the same buffer. The polymerase activity was eluted with a linear gradient 0 to 0.5 M KCl, in 0.05 M potassium phosphate pH 7.4, 5 *m*M 2-merca ptoethanol and 10% glycerol. Active fraction, Fraction Ⅵ were pooled and stored at −20°C.

Determination of molecular weight

SDS-polyacrylamide ge ellectrophoresis: The method was performed essentially according to the procedure described by Laemmli[22] with 8% polyacrylamide and 0.2% sodium dodecyl sulfate in the running gel. Proteins to be analyzed were denatured for 2 *min* at 95°C in a buffer containing 3% sodium dodecyl sulfate and 5% 2-mercaptoethanol. Standard proteins such as catalase (230,000), γ-globulin (160,000), bovine serum albumin (67,000), ovalbumin (45,000) and myoglobin (17,000) were run simultaneously with DNA polymerase α on the gel. After electrophoresis, the gels were stained with Coomasie brilliant blue, and then destained. The molecular weight of enzyme was calculated from

the plot of electrophoretic mobility vs. molecular weight of the standard proteins.

Gel filtration chromatography : Native molecular weight was estimated by gel filtration chromatography. The enzyme was applied on Sephadex G-200 column (1.4×80 *cm*) which was equilibrated with 50 *m*M potassium phosphate, pH 7.4, 50 *m*M KCl, 5 *m*M 2-mercapoethanol and 0.1 M NaCl. Calibration of the column for molecular weight determination was performed by determining the elution volume of proteins of known molecular weight including bovine serum albumin, ovalbumin and ribonuclease A. Elution of standard protein was monitored by measuring the absorbance at 280 *nm*. The partition coefficient (K_{av}) for individual standard proteins was then calculated as $(V_e-V_o)/(V_t-V_o)$, where V_e, V_o and V_t were the elution volume of the protein, the void volume and the total column volume, respectively. K_{av} for polymerase α was determined from the elution volume of the fraction having enzyme activity. The molecular weght of polymerase was then calculated from the plot of molecular weight vs. K_{av}.

Other methods : Protein was determined by the method of Lowry *et al*[23], using bovine serum albumin as a standard.

Results

The purification of DNA polymerase α

The purification of DNA polymerase α

DNA polymerase α was purified from sea urchin embryos as described in methods. The activity unit,

Table 1. Purification of DNA polymerase α from *Hemicentrotus pulcherrismus*

Fraction	Protein (*mg*)	Total activities (units)	Specific activity(units/*mg* protein)
Embryo	420.6	173.0	14.6
Ammonium sulfate	120.4	68.4	30.1
Phosphocellulose	33.2	43.0	69.3
DEAE-cellulose	10.8	17.6	276.0
Hydroxylapatite	2.7	10.3	729.0
Blue Sepharose	0.6	5.7	1800.0

specific activity of the fraction at each purification step are shown in Table 1. The purification relies on the sequential use of four step on phosphophocellulose (Fig. 1), DEAE-cellulose (Fig. 2), hydroxylatite (Fig. 3), and Blue Sepharose (Fig. 4). The ammonium sulfate fractionation enhanced the specific activity of the preparation by elimination about 29% of protein from the embryos. The final step in the purification procedure using Blue Sepharose column, which in a 130 fold enrichment step provides the pure enzymes. Fraction Ⅵ enzyme was relatively stable stored at −20°C in the presence of 20% glycerol. Different modifications or additions to this purification procedure, including polyethylene glycol precipitation[24], Sephadex G-200[25] have been tested but in all case, the specific activity of the most purified fraction was not matographic methods used in the present purifica-

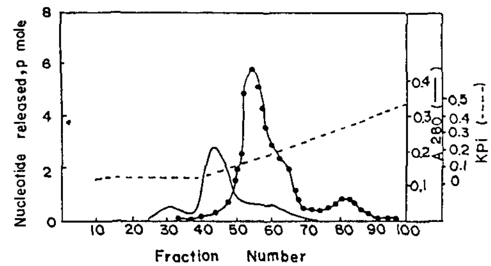

Fig. 1. Phosphocellulose column chromatography of DNA polymerase α. The column of 4.5×25 *cm* was used and fractions were collected through the graident. DNA polymerase activity(●), concentration of potassium phosphate (···), A_{280}(—).

