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Characterization and purification of a phage ϕ29-encoded DNA polymerase required for the initiation of replication

(phage ϕ29 DNA polymerase/phage ϕ29 replication/host factor)

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ABSTRACT **The phage ϕ29 protein p2, required for the formation of the protein p3–dAMP initiation complex, has been purified from *Escherichia coli* cells harboring a gene 2-containing recombinant plasmid. The purified protein p2, of molecular weight 68,000, had a specific DNA polymerase activity that elongated the p3–dAMP initiation complex when ϕ29 DNA–protein p3 was used as template. In addition, the purified protein p2 was active in catalyzing the initiation reaction when complemented with ϕ29 mutant *sus2*-infected *Bacillus subtilis* or plasmid-containing *E. coli* extracts providing protein p3, in the presence of ϕ29 DNA–protein p3 as template. However, when purified protein p3 was used in the complementation assay, a very low amount of initiation complex was formed; addition of extracts from uninfected *B. subtilis* or *E. coli* strongly stimulated the initiation reaction, indicating that, in addition to proteins p2 and p3 and the ϕ29 DNA–protein p3 template, some host factor(s) is required for the formation of the p3–dAMP initiation complex. The results show that phage ϕ29 encodes a DNA polymerase that is required at the initiation step of protein-primed DNA synthesis.**

The initiation of replication at the ends of the linear DNA of the *Bacillus subtilis* phage ϕ29 has been shown *in vitro* to take place by a mechanism of protein priming. A free molecule of p3, the protein covalently linked at the 5′ ends of ϕ29 DNA (1–4), in the presence of ϕ29 DNA–protein p3 complex as template, reacts with dATP, the nucleotide at both 5′ ends of ϕ29 DNA, and forms a protein p3–dAMP initiation complex that provides the 3′ hydroxyl group needed for elongation (5, 6). Since extracts of *B. subtilis* infected with *sus* or *ts* mutants in gene 2 were not active in the formation of the p3–dAMP initiation complex (7, 8), the gene 2 product was shown also to be required for the initiation reaction *in vitro*, in agreement with the *in vivo* role of gene 2 in the initiation of replication, as shown by shift-up experiments using mutant *ts2*(98) (9). With the aim of purifying the gene 2 product, we have cloned gene 2 in plasmid pPLc28 under the control of the P_L promoter of phage λ. After heat induction, a protein of molecular weight 68,000, the size expected for protein p2 (10), was synthesized in the cells transformed with the gene 2-containing recombinant plasmid pLBw2 but not in the cells transformed with the control plasmid pPLc28 (11). The protein p2 present in the *Escherichia coli* extracts was active in the *in vitro* formation of the p3–dAMP initiation complex when complemented with extracts of *B. subtilis* infected with a *sus* mutant in gene 2, with extracts from *E. coli* harboring gene 3-containing recombinant plasmids, or even with purified protein p3 (11).

In this paper, we report the characterization and purification of a ϕ29-specific DNA polymerase required for the protein-primed initiation of replication that was able to elongate the ϕ29 p3–dAMP initiation complex using ϕ29 DNA–protein p3 as template. The requirement of some host factor(s) when purified ϕ29 DNA polymerase (p2) and terminal protein (p3) are used in the initiation reaction is also reported. A preliminary account of some of these findings has been presented (12, 13).

