Purification and Characterization of a γ-like DNA Polymerase from *Chlamydomonas reinhardtii*†

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ABSTRACT: A crude in vitro system which initiates chloroplast DNA synthesis near the D-loop site mapped by electron microscopy [Wu, M., Lou, J. K., Chang, D. Y., Chang, C. H., & Nie, Z. Q. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 6761–6765] consists of soluble proteins and proteins extracted from purified thylakoid membrane. In this paper, a DNA polymerase activity was purified to near homogeneity from the soluble protein fraction of this in vitro system by sequential chromatographic separations on heparin–agarose, DEAE-cellulose, and single-stranded DNA–agarose columns and sedimentation in a glycerol gradient. In the glycerol gradient, the enzyme activity sedimented at a position corresponding to a 110-kDa protein. Electrophoretic analysis of the highly purified fraction on SDS–polyacrylamide gel revealed a major polypeptide band with an apparent molecular mass of approximately 116 kDa. In situ DNA polymerase activity assay shows that the DNA polymerization function is associated with the 116-kDa band and an 80-kDa band which could be a subunit of the enzyme. Polymerization activity is inhibited by *N*-ethylmaleimide, ethidium bromide, and dideoxycytosine triphosphate and is relatively resistant to aphidicolin. Poly(dA)·$(dT)_{10}$ and gapped double-stranded DNA are preferred templates. The purified enzyme contains no exonuclease activity and can initiate DNA replication in a supercoiled plasmid DNA template containing the chloroplast DNA replication origin.

The chloroplast (Cp) DNAs in *Chlamydomonas reinhardtii* have been found to be largely homogeneous in sequence organization and exist as multiple copies of circular molecules of 190 kb. Electron microscopy showed that DNA replication was initiated by the formation of D-loops at specific sites. Two sites were mapped on the physical map and designated Ori A and Ori B, respectively (Waddell et al., 1984). A 5.5-kb *Eco*Ri fragment, R-13, containing Ori A was cloned, and the Ori A site was further delimited in a 0.42-kb region (Wang et al., 1984). In an in vitro DNA replication system constructed from partially purified algal proteins, plasmid DNA containing R-13 was an effective template, and DNA replication was initiated within a restriction fragment containing Ori A (Wu et al., 1986). Protein components of this in vitro system consisted of soluble proteins and protein extracted from purified thylakoid membrane. DNA polymerase activity was located in the soluble protein fraction. In this paper, we report a purification procedure that results in a near-homogeneity preparation of a DNA polymerase from the soluble protein fraction of this in vitro system. The polypeptide responsible for the DNA polymerase activity was determined. The catalytic property of this purified enzyme was also examined.

Materials and Methods

Preparation of Crude Soluble Proteins from the Algae. The algal cells were harvested from an exponentially growing culture of *C. reinhardtii* CC125 during the early light period of the growing cycle by centrifugation at 4K rpm for 5 min in a Sorvell GS 3 rotor. The cells were resuspended at a concentration of 10^8 cells/mL in a freshly prepared cold extraction buffer containing 10% sucrose, 20 mM HEPES (pH 7.5), 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT, and the following protease inhibitors: 5 mM benzamidine, 10 mM δ-amino-*n*-caprioic acid, 1 mM phenylmethanesulfonyl fluoride (Sigma), and 10 mM sodium bisulfate. The cells were broked by sonification, and the extent of cell breakage was checked under phase-contrast light microscope. Cell debris was then removed by centrifugation at 12K rpm for 12 min in a Sorvell SS34 rotor. The supernatant was pooled for subsequent ammonium sulfate fractionation and column chromatography.

DNA Polymerase Assay. Reaction mixtures (0.05 mL) contained 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 400 μg/mL BSA, 60 μM each of dCTP, dATP, and dGTP, 30 μM [^{3}H]dTTP with specific activity of approximately 500 cpm/pmol, and 200 μg/mL activated calf thymus DNA (Aposhian & Kornberg, 1962). Incubation was carried out at 30 °C. Triplicate samples of 5 μL each were collected at 0-min incubation and again at 30-min incubation. Total nucleotide incorporation was measured by determining radioactivity after trichloroacetic acid precipitation. One unit of DNA polymerse activity is defined as the incorporation of 1 nmol of deoxynucleotide into acid-insoluble product in 60 min. When poly(rA)·$(dT)_{10}$ or poly(dA)·$(dt)_{10}$ (Pharmacia) was used as a template-primer, it was added to the reaction mixture at a concentration of 0.02 unit/0.05 mL. DNA polymerization activity using supercoiled plasmid DNA containing chloroplast DNA replication origin was carried out according to Wu et al. (1986) except that the only protein added to the system is the partially purified polymerase.