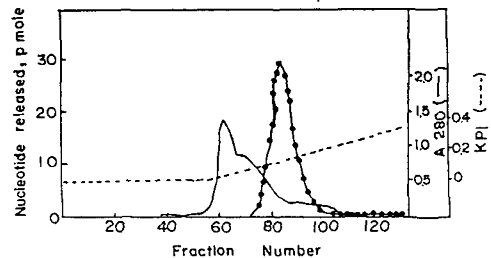

Fig. 2. DEAE-cellulose chromatography of DNA polymerase α. The column of 2.6×25 cm was used and fractions were collected through the gradient. Refer to Fig. 1.

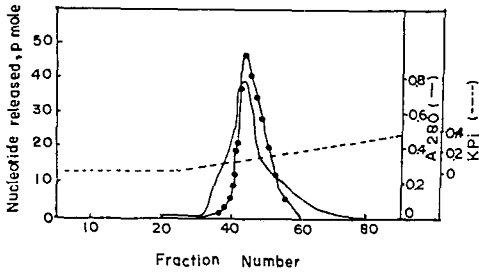

Fig. 3. Hydroxylapatite chromatography of DNA polymerase α. Fraction Ⅳ enzyme was chromatographed as described in "Methods". The column of $1.5\times15\,cm$ was used and fraction were collected through the gradient. Refer to Fig. 1.

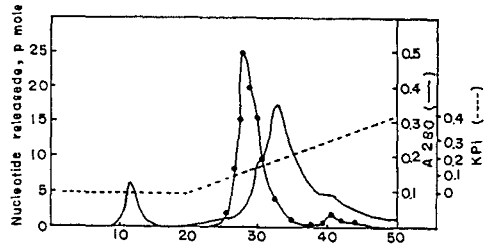

Fig. 4. Blue Sepharose chromatography of DNA polymerase α. Fraction Ⅴ enzyme was chromatographed as described in "Methods". The column of $1.0\times10\,cm$ was used and fractions were collected through the gradient. Refer to Fig. 1.

tion procedure.

Molecular weight determination : Molecular weight of Molecular weight of DNA polymerase α was determined by two different methods. In SDS-poly-acrylamide gel elctrophoresis, the mobility of the enzyme was compared with trypsin inhibitor, α- lacto-albumin and ribonuclease A (Fig. 5). The DNA polymerase α from sea urchin was found at the position of very similar with γ-globulin(M.W. 160,000). DNA polymerase α was determined the molecular weight of the active enzyme form by gel filtration chromatography on calibrated Sephadex G-200 column and estimated to be around 137,000 (Fig. 6).

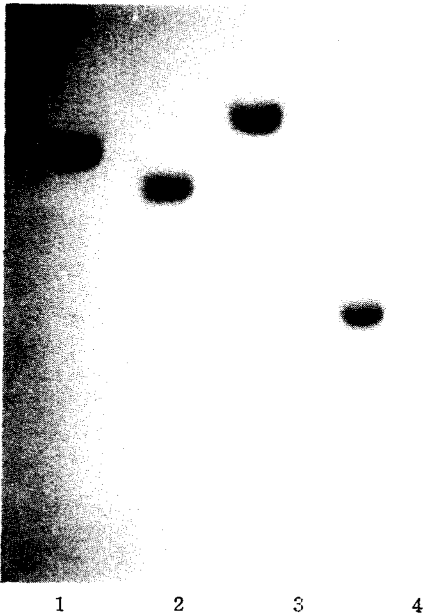

Fig. 5. SDS-polyacrylamide gel eletrophorsis patterns in 8.0% Lane 1. γ-globulin(Mw 160,000), 2. DNA polymerase, 3. catalase (Mw 230,000), 4. bovine serum albumin (Mw 67,000).

Requirements for reaction

(a) Effect of pH : The various pH ranges were important of regulating their activity. The pH depency of DNA polymerase α was investigated (Fig. 7a). The optimum pH was confirmed to be pH

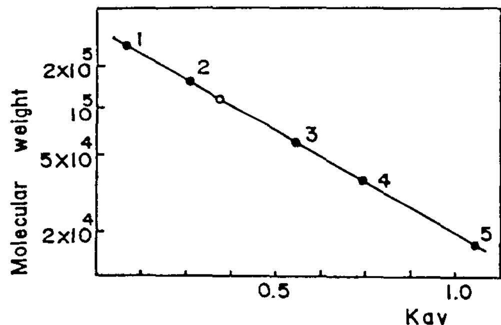

Fig. 6. Estimation of the molecular weight of DNA polymerase α by gel filtration on Sephadex G-200. Marker proteins 1. catalase(Mw. 230,000), 2. γ-globulin(Mw. 160,000), 3. bovine serum albumin(Mw. 68,000), 4. ovalbumin(Mw. 45,000), 5. myoglobin(Mw. 17,200). The open circle indicated the position of DNA polymerase α. partition coefficient.

