BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **238,** 33–38 (1997)
ARTICLE NO. RC977234

Strand Displacement Associated DNA Synthesis Catalyzed by the Epstein-Barr Virus DNA Polymerase

Tatsuya Tsurumi,*,†,[1] Hiroshi Yamada,† Tohru Daikoku,†
Yasuo Yamashita,† and Yukihiro Nishiyama†

**Laboratory of Viral Oncology, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464, Japan; and †Laboratory of Virology, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan*

Received July 25, 1997

The Epstein-Barr virus (EBV) DNA polymerase (Pol) holoenzyme is an essential enzyme required for ori-Lyt dependent EBV DNA replication. Using singly primed M13ssDNA circles as template, the EBV DNA Pol holoenzyme synthesized DNA chains greater than the unit length of M13 ssDNA in addition to full length products even at a low ratio of polymerase molecule per templates. The long replication products consisted of circular double-stranded DNA with single-stranded tails that were sensitive to mung bean nuclease. Reconstitution of the EBV Pol holoenzyme by preincubation of BALF5 Pol catalytic subunit and BMRF1 Pol accessory subunit in vitro resulted in reproduction of the strand displcement DNA synthesis. Thus, the EBV DNA Pol holoenzyme by itself is able to produce strand displacement coupled to the polymerization process in a highly processive way in the absence of any other protein.

Epstein-Barr virus (EBV) is a human herpesvirus with a linear double stranded DNA, 172 kbp in length (1). The EBV has both a latent state and a lytic replicative cycle in the infected lymphoblastoid cells. After induction of the viral productive cycle, the EBV replication proteins are induced and the EBV genome is amplified 100 to 1000-fold. Intermediates of viral DNA replication are found as large head to tail concatemeric molecules which could result from rolling-circle DNA replication initiated from the lytic phase replication origin, ori-Lyt (2). The viral replication proteins that are essential for the ori-Lyt dependent EBV DNA replication are determined (3): the DNA Pol catalytic subunit (BALF5), the DNA Pol accessory subunit (BMRF1), the single-stranded DNA binding protein (BALF2), helicase and primase (BBLF4 and BSLF1), helicase-primase associated protein (BBLF2/3) and lytic transactivator and ori-Lyt-binding protein (BZLF1). These viral replication proteins except BZLF1 protein are conceivable to work together at replication fork to synthesize leading and lagging strands of the concatemeric EBV genome.

The BALF5 Pol catalytic polypeptide copurifies with the BMRF1 Pol accessory subunit from EBV-producing lymphoblastoid cells (4, 5, 6). The BALF5 protein appears to form a tight complex with the BMRF1 protein to function as Pol holoenzyme. The EBV Pol holoenzyme exhibits both 5′-to-3′ DNA polymerase and 3′-to-5′ exonuclease activities (6). Besides these activities, the EBV DNA Pol holoenzyme is characterized by its strikingly high polymerase processivity (7, 8). The ori-Lyt dependent DNA replication would be best served by a highly processive DNA polymerase which could synthesize long DNA chains without dissociating from the template. Although the BALF5 Pol catalytic subunit by itself is a quasi-processive enzyme (9, 10), the BMRF1 Pol accessory subunit has been presumed to form a complex with the BALF5 Pol catalytic subunit to stabilize the interaction of the holoenzyme complex with the 3′-OH end of the primer on the template DNA during polymerization and exonucleolysis (9, 11).

In a study of the EBV Pol holoenzyme by using singly primed M13 ssDNA circles as template we have observed that the EBV Pol holoenzyme synthesizes DNA chains greater than unit length of M13 ssDNA in addition to full length products. Here we reports that the EBV DNA Pol holoenzyme by itself is able to carry out strand displacement DNA replication in a highly processive way under some limited condition.

MATERIALS AND METHODS

Materials. ATPγ-S, adenosin 5′-O-(3-thiotriphosphate), was purchased from Boehringer Mannheim Inc. [α-^{32}P]dCTP (3000Ci/mmol) and [α-^{32}P]dGTP (3000Ci/mmol) were from Amersham Corp.. *E.coli*

[1] Corresponding author. Fax: 81-52-744-2428.