Ammonium Sulfate Fractionation and Column Chromatography. Total algal soluble protein was divided into several fractions by ammonium sulfate precipitation. Material precipitated before 20% saturation and at 20–30%, 30–40%, 40–50%, 50–60% saturation and the 60% supernatant were collected. The redissolved precipitate or the supernatant was dialyzed against the extraction buffer except that the 10%

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sucrose was substituted by 5% (v/v) glycerol. The protein concentration in each fraction was measured by a protein assay system from Bio-Rad (Bradford, 1976) according to the manufacturer's instructions. Enzyme activity in each fraction was determined. Fractions containing high enzyme activities were used for column chromatography.

The heparin–agarose (Sigma) column was washed extensively with 1 mg/mL BSA, equilibrated with column buffer containing 10 mM Tris-HCl (pH 8.0), 5% (v/v) glycerol, 0.1 M KCl, 0.1 mM dithiothreitol, 5 mM $MgCl_2$, and protease inhibitors at the same concentrations as in the extraction buffer. For a typical column procedure, approximately 60 mg of protein was dissolved in 0.2 mL of column buffer and loaded on to a 1.0 × 10 cm heparin–agarose column in a cold room. Protein which did not bind to the column was washed with 40 mL of column buffer and collected. The column was then washed with a 50-mL linear gradient consisting of 0–0.7 M ammonium sulfate in column buffer, and 1-mL fractions were collected. Enzyme activity and protein concentration in each fraction were determined. Active fractions were pooled, concentrated by ammonium sulfate precipitation, dialyzed against column buffer, and used for subsequent column chromatography.

DEAE-cellulose (DE-52) and cellulose phosphate (P11) were obtained from Whatman. Hydroxyapatite (HAP) was obtained from Bio-Rad, and each was used according to the manufacturer's instructions. Single-stranded (SS) DNA–agarose was obtained from BRL. After being packed, the column was washed extensively with 1 mg/mL BSA to block nonspecific binding sites. Proteins bound to the column were eluted with column buffer containing 1 M NaCl.

Glycerol Gradient Sedimentation and Protein Gel Electrophoresis. About 250 μg of protein in 0.1 mL of column buffer was layered onto a performed, linear 15–35% glycerol gradient. Centrifugation was at 40000 rpm for 24 h at 4 °C in a Beckman SW 50.1 rotor. Fractions of 0.1 mL each were collected. The enzyme activity and polypeptide content of each fraction were determined. Protein markers, bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), and β-amylase (200 kDa), were separated on parallel gradients.

The polypeptide pattern of the partially purified fraction was analyzed by electrophoresis on 12% polyacrylamide or a 5–10% linear gradient polyacrylamide gel containing 1% SDS. The stacking gel was 5% polyacrylamide. The electrophoresis buffer contained 25 mM Tris–glycine, pH 8.3. Electrophoresis was performed at a constant current of 30 mA for 3–4 h or until the tracking dye was 1.0 cm from the bottom. Proteins used as molecular weight standards were purchased from BRL. Peptide bands were visualized after silver staining (Giulian et al., 1983).