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MATERIALS AND METHODS

Assay for Formation of the Initiation Complex. Extracts of *E. coli* K-12 *ΔH1Δtrp* cells harboring the gene 3-containing recombinant plasmid pKC30 A1 (14), of *B. subtilis* HA101(59)F, lacking DNA polymerase I activity (15), infected with mutant *sus2*(513) (16), and of *E. coli* NF2690 (obtained from T. Atlung) or *B. subtilis* HA101(59)F uninfected or infected with mutant *sus2*(513) *sus3*(91), prepared by J. Gutierrez, were as described (5, 6, 14). The incubation mixture for the initiation reaction contained, in 0.05 ml, 50 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM ATP, 0.25 μM [α-^{32}P]dATP (5 μCi; 410 Ci/mmol; 1 Ci = 37 GBq), 0.5 μg of ϕ29 DNA–protein p3, and ≈24 μg of protein from plasmid pKC30 A1-containing *E. coli* extracts to provide protein p3 and an amount of the p2-containing fraction giving a linear response for p3–dAMP complex formation. When indicated, extracts from *B. subtilis* infected with mutant *sus2*(513) or highly purified protein p3, obtained from I. Prieto (17) were used as a source of free protein p3. In the latter case, extracts of *B. subtilis* uninfected or infected with mutant *sus2*(513) *sus3*(91) or *E. coli* were added when indicated. After incubation for 20 min at 30°C, the samples were processed as described by Peñalva and Salas (5). Quantitation was done as described (17).

DNA Polymerase Assay: *In Situ* Gel Analysis. The incubation for the DNA polymerase assay contained, in 0.05 ml, 50 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/1 mM ATP/1 mM spermidine/1 mM dithiothreitol/5% glycerol (vol/vol)/5 μg of bovine serum albumin/10 μM [^{3}H]dTTP (2 μCi; 43 Ci/mmol)/0.5 μg of poly(dA)·$(dT)_{12-18}$ as template-primer and an amount of the fraction to be assayed giving a linear response in [^{3}H]TMP incorporation. When other DNAs were used as template, dATP, dGTP, and dCTP (20 μM each) were also added. After incubation for the indicated times at 30°C, the acid-insoluble radioactivity was determined.

The *in situ* DNA polymerase gel analysis was carried out essentially as described by Karawya *et al.* (18). The samples were subjected to $NaDodSO_4$/PAGE using a DNA-containing gel, followed by *in situ* renaturation of proteins and incubation of the gel in a DNA polymerase assay mixture. The incubation was for 12 hr at 30°C. After washing unincorporated [^{32}P]dNTP from the gel, the activity bands were detected by autoradiography.

RESULTS

Initiation and DNA Polymerase Activities Associated with Protein p2. Extracts of *E. coli* K-12 *ΔH1Δtrp* cells transformed with the gene 2-containing recombinant plasmid pLBw2 (11) or the control plasmid pPLc28 were precipitated

with ammonium sulfate, treated with DNase, and passed through columns of blue dextran-agarose essentially as will be described in Table 1. As can be seen in Fig. 1*A*, a protein with the electrophoretic mobility expected for protein p2 (M_r, 68,000) was present in the 0.5 M NaCl eluate of the column corresponding to the gene 2-containing fractions but not in the control column; all the other proteins present in this fraction and in the other fractions from the blue dextran-agarose column were similar in the gene 2-containing and in the control samples. The initiation activity of the different fractions is shown in Fig. 1*B*. Extracts of *E. coli* transformed with the recombinant plasmid pLBw2 (lane a), but not with the control plasmid pPLc28 (lane b), were active in the formation of the p3–dAMP initiation complex when complemented with extracts of *E. coli* transformed with the gene 3-containing recombinant plasmid pKC30 A1 (11, 12). The