0006-291X/97 $25.00

Pol III holoenzyme was purchased from Toyobo Inc. Japan. Primer 1, an oligodeoxynucleotide CACAATTCCACACAAC, complementary to nucleotides 6170 to 6185 of M13mp18 single-stranded DNA was purchased from New England Biolabs, Inc.. Circular single-stranded M13 mp18 DNA was from Pharmacia LKB Biotechnology Inc..

Preparation of the EBV DNA Pol holoenzyme, the BALF5 Pol catalytic subunit, and the BMRF1 Pol accessory subunit. The EBV DNA Pol holoenzyme was purified to homogeneity from nuclear extract of B95-8 cells treated by 100 ng/ml TPA and 5 mM sodium n-butyrate as described previously (6). The purified EBV DNA Pol holoenzyme contained 110 k and 52∼48 k polypeptides which were identified as the BALF5 gene product and the BMRF1 gene products, respectively, by westernblot analysis (data not shown). The specific activity of the purified EBV Pol holoenzyme was 1,400,000 U/mg. One unit of enzyme activity was defined as the amount required for incorporation of 1 pmol of deoxynucleoside monophosphates into acid-insoluble material in 60 min at 35 °C in the presence of 80 mM ammonium sulfate by using 80 μg/ml of activated DNA as template (6). The enzyme was devoid of DNA dependent ATPase, AdATPase, primase, and DNA ligase activities which were done as described (12). Furthermore, in order to exclude the possibility of the contamination of DNA helicases, DNA helicase assay was performed using the enzyme preparation and M13 ssDNA circles hybridized ^{32}P-labelled oligonucleotide (20 mer) as substrate in the presence of 1 mM ATP or dATP essentially as described (13). However, no labelled oligonucleotide was displaced even when large amount of the enzyme was contained in the reaction (data not shown).

The overexpression and purification systems of the individual components of the EBV Pol holoenzyme have been developed by using a baculovirus expression system (10, 14). The EBV DNA Pol catalytic subunit (BALF5 protein) was purified to homogeneity from cytosolic extracts of recombinant baculovirus AcBALF5-infected Sf9 cells as described previously (10). The specific activity of the BALF5 Pol catalytic subunit was 5,900,000 U/mg. One unit of enzyme activity was defined as the amount required for incorporation of 1 pmol of deoxynucleoside monophosphates into acid-insoluble material in 60 min at 35 °C in the absence of ammonium sulfate by using 80 μg/ml of activated DNA as template (10). The purified BALF5 protein (110 k) was devoid of DNA dependent ATPase, dATPase, DNA helicase and primase activities. Incidentally, the Pol assay condition for determination of the specific activity for the EBV Pol holoenzyme and that for the BALF5 Pol catalytic subunit are different in salt concentration. The polymerase activity associated with the EBV Pol holoenzyme is stimulated by ammonium sulfate when activated DNA is used as template-primer, whereas that of the BALF5 Pol catalytic subunit is inhibited drastically by 80 mM ammonium sulfate (9, 10).

The EBV DNA Pol accessory subunit (BMRF1 protein) was purified to homogeneity from nuclear extracts of recombinant baculovirus AcBMRF1-infected Sf9 cells as described previously (14). The purified BMRF1 protein (48∼50 k) was devoid of detectable DNA polymerase, DNase, DNA dependent ATPase, dATPase, DNA helicase and primase activities (12).