Analysis of DNA Polymerase Activity in Situ. The analysis was carried out essentially according to the procedure reported by Hubscher (1983). The enzyme sample was heated at 37 °C for 3 min and then electrophoresed in 6.6% (w/v) polyacrylamide gel containing 0.1% SDS, 2 mM EDTA, and activated calf thymus DNA at a final concentration of 150 μg/mL. Electrophoresis was at 5 mA for 4.5 h, and SDS was removed by immersing the gel three times in a buffer containing 50 mM Tris-HCl (PH 8.5), 1 mM EDTA, and 10 mM β-mercaptoethanol for 10 min. The gel was then treated with five gel volumes of 50 mM Tris-HCl (PH 8.0), 5 mM $MgCl_2$, 70 mM KCl, 400 μg/mL BSA, 16% glycerol, 0.01 mM EDTA, and 5 mM β-mercaptoethanol at 37 °C for 30 min and then at 4 °C for 16 h. For in situ activity assay, the gel was incubated in five volumes of the same buffer containing 15 μM dGTP, 15 μM dTTP, 15 μM dCTP, 2 μM dATP, and 2.5 μM [α-^{32}P]dATP at 30 °C for 20 h. After the unincorporated dNTPs were removed by washing with 5% (w/v) TCA containing 1% (w/v) sodium pyrophosphate for 26 h, the gel was dried for autoradiography. *Escherichia coli* DNA polymerase I was obtained from New England Biolab.

Table I: Ammonium Sulfate Fractionation of Enzyme Activity[a]

fraction	total protein (mg)	polymerase activity (unit/mg of protein)
20% precipitate	140	ND
20–30% precipitate	52	0.89
30–40% precipitate	67	0.97
40–50% precipitate	132	ND
50–60% precipitate	90	ND
60% soluble	187	ND

[a] ND = not detectable. Total soluble protein was isolated from 9 × 10^{10} cells.

Inhibitor Studies and Exonuclease Assay. *N*-Ethylmaleimide (NEM) (Sigma) and ethidium bromide (Sigma) were dissolved in water immediately before use. A fresh stock solution of aphidicolin (Sigma) was prepared in dimethyl sulfoxide (DMSO). The effect of a small amount of DMSO added to the assay mixture (2.5 μL/100 μL) on the enzyme activity was corrected by controls. d_2CTP was purchased from P-L Biochemicals Inc.

Exonuclease activity was assayed essentially as described by Lehman and Richardson (1964). One microgram of single-stranded ^{3}H-labeled DNA was added to the assay mixture containing 0.05 M Tris-HCl (pH 8.0), 10 mM 2-mercaptoethanol, and 10 mM $MgCl_2$. The amount of protein used in each assay is listed in Table IV. After incubation for 30 min at 30 °C, radioactivities in both the acid-soluble and the acid-precipitable fractions were measured.

Results

Purification of the DNA Polymerase. The crude soluble protein contained low DNA polymerase activity when the activated calf thymus DNA was used as template in the assay system. The total soluble protein was then divided into different fractions by ammonium sulfate fractionation, and the enzyme activity in each fraction was determined. Most enzyme activity was recovered in the protein precipitated between 20% and 40% saturated ammonium sulfate (Table I). This fraction was used for subsequent purification procedure.

Several column and buffer combinations were tested. The system that could effectively remove other algal proteins and provide rapid and quantitative recovery of DNA polymerase enzyme activity was used in the final purification procedure. The test showed that heparin–agarose, DE-52, SS DNA–agarose, P11, and HAP columns could be used to enrich the enzyme activity to some extent. However, P11 and HAP columns were not used in the final procedure due to the following reasons. The buffer system used for P11 column chromatography was quite different from that used for other columns, and the specific activity was not enriched significantly by this procedure. DNA polymerase binds to the HAP column equilibrated with column buffer while a lot of other algal proteins flowed through under this condition, and enzyme activity could be recovered by washing the column with buffer containing 0.5 M phosphate. However, the yield of recovery varied when different batches of HAP were used. Therefore, sequential chromatographic separations on heparin–agarose, DE-52, and SS DNA–agarose columns and glycerol gradient sedimentation were used to purify the enzyme.

Table II: Purification of the DNA Polymerase Enzyme Activity[a]

fraction	purification step	protein (mg)	activity (units)	sp act. (units/mg)	yield (%)	purification (x-fold)	rel act. in the in vitro system[b]
1	total soluble protein	680	163.2	0.24	100	1	ND
2	ammonium sulfate fractionation	118.8	110.5	0.93	68	4	1
3	heparin–agarose column	15.9	63.9	4.01	39	17	1.8
4	DE-52 column	10.3	54.4	5.18	33	22	ND
5	SS DNA–agarose	0.34	40.1	117.9	24.5	491	17
6	glycerol gradient	0.026	13.8	518.5	8.4	2160	52

[a] Total soluble protein was isolated from 9×10^{10} cells. 1 unit is 1 nmol of nucleotide incorporated in 60 min at 30 °C. [b] DNA synthesis activity was measured for 60 min at 30 °C. 1 equals 9.0 pmol of dNMP incorporated.