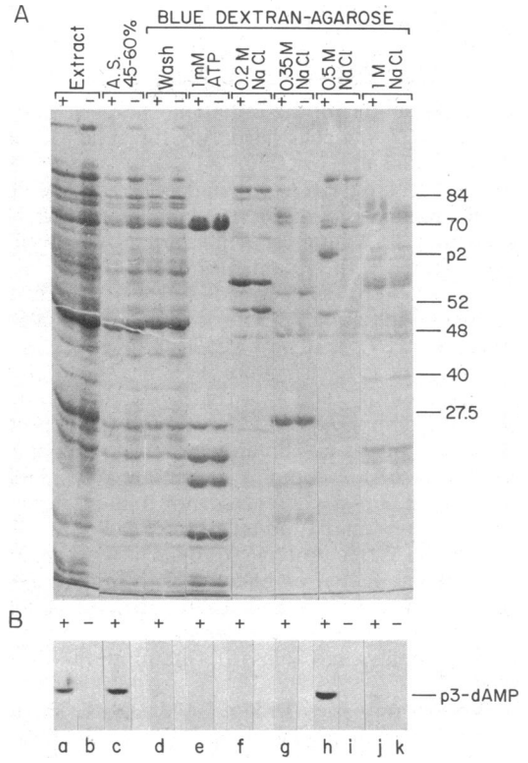

FIG. 1. Initiation activity associated with a partially purified protein p2-containing fraction. (*A*) Extracts of *E. coli* K-12 *ΔH1Δtrp* cells harboring the gene 2-containing plasmid pLBw2 or the control plasmid pPLc28 were partially purified essentially as described in Table 1. The proteins at the different purification steps were subjected to $NaDodSO_4$ electrophoresis in slab gels containing a 10%–20% acrylamide gradient and stained. + and − indicate the gene 2-containing fractions and the control fractions, respectively. The amount of protein used from the different fractions was as follows: extract, 65 μg; ammonium sulfate, 45%–60%, 21 μg; blue dextran-agarose, flow-through, 25 μg; 1 mM ATP eluate, 50 μg; 0.2 M NaCl eluate, 13 μg; 0.35 M NaCl eluate, 13 μg; 0.5 M NaCl eluate, 9 μg; 1 M NaCl eluate, 15 μg. The mobility of the φ29 structural proteins and the position of protein p2 are indicated on the right. (*B*) The different fractions indicated above were used as protein p2 donors in a complementation assay with extracts of *E. coli* transformed with the gene 3-containing recombinant plasmid pKC30 A1, to analyze for the formation of the p3–dAMP initiation complex. As in *A*, + and − indicate the gene 2-containing fractions and the control fractions, respectively. Lanes: a and b, extract (32 μg); c, ammonium sulfate (3.5 μg); d–j, blue dextran-agarose column; d, flow-through (10 μg); e, 1 mM ATP eluate (0.5 μg); f, 0.2 M NaCl eluate (0.7 μg); g, 0.35 M NaCl eluate (0.1 μg); h and i, 0.5 M NaCl eluate (0.08 μg); j and k, 1 M NaCl eluate (0.13 μg). The position of the p3–dAMP complex is indicated on the right.

initiation activity, precipitated with ammonium sulfate between 45% and 60% saturation (lane c), was eluted from the blue dextran-agarose column with 0.5 M NaCl (lane h) and to a lesser extent with 1 M NaCl (lane j). Fig. 2*A* shows that a specific DNA polymerase activity was also eluted, like the initiation activity, with 0.5 M NaCl and to a lesser extent with 1 M NaCl from the blue dextran-agarose column corresponding to the gene 2-containing fractions. This DNA polymerase activity was not present in the control fractions (Fig. 2*B*). As also shown in Fig. 2, the rest of the DNA polymerase activity was present in the flow-through fractions to a similar extent in the gene 2-containing and in the control samples. By *in situ* gel analysis, the DNA polymerase activity present in the fraction from the gene 2-containing sample eluted with 0.5 M NaCl from the blue dextran-agarose column was shown to be associated with a M_r 68,000 band (Fig. 2*C*, lane a); no such activity band was present in the corresponding fraction from the control sample (lane b).

Purification of Protein p2. The finding that the fraction eluted with 0.5 M NaCl from the blue dextran-agarose column contained a protein with the electrophoretic mobility of p2 and had initiation and DNA polymerase activities associated with a M_r 68,000 band suggested that protein p2 has an intrinsic DNA polymerase activity. Protein p2 was further purified, as described in Table 1. Most of the initiation activity was eluted from blue dextran-agarose with 0.7 M