Replication assay with singly primed circular M13: Strand displacement assay. Measurements of the strand displacement activity of the EBV DNA polymerase were usually performed in Buffer C (30 μl) (20 mM Hepes-Na [pH 7.4], 2 mM $MgCl_2$, 1 mM DTT, 5 % glycerol, 50 mM NaCl, 100 μg of BSA per ml) containing 40 μM each of dTTP and dATP, and 50 ng of primer 1-hybridized M13 single-stranded DNA circle along with the EBV DNA Pol complex (0.8 ng), or the BALF5 Pol catalytic subunit (0.5 ng), or the reconstituted Pol complex composed of the BALF5 protein and the BMRF1 Pol accessory subunit (0.5 ng each of) at the concentration and amounts indicated in each case. Reactions were initiated by the addition of 40 μM dGTP, 4 μM [α-^{32}P] dCTP (5 μCi). The samples were removed at the times indicated and quenched by addition of 5 μl of 200 mM EDTA. Six μl of aliquotes were analysed for DNA synthesis by measurement of the radioactivity incorporated into acid-insoluble material as described (6). When the replication products were analyzed by alkaline agarose gel electrophoresis (15), the remaining replication products were precipitated with ethanol to remove unincorporated nucleotides. The precipitates were resuspended in gel loading buffer consisting of 50 mM NaOH, 1 mM EDTA, 20 % glycerol, and 0.1 % bromophenol blue. The DNA size markers (High molecular weight DNA markers from BRL and λ DNA/Hind III fragments) were 3′-end labeled by T4 DNA polymerase. The alkaline agarose vertical gel electrophoresis was run at 80 mA for 15 h. After electrophoresis, the gels were washed in 7.5 % trichloroacetic acid and then dried on DE81 paper under vacuum. The dried gels were exposed to Fuji imaging plate and analysed by Fuji Image Analyser BAS 2000.

When the products were treated with mung bean nuclease, the reactions were stopped by addition of an equal volume of 0.6 % SDS, 16 mM EDTA, and 0.16 mg of proteinase K per ml, followed by further incubation for 15 min at 37 °C. The DNA was extracted with equal volume of phenol and chroloform-isoamylalcohol (24:1), precipitated with ethanol and suspended in mung bean nuclease buffer (30 mM Sodium Acetate, pH 5.0, 100 mM NaCl, 1 mM Zinc Acetate, and 5 % glycerol). The replication products were treated with indicated amounts of mung bean nuclease for 3 h at 35°C, followed by addition of 0.1 % SDS and 50 mM EDTA to inactivate the nuclease. The DNA was precipitated with ethanol and resuspended in the gel loading buffer. The samples were heated to 100 °C for 3 min and then loaded onto the 0.7 % alkaline agarose gels containing 50 mM NaOH and 1 mM EDTA alongside DNA size markers.

RESULTS

Strand Displacement-Associated Replication of Singly Primed ssDNA Circle by the EBV DNA Pol Holoenzyme

The ability of the enzyme to carry out strand displacement was analysed by performing primer-extention experiments at a molar ratio of enzyme/primer 1 hybridized M13 ssDNA circles of 1 : 4. The replication products were denatured and the newly synthesized strands were analysed in alkaline 0.7 % agarose gels (Fig. 1). Alkaline agarose gels are used to analyse the size of the single stranded DNA.

For comparison, the E.coli Pol III holoenzyme was used. Although E.coli Pol III holoenzyme is capable of very rapid (> 500 nucleotides/sec) and highly processive primer elongation (> 5000 nucleotides), it is easily dissociated by encountering the 5′ ends of primers (16, 17). Total nucleotide incorporation and the alkaline analyses of the in vitro labeled replication products shows that the Pol III holoenzyme stopped synthesis within 5 min when the M13 DNA circle was completed. The Pol III holoenzyme synthesized full length M13 DNA in addition to a band of pausing site at 3.5 kb. However, DNA species greater than unit length M13 ssDNA were not generated even after 40 min incubation.

In contrast, the EBV Pol holoenzyme incorporated nucleotides with time and produced DNA molecules of approximately 7200 nucleotides, corresponding to the entire length of M13mp18 DNA in an 10 min incubation, in addition to a specific band of pausing site (3 Kb). The regions that block DNA Pol from passing through is known to form particular stable duplex hair-