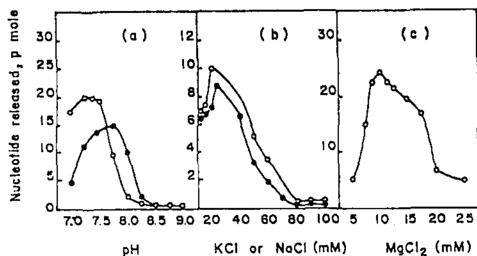

Fig. 7. Effects of pH, K^+, Na^+ and Mg^{2+} on the activities of DNA polymerase α. Each assay contained 50μg protein of polymerase α. (a) Effect of pH. Various pH values of potassium phosphate buffer of Tris maleate buffer (25 *mM*) were used. Enzyme fraction which had been dialyzed against various pH values of potassium phosphate buffer of Tris-maleate buffer was used for assay.

DNA polymerase α activity was assayed in reaction mixturewith phosphate buffer (○) or with Tris-maleate buffer. (b) Effects of K^+ or Na^+. KCl or NaCl was added to reaction mixture to give the final concentration shown on the abscissa. Polymerase α activity was assayed in reaction mixture with various concentration of KCl(○) or NaCl (●). (c) Effect of Mg^{2+} $MgCl_2$ was added to reaction mixture to give the final concentration shown on the abscissa. Polymerase α activity was assayed in reaction mixture with various concentration of $MgCl_2$.

7.2-7.4 when phosphate buffer was used for the enzyme assay system. On the other hand, polymerase α was active around at pH 7.6-7.8, when Tris-HCl buffer was used for the enzyme assay.

(b) Effect of K^+, Na^+ and Mg^{2+} : DNA polymerase α activity depends on addition of K^+, Na^+ and Mg^{2+}. In order to confirm these findings the addition of K^+ or Na^+ to the assay reaction mixture was investigated at pH 7.4 or 7.6. As shown in Fig. 7b, the optimum K^+ or Na^+ concentration was found to be 20*m*M KCl and 25 *m*M NaCl(Fig. 7b). Otherwise, Mg^{2+} for their activity, optimum Mg^{2+} concentration of DNA polymerase α was markedly stimulated at the ionic strength of various concentration of $MgCl_2$ (Fig. 7c). Optimum Mg^{2+} concentration was about 10 *m*M for their activity.

Effects of inhibitors on polymerase α

(a) N-ethylmaleimide (NEM) : NEM was known potent inhibitor of DNA polymerase α and γ, but not of DNA polymerase β[7] was tested first. DNA polymerase α was inhibited about 60% by 0.1 *m*M NEM and about 90% by 0.6 *m*M NEM (Fig. 8a).

(b) Aphidicolin : This antibiotic arrests growth of all eukaryotic cells which are permeable to it and inhibits the replication of DNA. As shown in Fig. 8b, when polymerase α was exposed to aphidicolin the incorporation of ³H-thymidine into DNA was almost completely inhibited by treatment with 1.0 *μg/ml* of aphidicolin. The concentration of aphidicolin which caused 50% inhibition of polymerase α activity was 0.1 *μg/ml*.

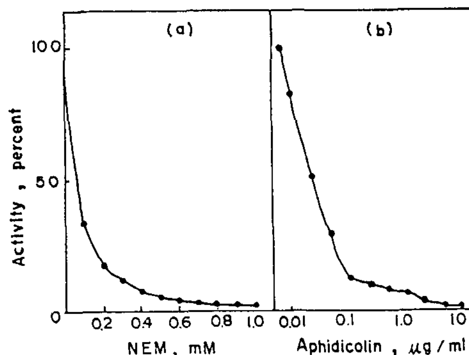

Fig. 8. Effcets of inhibitors on the activities of polymerase α. Each assay contained 50 *μg* protein of polymerase α The inhibitor was added to the reaction mixture to give the final concentration shown on the abscissa. (a) Effect of N-ethylmaleimide(NEM). Each enzyme was preincubated at 4°C for 30 *min* with various concentrations of NEM. (b) Effect of aphidicolin.