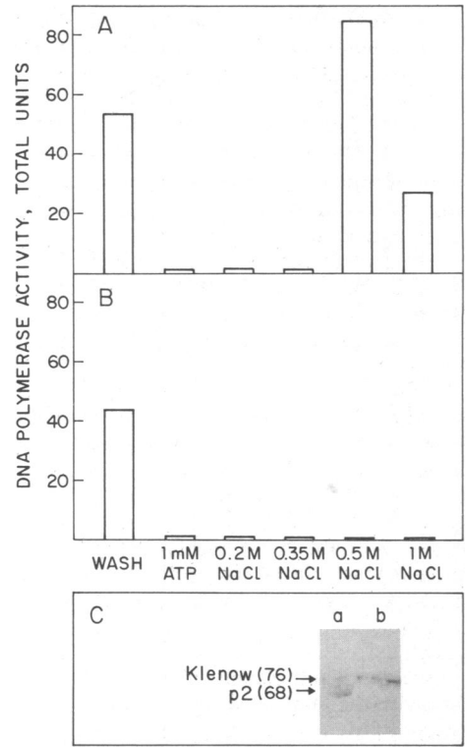

FIG. 2. Extracts of *E. coli* K-12 *ΔH1Δtrp* cells harboring the gene 2-containing plasmid pLBw2 (*A*) or the control plasmid pPLc28 (*B*) were subjected to affinity chromatography in the blue dextran-agarose column described in Fig. 1. DNA polymerase activity was assayed in the various fractions using poly(dA)·$(dT)_{12-18}$ as template. One unit of DNA polymerase catalyzed the incorporation of 1 nmol of dTMP into acid-insoluble material in 10 min at 30°C. (*C*) *In situ* DNA polymerase gel analysis of the fractions eluted with 0.5 M NaCl from dextran-blue agarose in the gene 2-containing (lane a) and control (lane b) samples. The position expected for p2 and that of the Klenow fragment of *E. coli* DNA polymerase I, determined by its activity in the *in situ* assay, are indicated with arrows. Numbers in parentheses represent $M_r \times 10^{-3}$.

Table 1. Purification of protein p2

Fraction	Initiation activity			DNA polymerase activity	
	Total protein, mg	Total units, pmol	Specific activity, pmol/mg	Total units, nmol	Specific activity, nmol/mg
Extract	354	117	0.33	1035	2.9
Ammonium sulfate	35	123	3.5	569	16.2
Blue dextran-agarose (0.35 M)	10.6	0	0	193	18.2
Blue dextran-agarose (0.7 M)	1.5	35	23	54	36
Phosphocellulose (0.4 M)	0.05	6.8	136	7.2	144

E. coli NF2690 cells (5 g) harboring the gene 2-containing recombinant plasmid pLBw2 (11) were ground with alumina and extracted with buffer A [50 mM Tris·HCl, pH 7.5/50 mM NaCl/5% (vol/vol) glycerol]. After centrifugation at 20,000 × *g* for 15 min the extract was treated with polyethylene glycol 8000/dextran 500 in 4 M NaCl as described (19). The polyethylene glycol was removed by precipitaton with ammonium sulfate to 35% saturation, and the resulting aqueous phase was further precipitated with ammonium sulfate up to 60% saturation. The precipitate, dissolved in buffer B (50 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$) was passed through a blue dextran-agarose column (15 × 1.8 cm) saturated with 10 vol of lysozyme (150 μg/ml) and equilibrated in buffer B. After washing with buffer B, the column was eluted with buffer C (50 mM Tris·HCl, pH 7.5/1 mM $MgCl_2$/1 mM ATP) and then with the latter buffer containing 0.35 M, 0.7 M, or 1 M NaCl. A 20% sample of the fraction eluted with 0.7 M NaCl, containing protein p2, was passed through a phosphocellulose column (1.8 × 1.2 cm) equilibrated with buffer A. The column was washed with buffer A and then eluted with a buffer containing 50 mM Tris·HCl, pH 7.0/1 mM dithiothreitol/5% (vol/vol) glycerol/0.4 or 0.6 M NaCl. Initiation activity was assayed. The amount of p3–dAMP complex was quantitated by excising the ^{32}P-labeled band from the gel and counting the Cerenkov radiation. Similar values were obtained by densitometry of the autoradiographs. One unit of initiation activity catalyzed the formation of 1 pmol of p3–dAMP complex in 20 min at 30°C. One unit of DNA polymerase activity, assayed with poly(dA)·$(dT)_{12-18}$ as template, catalyzed the incorporation of 1 nmol of dTMP into acid-insoluble material in 10 min at 30°C. Protein concentration was determined by the method of Bradford (20). The amount of protein in the phosphocellulose fraction was determined by densitometry of the stained band, using bovine serum albumin as standard. The values summarized in this fraction are those expected if the entire fraction had been subjected to the phosphocellulose chromatography.