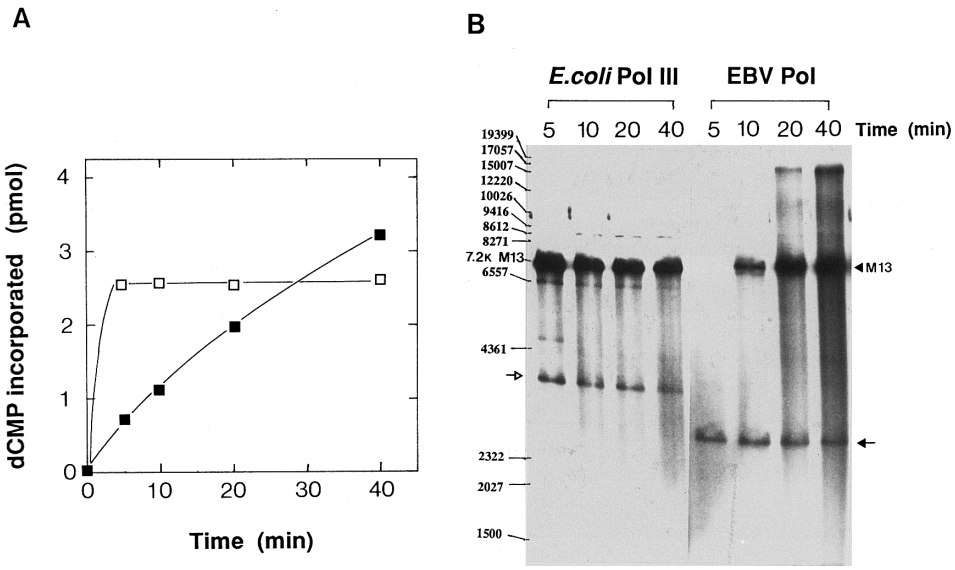

FIG. 1. DNA replication of singly primed M13 ssDNA by the EBV DNA Pol holoenzyme and E.coli Pol III holoenzyme. The replication assay was carried out as described under Materials and Methods. The incubation mixture contained 20 μM concentration each of dATP, dGTP, dTTP, and 4 μM [α-^{32}P]dCTP (5 μCi), 50 ng of singly primed M13 ssDNA, and 0.8 ng of the EBV Pol holoenzyme purified from B95-8 cells or 5 units of the E.coli Pol III holoenzyme (Toyobo, Inc.). After incubation at 35 °C for the time indicated, the reactions were stopped. Six μl of the reactions of the EBV DNA Pol holoenzyme (■) or of E.coli Pol III holoenzyme (□) were measured dCMP incorporation into acid-insoluble material (A). The remaining reactions were processed as described and the replication products were analysed by alkaline 0.7% agarose gel electrophoresis (B). The positions of nucleotides length markers were indicated at the left of the panel B as well as unit-size of M13 ssDNA. Major bands of pausing sites were represented by arrows. E.coli Pol III holoenzyme had a different pausing site on M13 ssDNA circle from that of EBV DNA Pol holoenzyme.

pin structures (8, 18). But it is unclear why the sites of pausing are distinct between the EBV DNA Pol and E.coli. Pol III holoenzymes. After longer incubation periods, single stranded DNA molecules over 7200 nucleotides were detected. Thus, it is conceivable that the EBV DNA Pol holoenzyme fully replicated a primed M13 ssDNA in 10 min, proceeding further through strand displacement associated DNA replication. Some of DNA chains produced after 20 min reached approximately 15 Kb, more than twice the length of M13 ssDNA. Maximum strand displacement activity was obtained at 20 mM Hepes-Na, pH 7.4 and 50mM NaCl, and 2 mM $MgCl_2$ (data not shown).

Processive DNA Synthesis by the EBV DNA Pol Holoenzyme during Strand Displacement

To test the processivity of the EBV DNA Pol holoenzyme during the strand displacement DNA replication, the synthesis of DNA chains with M13 ssDNA circles hybridized with primer 1 was studied using increasing dilutions of the enzyme. The replication products synthesized in vitro were denatured and analysed by alkaline 0.7% agarose gel electrophoresis (Fig.2). A nonprocessive pattern of elongation should give rise to a decrease in the DNA size concomitant with the polymerase dilution. A 32-fold decrease in DNA polymerase/primer terminus molar ratio decreased about 30 fold the amount of nucleotide incorporation (data not shown). However, high molecular weight DNA chains longer than unit length of M13ssDNA was still synthesized, indicating that the EBV Pol holoenzyme elongates the DNA chains without dissociation even during the strand displacement DNA replication.