(c) Cytosine β-D arabinofuranoside 5′-triphos-phate (*ara* CTP) : The competitive inhibition by *ara* CTP of DNA synthesis has been described earlier and *ara* CTP markedly inhibited DNA polymerase α[26]. Thus the effect of inhibitors on the rate of enzyme activity by *ara* CTP was investigated with the various concentration, As shownin Fig. 9a, polymerase α was considerably sensitive to *ara* CTP. Especially at about 0.5 *m*M *ara* CTP, the enzyme

activity decreased notably up to 80%.

(d) Phosphonoacetic acid : An analogue of pyrophosphate was inhibitory for *Ad* 5 DNA *RC* activity as for purified DNA polymer ase α[27]. Phosphonoacetic acid showed to inhibit sea urchin DNA polymerase α (Fig. 9b). This enzyme was sensitive to phosphonoacetic acid and its activity was considerably inhibited by 30 $\mu g/ml$ of phosphonoacetic acid, 100 $\mu g/ml$ of phosphonoacetic acid concentration inhibit 75% DNA polymerase α activity.

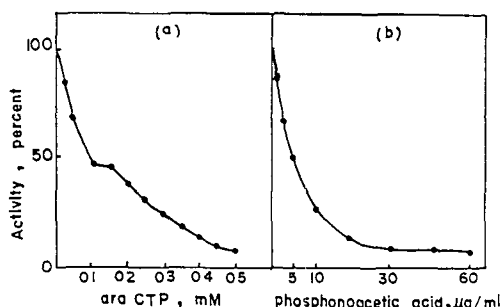

Fig. 9. Effects of inhibitors on the activities of polymerase α. Each assay contained 50 μg protein of polymerase α. The inhibitor was added to the reaction mixture to give the final concentration shown on the abscissa. Inhibitors were preincubated with the enzyme at 4°C for 30 *min*. (a) Effect of *ara* CTP. (b) Effect of phosphonoacetic acid.

Discussion

The present results in this paper demonstrate that highly purified preparation of sea urchin embryo DNA polymerase α. Properties of this enzyme were shown that rechromatography of the final fraction on Blue Sepharose yielded a single symmetrical peak demonstrating uniform specific activity. DNA polymerase α has been found in calf thymus [28], human[5], hamster cells[29] and sea urchin[4] and yeast[6] among others. DNA polymerase α has been purified over 300 fold with respect to sonically disrupted preparations of whole embryos. The final separation step, which gives a 130 fold enrichment involves affinity chromatography on the Blue Sepharose column used in the purification of DNA polymerase α. The molecular weight of the enzyme has been estimated by its elution volume on gel filtration colum. Using marker, the calculated molecular weight of DNA polymerase α was 137,000, We estimate the molecular weight to be 137,000~138,000. DNA polymerase α of calf thymus is a single polypeptide of molecular weight to be 155,000 – 170,000[28]. Brun *et al*[30] found the bulk of the chick embryo α-polymerase to have a molecular weight of 148,000 but also found higher molecular weight forms. Craig *et al*[29] have investigated the DNA polymerase of BHK-21/C13 cells and tentatively identified four subspecies of the α-polymerase rangin gin aparent molecular weight from 140,000 to 1,000,000. They have noted, as did Holmes *et al.* previously[31], since the molecular homogeneity of the α-polymerase appeared to be a general phenomenon. It is not always possible to assign a simple relationship between the various molecular weight and chromatographic forms of the α-polymerase and their enzymatic activity. The optimum of DNA polymerase α was estimated to be around 7.4. In addition, polymerase α showed high activity when it was assayed at neutral pH (pH 7.4). The pH dependency of polymerase α was investigated (Fig. 7a). The optimum pH was confirmed to be pH 7.2 when phosphate buffer or Tris-HCl buffer were used for the enzyme activity. Variation of monovalent metal cation in DNA polymerase activity is required for an optimum concentration (Fig. 7 b. c). The optimum K^+, Na^+ and Mg^{2+} concentration were about 20 *m*M, 25 *m*M and 10 *m*M, respectively. Sakai *et al*[32] reported that the optimum Mg^{2+} concentration was 15 *m*M, the optimum K^+(or Na^+) concentration was 20 *m*M. Fansler *et al*[33] reported that optimal Mg^{2+} concentration is about 9 *m*M. In the absence of Mg^{2+}, added Zn^{2+} gives maximum polymerase activity at 0.3 – 0.5 *m*M, which is 10 – 13% of the activity achieved at optimal Mg^{2+} concentration. DNA polymerase α is charcteristically inhibited by N-ethyl-maleimide (NEM)[7]. This DNA polymerase α is very sensititive to the sulfhydryl inhibitor N-ethylmaleimide[34]. Sea urchin DNA polymerase α activity redund 80% by 0.2 *m*M of N-ethylmaleimide. Therefore, nulcear extracts