NaCl. No initiation activity was present in the flow-through fraction (not shown) or in the 0.35 M (Table 1) or 1 M (not shown) NaCl eluates. The DNA polymerase activity in the ammonium sulfate cut fractionated in two peaks, one eluting at 0.35 M NaCl and the other at 0.7 M NaCl, the latter together with the initiation activity. The two DNA polymerase activities were clearly different. The one eluting at 0.35 M NaCl, like purified *E. coli* DNA polymerase I, had an activity with activated salmon testes DNA about one-half that with poly(dA)·$(dT)_{12-18}$; the K_m value for dTTP was ≈2.5 × 10^{-6} M, with activated DNA as template. The DNA polymerase eluting at 0.7 M NaCl had an activity with activated salmon testes DNA ≈15% of that with poly(dA)·$(dT)_{12-18}$; the K_m value for dTTP was ≈1 × 10^{-6} M with activated DNA as template. Fig. 3 (lane a) shows that the 0.7 M NaCl fraction had a protein band with the electrophoretic mobility expected for protein p2. To further purify protein p2, the latter fraction was passed through a phosphocellulose column. Fig. 3 (lane c) shows the presence of a major band at the position expected for protein p2 in the fraction eluted with 0.4 M NaCl. Densitometric analysis of the gel indicated that the protein was ≈86% pure. No protein band at the position of p2 was present in the flow-through fractions (lane b) or in the 0.6 M NaCl eluate (lane d). As shown in Table 1, the protein at the final purification step had initiation and DNA polymerase activities. Protein p2, stored at −20°C in 50% (vol/vol) glycerol in the presence of bovine serum albumin (1 mg/ml), was stable for at least 3 months. However, protein p2 was inactivated when stored at 4°C or at low ionic strength.

Formation of the p3–dAMP Initiation Complex with Purified Protein p2: Requirement of a Host Factor. Purified protein p2 was active in the formation of the p3–dAMP initiation complex when complemented with extracts of *B. subtilis* infected with a *sus2* mutant (Fig. 4, lane b) or with extracts of *E. coli* transformed with the gene 3-containing recombinant plasmid pKC30 A1 (lane a). Protein p2 by itself was inactive (lane c). When purified protein p2 was complemented with purified protein p3 for the initiation reaction, little activity was observed (lane d). However, addition of extracts of either uninfected *B. subtilis* (lane e), *B. subtilis* infected with a *sus2 sus3* double mutant (lane f), or *E. coli* (results not shown) stimulated ≈20-fold the formation of the initiation complex, indicating that, in addition to the viral proteins p2 and p3, some host factor(s), but not other viral proteins, is essential for the initiation reaction. No reaction took place when, in the presence of purified proteins p2 and p3 and uninfected *B. subtilis* extracts, the φ29 DNA–protein p3 complex was omitted (lane g). Addition of bovine serum albumin did not replace the *B. subtilis* or *E. coli* extracts (lane h). The finding that *E. coli* extracts can provide the host factor(s) is in agreement with the fact that purified protein p2 could be complemented with plasmid-containing *E. coli* extracts providing protein p3 (lane a).