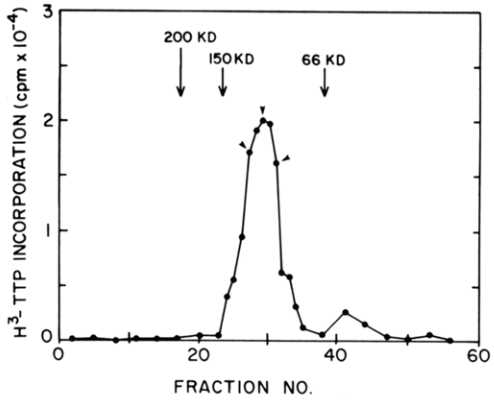

FIGURE 1: Glycerol gradient sedimentation of algal DNA polymerase purified by column chromatography. Enzyme activity of each fraction was plotted. Triangles mark the fractions containing the peak of activity. These fractions were used for the gel electrophoretic analysis shown in Figure 2. Protein markers run in parallel gradients were bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), and β-amylase (200 kDa). Peak fraction for each marker is indicated by an arrow.

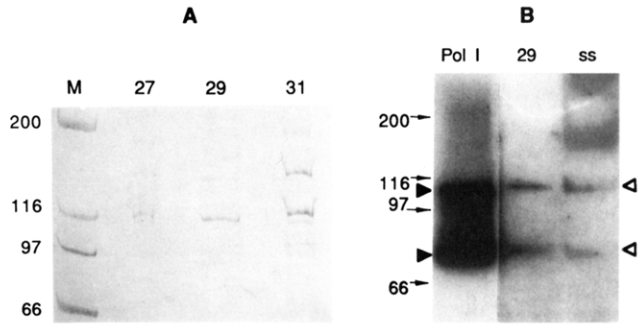

FIGURE 2: (A) The polypeptide pattern of the fractions containing peak enzyme activity is shown in Figure 1. Molecular masses (kDa) of the marker proteins (lane M) are indicated at the corresponding bands. Electrophoretic separation was carried out on a 6.6% polyacrylamide gel containing 0.1% SDS. Proteins were detected by silver staining. (B) Autoradiograph showing DNA polymerization activity in situ. *E. coli* DNA polymerase I was used as a DNA polymerization standard and an internal molecular mass marker (lane Pol I). DNA polymerization by protein(s) in fraction 29 which contains near-homogeneous DNA polymerase and by protein preparation purified through single-stranded DNA–agarose column chromatography is shown in lane 29 and lane SS, respectively. Open triangles point to the active bands in lanes 29 and SS; filled triangles point to the center of the active bands in lane Pol 1. The positions of other protein size markers separated in a parallel gel are indicated. Details are described under Materials and Methods.

A typical purification procedure was performed as follows. About 60 mg of algal protein which precipitated between 20% and 40% ammonium sulfate was chromatographed on a 1 × 10 cm heparin–agarose column as described under Materials and Methods. No significant DNA polymerase activity was detected in the fraction which flowed through the column. When the column was eluted with the ammonium sulfate gradient, a large single peak was detected. This fraction contained enriched enzyme activity and was used for further purification. The protein peak was concentrated, equilibrated with the column buffer, and loaded onto a 1 × 9 cm DE-52 column. All enzyme activity was recovered in the protein fraction which did not bind to the column. This fraction was then loaded onto a 1 × 5 cm SS DNA–agarose column equilibrated with the same column buffer. After the column was washed copiously with column buffer, enzyme activity was recovered in a small peak obtained by washing the column buffer containing 1 M NaCl. Results of the purification procedure are summarized in Table II. After each step of purification, the polypeptide pattern of the fraction which contained the enzyme activity was visualized by SDS–polyacrylamide gel electrophoresis (data not shown). After the SS DNA column chromatography, multiple polypeptide bands were detected in the enzyme active fraction. The fraction was further purified by glycerol gradient sedimentation as described under Materials and Methods. Enzyme activity of each fraction was assayed. The major activity was detected in a peak sedimented between the 150-kDa alcohol dehydrogenase and the 66-kDa BSA protein markers, which were run in a parallel gradient (Figure 1). The molecular mass of the peak fraction was estimated to be 110 kDa. The polypeptide pattern of the peak activity fractions was visualized by SDS–polyacrylamide gel electrophoresis (Figure 2A). The enzyme activity corresponds well to a polypeptide band of apparent molecular mass of 116 kDa. In fraction 29 which showed the highest specific activity, sometimes, this was the only band detected by silver staining. At this stage of purification, the enzyme was enriched 2160-fold with an overall yield of 8.4% (Table II). Corresponding fractions from several gradients were pooled and used for the characterization studies described in the next section.