were inactivated by treating them with 3 mM N-ethylmaleimide for varying duration of time[35]. A large number of subsequent investigations had established that the activity of α-polymerase is abolished by N-ethylmaleimide whereas polymerase was usually resistant to this sulfhydryl group blocker[7,8]. Experiments have shown that aphidicolin selectively inhibited the activity of DNA polymerase α obtained from regenerating rat liver[13] and embryos of the sea urchin[11]. The main interest in aphidicolin stems from the finding that it acts as a effective and selective inhibitor of animal cell DNA polymerase α[11]. We also found that inhibition of aphidicolin by various concentration markedly reduced the DNA polymerase α activity. Hence these different lines of evidence indicates that the target within the nucleus for the inhibitory action of aphidicolin is DNA polymerase α. The mechanism of inhibition of polymerase α by aphidicolin is not completely clear[36]. Aphidicolin has been shown to reversibly inhibit animal cell growth and DNA polymerase α-like activity in extracts of animal cells [14]. *Ara* CTP at the concentrations used, inhibited polymerase α rather selectively. The analogue *ara* CTP effectively inhibits eukaryotic and viral DNA polymerases by direct competition with dCTP[26]. It was observed by Shrecker *et al*[37] that the inhibition of polymerase α from normal human lymphocytes by *ara* CTP varied changes in primer template and substrate. *Ara* CTP inhibited copying of activated DNA by CV-1 cell polymerase α 20 to 30 times more effectively than either polymerase β or γ, respectively[35]. Yoshida *et al*[26] reported that calf thymus polymerase α was more sensitive to *ara* CTP than polymerase α. This is in accordance with the results of Yoshida *et al*[26].

Phosphonoacetic acid, an analogue of pyrophosphate was as inhibitory for *Ad*5 DNA *RCs*(replication complexes)activity as for purified DNA polymerase α[27] suggesting that the latter was functional enzyme involed in the replication of *Ad*5 DNA in viral *RCs*. Phosphonoacetic acid had been shown to inhibit DNA polymerases induced herpes simplex virus, human cytomegalovirus, Marek disease virus. Epstein Barr virus and Vaccinia virus[8]. Eukaryotic cell DNA polymerases were initially considered to be resistant to phosphonoacetic acid[38]. Leinbach *et al*[39] demonstrated that phosphonoacetic acid inhibited DNA polymerase by interacting with its pyrophosphate binding site. It appears from our studies that under conditions where polymerase is strongly inhibited such as inhibitors. It is notew orthy that enzyme activity has same sensitivity to these inhibitors as polymerase α[8]. Whether any of these species of DNA polymerase α is identical with or related to DNA polymerase β or γ will have to a wait further characterization of these enzyme and immunological studies.

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말똥 성게의 DNA Polymerase α 의 정제와 특성

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말똥 성게를 인공 수정시킨 후 column chromatography 법으로 DNA polymerase α 를 분리 정제하였다. Sephadex G-200과 SDS polyacryamide gel electophoresis 에 의한 DNA polymerase α 의 분자량은 약 137,000~138,000 이었다. 효소활성의 최적 pH 는 7.4였고, 칼슘이온 20 mM, 나트륨이온 25 mM 에서 활성이 높았고, 마그네슘 이온은 10 mM 일 때 활성이 높았다. DNA polymerase α 는 N-ethylmaleimide, aphidicolin, cytosin β-D-arabinofuranoside 5′-triphosphate (ara CTP)와 phosphonoacetic acid에 의하여 활성이 크게 저하되었다.