Requirements of the φ29 DNA Polymerase. The requirements for the DNA polymerase activity of protein p2 were studied using the phosphocellulose fraction. The DNA template and Mg^{2+} were absolutely needed. The activity with poly(dA)·$(dT)_{12-18}$ was ≈7-fold higher than that obtained with activated salmon testes DNA. Removal of ATP decreased the DNA polymerase activity to 40%. Addition of 0.15 M and 0.25 M KCl decreased the activity to 28% and 4%, respectively. Aphidicolin, 6-(*p*-hydroxyphenylazo)-uracil (gifts from the Imperial Chemical Industries) or antiserum against the *B. subtilis* DNA polymerase III (gift from N. C. Brown) had no effect on the p2 DNA polymerase activity. The K_m value for dTTP, assayed with activated salmon testes DNA, was 0.7 × 10^{-6} M, in agreement with the value obtained with the 0.7 M NaCl eluate from the blue dextran-agarose column. When a template-primer, such as poly(dA)·$(dT)_{12-18}$, was used there was a sigmoid kinetics and no dTMP incorporation occurred up to 2 μM dTTP. This is in agreement with the fact that φ29 DNA replication using extracts of φ29-infected *B. subtilis*, in the presence of 0.5 μM [α-^{32}P]dATP, gave rise to the formation of p3–dAMP initiation complex, and not the expected elongation product p3-AAA, which was only produced when the dATP concentration was increased (5).

φ29 DNA–Protein p3 Is a Template for the Protein p2 DNA Polymerase. Fig. 5*A* shows that, in the presence of purified protein p3, a partially purified protein p2 containing the host factor(s) required for initiation and free of host DNA polymerase activity, gave rise to [^3H]TMP incorporation when φ29 DNA–protein p3 was used as template. No incorpo-

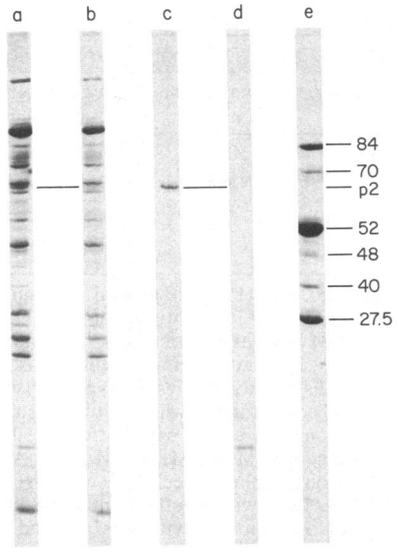

FIG. 3. NaDodSO$_4$/PAGE of purified protein p2. Proteins at the last purification steps were subjected to NaDodSO$_4$ electrophoresis in slab gels containing a 10%–20% acrylamide gradient and stained as described by Fairbanks *et al.* (21). Lanes: a, 0.7 M NaCl eluate from the blue dextran-agarose column (70 μg); b–d, phosphocellulose fractions; b, flow-through (50 μg); c, 0.4 M NaCl eluate (2 μg); d, 0.6 M NaCl eluate (2 μg); e, structural proteins of phage ϕ29 (8 μg).

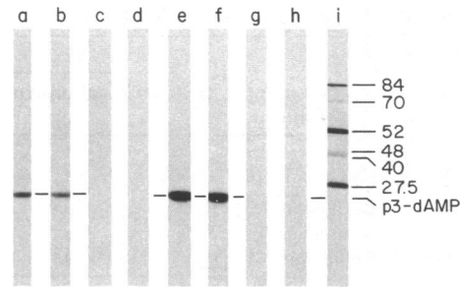

FIG. 4. Activity of purified protein p2 in the formation of the p3–dAMP initiation complex. Purified protein p2 (10 ng) was incubated with extracts of *E. coli* cells transformed with the gene 3-containing recombinant plasmid pKC30 A1 (lane a), with extracts of *B. subtilis* infected with mutant *sus2*(513) (lane b), or without extract (lane c) and assayed for formation of p3–dAMP complex in the presence of ϕ29 DNA–protein p3 as template; lanes d–h, purified protein p2 (10 ng) was complemented with purified protein p3 (20 ng) in the presence of ϕ29 DNA–protein p3 as template with the following additions; lane d, none; lane e, extract of uninfected *B. subtilis* (3 μg); lane f, extract of *B. subtilis* infected with mutant *sus2*(513) *sus3*(91) (1.5 μg); lane g, as in lane e but without ϕ29 DNA-protein p3 template; lane h, bovine serum albumin (3 μg); lane i, ^{35}S-labeled ϕ29 structural proteins.