After each purification step, the partially purified enzyme was also tested for its activity in the in vitro DNA replication system. The supercoiled plasmid DNA of pSC 3-1 which contains the 0.42-kb D-loop sequences in pBR 322 was a preferred template. When the supercoiled form of pBR 322 or the linear or open circles of pSC 3-1 was used as a template, the replication activity was very low. The low activity obtained from pBR 322 was subtracted in the results summarized in Table II. The enzyme was increasingly active in promoting in vitro DNA replication upon further purification. The purified enzyme (after glycerol gradient) also displayed greater specificity than the crude enzyme preparation (after ammonium sulfate fractionation) with plasmid DNA containing the D-loop sequences, pSC 3-1. The vector plasmid alone produced 30% and 10% of the replication activity detected in pSC 3-1 when the crude enzyme preparation and purified enzyme were used, respectively. After the final step of purification, the enzyme was still active in initiating DNA polymerization on supercoiled DNA containing replication origin. However,

the increase of replication activity in the in vitro system is not proportional to the increase of DNA polymerization activity measured with activated calf thymus DNA as template.

The polypeptide components of the purified enzyme were further investigated on a 5–15% linear gradient polyacrylamide gel containing 1% SDS. In this electrophoretic condition, sometimes, a 80-kDa band was resolved in addition to the 116-kDa band (data not shown). Polymerization in situ demonstrated that the polymerization function is associated with both the 116-kDa and the 80-kDa bands in the purified enzyme preparation (Figure 2B). In glycerol gradient, the molecular mass of the polymerization activity peak was estimated to be around 110 kDa, and no polymerization band was detected in fractions sedimented around 80 kDa region in the gel in situ assay (data not shown). The 116-kDa and the 80-kDa activity bands were always detected in the same fraction from a column separation procedure or the glycerol gradient. These observations led us to hypothesize that the purified enzyme could be dissociated into subunits in the gel separation condition used for the gel in situ activity assay and the 80-kDa subunit still possesses the DNA polymerization function.

We were also interested in determining whether the polymerization in situ assay could be used to localize polypeptide bands containing polymerization activity in a crude preparation. Protein fractions from each of the purification steps listed in Table II were used for this assay. Polypeptide bands containing polymerization activity were consistently detected in a protein preparation purified through the single-stranded DNA–agarose column chromatographic procedure (lane SS in Figure 2B). In addition to the two polypeptides which correspond to the 116-kDa and 80-kDa bands detected in the purified enzyme preparation, two broad smears with weak polymerization activity were consistently detected in the higher molecular mass regions.

Characterization of Enzyme Activity. Three distinctive DNA polymerases have been isolated from eucaryotic animal cells and are designated α, β, and γ. DNA polymerases α and β are associated with nuclear DNA replication and repair processes, respectively. DNA polymerase γ has been purified from mitochondria (Wernette & Kaguni, 1986; Yamaguchi et al., 1980; Bolden et al., 1977). The three polymerases are readily identified by several features, especially by their different sensitivities to various inhibitors (Kornberg, 1980). Therefore, the enzyme activity of this near-homogeneous preparation in the presence of different concentrations of monovalent cation and various inhibitors was determined (Figure 3). When the activated calf thymus DNA was used as template, optimal enzyme activity was obtained in the absence of KCl and 50% inhibition was detected in the presence of 150 mM KCl (Figure 3A). Subsequent enzymatic assays using activated calf thymus DNA as template were conducted in the absence of KCl. When the purified enzyme was added to the crude in vitro system with supercoiled form of R-13 plasmid DNA as template, optimal replication activity was detected in the presence of 100 mM KCl (data not shown). Therefore, replication activity in the in vitro system (Table II) was assayed in this salt concentration.