ration took place with proteinase K-treated ϕ29 DNA–protein p3. Protein p2 by itself, although active with the template-primer poly(dA)·(dT)$_{12-18}$ (see Fig. 2*A*), was not active with ϕ29 DNA–protein p3 whether proteinase K-treated or not. These results indicate that elongation is taking place from the p3–dAMP initiation complex, and it is not due to a repair reaction. To further show the specificity of the elongation reaction, proteins p3 and p2 (containing host factor) were incubated with ϕ29 DNA–protein p3 for 5 min in the presence of 0.25 μM [α-^{32}P]dATP to allow the formation of the p3–dAMP initiation complex. Then, by addition of an excess of dATP, dTTP, dGTP, and dideoxy-CTP, elongation was allowed to occur up to nucleotides 9 and 12 from the left and right DNA ends, respectively (5). Fig. 5*B* shows that, after the chase, in addition to the protein p3–dAMP band, there were two slower bands at the expected positions.

DISCUSSION

A ϕ29-specific DNA polymerase has been purified and characterized by using *E. coli* cells transformed with a gene 2-containing recombinant plasmid (11, 12). The purified ϕ29 DNA polymerase was shown to be required for the formation of the p3–dAMP initiation complex, in agreement with the *in vivo* (9) and *in vitro* (7, 8) role of the gene 2 product in the initiation of ϕ29 DNA replication. The purified protein p2 has a molecular weight of 68,000, the size reported by McGuire *et al.* (10) for the gene 2 product. The open reading frame in the ϕ29 DNA sequence to the left of the one shown

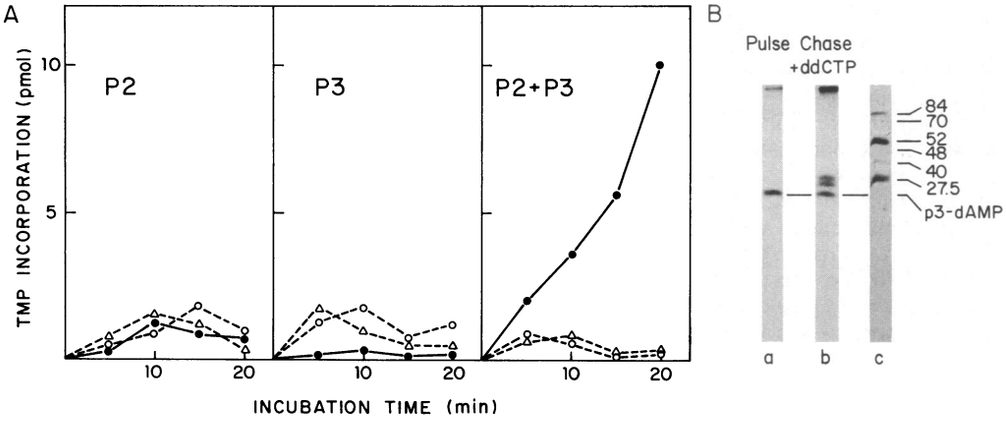

FIG. 5. DNA polymerase activity of protein p2 with ϕ29 DNA-protein p3 as template. (*A*) Partially purified fraction of protein p2 (0.4 μg) eluting from the blue dextran-agarose column with 0.5 M NaCl (Fig. 1) was incubated with ϕ29 DNA-protein p3 complex (●), with proteinase K-treated ϕ29 DNA (○), or without DNA (△) in the absence or presence of purified protein p3 (20 ng). A control of purified protein p3 in the absence of protein p2 was also incubated with the same DNA templates. After 15 min at 30°C, the incorporation of [^{3}H]dTMP in trichloroacetic acid-insoluble material was determined. (*B*) Partially purified fraction of protein p2 (0.5 μg) described in *A* was incubated with purified p3 (20 ng) and ϕ29 DNA–protein p3 complex in the presence of 0.25 μM [α-^{32}P]dATP (5 μCi) for formation of the p3–dAMP initiation complex. After 5 min at 30°C (lane a), 40 μM each dATP, dTTP, and dGTP and 100 μM dideoxy-CTP were added, and the incubation continued for 15 min (lane b). The samples were passed through a Sephadex G50 column to remove the unincorporated [^{32}P]dATP, precipitated with trichloroacetic acid, and subjected to NaDodSO$_4$/PAGE as described (5). The ^{35}S-labeled structural proteins of ϕ29 were used in (lane c) as molecular weight markers (numbers on right represent $M_r \times 10^{-3}$).

to be that of gene 3 (22, 23) corresponds to a protein of M_r ≈66,000 (24), in good agreement with the molecular weight of the purified protein p2. The φ29 DNA polymerase activity, shown by *in situ* gel analysis to be associated with a M_r 68,000 protein, in addition to being involved in the initiation reaction, is able to elongate *in vitro* the p3–dAMP initiation complex.

The role of protein p2 in initiation is likely to be to catalyze the formation of the covalent linkage between the hydroxyl group of a serine residue in protein p3 (25) and 5′-dAMP, probably acting as a DNA polymerase that is able to recognize, in addition to the 3′ hydroxyl group provided by a nucleotide in the DNA chain, the hydroxyl group of a serine residue in protein p3.

The role of φ29 protein p2 in elongation *in vivo* remains to be elucidated. *In vivo* shift-up experiments using mutant *ts2*(98) indicated that the mutation affects an initiation step in φ29 DNA replication (9); however, one would expect also a role of protein p2 in elongation because of its DNA polymerase activity *in vitro*. The above results can be explained by assuming that (*i*) protein p2 has two active centers, one for initiation and another for elongation, and that the *ts2*(98) mutation affected the first one or (*ii*) the DNA polymerase activity of protein p2 is protected from heat inactivation when it is present in the replication complex. An *in vivo* behavior similar to that of mutant *ts2*(98) has been reported for the adenovirus-5 mutant *ts149* (26), which affects the adeno-DNA polymerase involved in the protein-primed initiation of replication (reviewed in ref. 27).

When purified φ29 DNA polymerase (p2) and terminal protein (p3) were used, the initiation reaction was strongly stimulated by addition of extracts of *B. subtilis* or *E. coli*, indicating that some host factor(s) is required in addition to proteins p2 and p3 and φ29 DNA-protein p3 template for the formation of the initiation complex. If the host factor is a DNA replication protein, the fact that it is also present in *E. coli* may help elucidate its role in the initiation of φ29 DNA replication because of the knowledge of the function of replication proteins in *E. coli* and the availability of mutants.

Linear double-stranded DNAs with protein covalently linked at their 5′ ends have been reported, in addition to φ29, in adenovirus and the φ29-related phages φ15, M2, Nf, and GA-1 (28); in plasmid pSLA2 from *Streptomyces* (29); in the S1 and S2 mitochondrial DNA from maize (30); in the *Streptococcus pneumoniae* phage Cp-1 (31); and in the *Salmonella typhimurium* phage PRD1 (32). In addition to φ29 and adenovirus, a covalent complex between the terminal protein and the terminal nucleotide 5′-dAMP, has been found *in vitro* when extracts from cells infected with phages M2 (8) or Cp-1 (unpublished results) were incubated with [α-^{32}P]dATP. The mechanism of protein priming is likely to be a way to initiate replication at the ends of protein-containing linear DNAs, and a specific DNA polymerase, able to recognize the hydroxyl group from an amino acid residue in the terminal protein, is probably required in each case.

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