The enzyme activity was strongly inhibited by d_2CTP; 55% inhibition was detected when equal amounts of d_2CTP and dCTP were added to the reaction mixture. When the ratio of d_2CTP/dCTP was adjusted to 5, 90% inhibition was detected (Figure 3B). The enzyme was sensitive to ethidium bromide; 50% inhibition was detected in the presence of 20 μM ethidium bromide (Figure 3C). The sulfhydryl group blocking agent NEM also inhibited the enzyme activity; 82% inhibition was detected in 2 mM NEM (Figure 3D). Figure 3E shows that the presence of aphidicolin up to a concentration of 0.1 mM had no apparent effect on the enzyme activity. These features show more similarities with animal DNA polymerase γ (Scovassi et al., 1980).

Table III: Template-Primer Specificity of Purified DNA Polymerase[a]

template-primer	relative enzyme activity (%) 0 mM KCl	100 mM KCl	200 mM KCl
activated calf thymus DNA	100	64.3	33
poly(rA)·$(dT)_{10}$	ND	14.7	8
poly(dA)·$(dT)_{10}$	688	451	125.2
form II ds DNA (pBR322)	ND	ND	7

[a] Enzyme activity was assayed as described under Materials and Methods. Each assay (50 μL) contained 0.1 μg of the enzyme obtained after glycerol gradient. 100% was 0.02 nmol of nucleotide incorporated in 30 min at 30 °C. ND is not detectable.

Table IV: Exonuclease Activity of Different Enzyme Fractions[a]

enzyme fraction	protein used for each assay (μg)	acid-soluble radioactivity
20–40% ammonium sulfate precipitated	16	3.6×10^4
heparin–agarose column purified	4	9×10^3
DE-52 column purified	1.6	1.1×10^3
SS DNA–agarose purified	0.05	1.5×10^3
glycerol gradient purified	0.03	ND

[a] Total acid-precipitable radioactivity in the initiation assay mixture was 8.5×10^4 cpm. ND is not detectable.

Template-Primer Specificity and Exonuclease Activity. In the assay condition used, poly(dA)·$(dT)_{10}$ was the prefered template rather than activated calf thymus DNA for the purified enzyme. The assay reaction mixtures contained saturated levels of the indicated template-primers. The relative activity of the enzyme was about 7-fold greater on poly(dA)·$(dT)_{10}$ as compared to activated calf thymus DNA at optimal salt concentration (Table III). Poly(rA)·$(dT)_{10}$ was used at a very low level in the presence of 100 mM KCl. The fact that singly nicked double-stranded DNA was not used as template suggested the enzyme does not contain an exonuclease activity to catalyze nick translation (Table III).

Nuclease activities of the purified enzyme and the enzyme-active fractions at various stages of purification were analyzed. The result is summarized in Table IV. Most nuclease activity was removed after heparin–agarose column chromatography. The purified enzyme lacks any associated nuclease activity.

Discussion

In higher plants, DNA polymerase activity has been purified from isolated chloroplasts of spinach (Sala et al., 1980) and pea (McKown & Tewari, 1984). Both enzyme activities were inhibited by NEM and were resistant to aphidicolin. Therefore, they were classified as γ-like. In view of the resemblance of chloroplast DNA to mitochondrial DNA in its lack of nucleosomal organization and in the presence of displacement loops in its replicative forms, the similarities in physical and functional properties of the chloroplast DNA polymerase to polymerase γ are not surprising.

The relative molecular mass of the spinach chloroplast DNA polymerase was determined to be 105 kDa on a glycerol density gradient (Sala et al., 1980). The chloroplast DNA polymerase of pea has been identified with a single protein band of 90 kDa on polyacrylamide gel containing 1% SDS

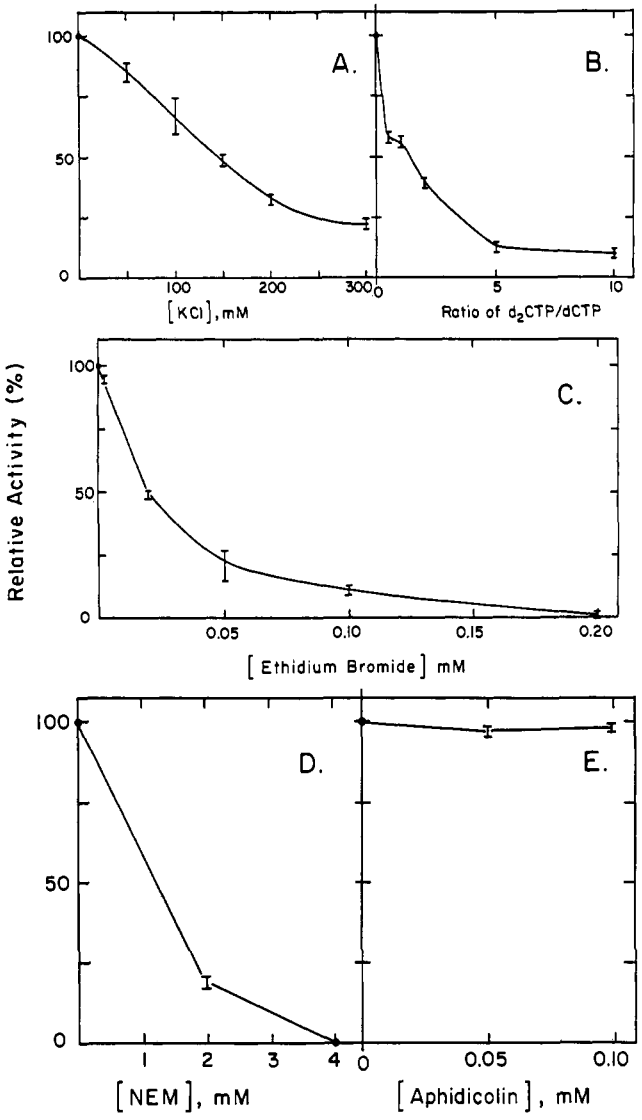

FIGURE 3: (A) Dependence of DNA polymerase activity on monovalent cation concentration. (B–E) Inhibitions of the enzyme activities by d_2CTP, ethidium bromide, NEM, and aphidicolin, respectively. The assay conditions are described under Materials and Methods.

(McKown & Tewari, 1984). Both the algal γ-like enzyme and the pea enzyme could not use poly(rA)·oligo(dT) as a template. This feature is distinctive from polymerase γ isolated from animal cell mitochondria. Although the apparent molecular mass of the algal γ-like DNA polymerase was determined to be 116 kDa, SDS–PAGE and the polymerization in situ assay demonstrated that the 116-kDa protein could be dissociated into subunits. The polymerization function is associated with an 80-kDa subunit as well as the 116-kDa form purified by glycerol gradient. DNA polymerase activity purified from isolated chloroplasts of *C. reinhardtii* CW 15 was reported previously by Keller and Ho (1981). They reported that chloroplast DNA polymerase activity migrates at 180 kDa on the G-150 column when NaCl is present at 0.8 M. At the low ionic strength of 0.05 M NaCl, the polymerase activity separates into peaks on a G-150 column at 180, 80, 40 kDa (Keller & Ho, 1981). The result of our in situ activity assay also suggested that the γ-like DNA polymerase could exist in a higher molecular mass form than the 116-kDa protein detected and purified in the glycerol gradient. A preliminary Western blot analysis showed that the 116-kDa band cross-reacts with an antiserum raised against the DNA polymerase isolated from purified tobacco chloroplasts. However, at this stage, we do not know whether the purified γ-like enzyme is the holoenzyme responsible for chloroplast DNA replication in vivo or is a stable subassembly of the holoenzyme. The location of this enzyme inside the algal cell also has not been determined.

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