Susceptibility of the Replication Products to Mung Bean Nuclease

We examined the effect of mung bean nuclease on the high molecular weight DNA synthesized by the EBV Pol holoenzyme to verify that the products were generated by strand displacement associated DNA replication (Fig.3 lanes 1~3). After 30 min incubation, the reactions were removed and treated with proteinase K, followed by phenol/chroloform. The replication products were precipitated by ethanol and then treated with mung bean nuclease. Mung bean nuclease is a single stranded DNA specific endonuclease and does not digest double stranded DNA (15). The nuclease treated samples were denatured and applied to alkaline 0.7 % agarose gel electrophoresis. Mung bean nuclease treatment did not affect the alkaline agarose gel pattern of the replication products synthesized by E.coli Pol III holoenzyme at all even in longer incubation (data not shown). On the other hand, single stranded DNA bands greater than 7.2 kb which were synthesized by the EBV

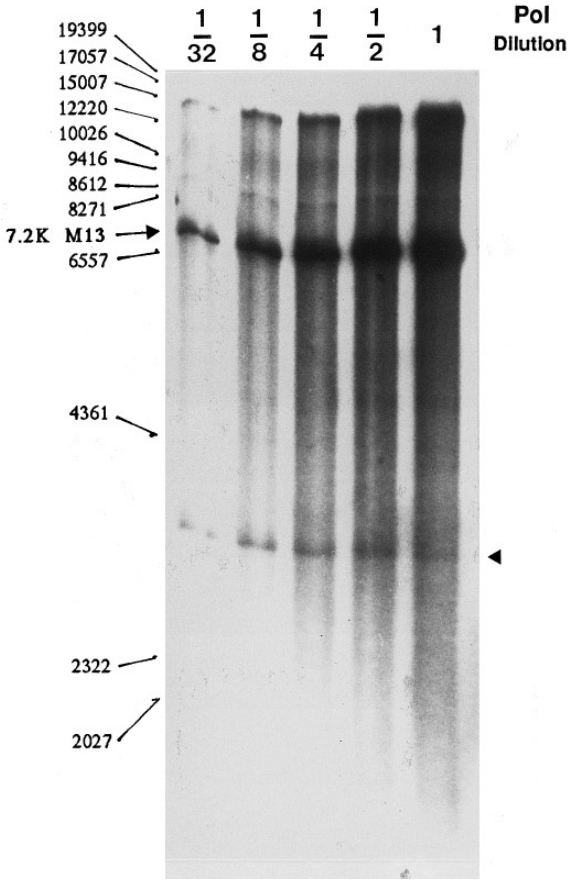

FIG. 2. Processive strand-displacement DNA synthesis by the EBV DNA Pol holoenzyme. The replication assay was carried out as described under Materials and Methods using 50 ng of singly primed M13 ssDNA, 20 μM concentration each of dATP, dCTP, dTTP, [α-^{32}P]dGTP (5 μCi), and the indicated dilutions of the EBV DNA Pol holoenzyme; dilution 1 corresponds to 3.2 ng of the EBV Pol holoenzyme (primer/enzyme ratio = 1). After incubation for 20 min at 35°C, the reactions were stopped. Six μl of the reactions were measured dCMP incorporation into acid-insoluble material (data not shown). The remaining samples were processed as described and the replication products were analysed by alkaline 0.7% agarose gel electrophoresis. The positions of nucleotides length markers were indicated at the left of the panel B as well as unit-size of M13 ssDNA. The position of pausing site was marked by closed triangle.

DNA Pol holoenzyme were disappeared by mung bean nuclease treatment (Fig.3. lanes 2 and 3). Also, the products of extended synthesis after alkaline denaturation and neutralization are fully sensitive to digestion with mung bean nuclease, ruling out the possibility of snap back DNA synthesis (data not shown). These observations mean that single stranded DNA region of the high molecular-weight replication products synthesized by the EBV DNA Pol holoenzyme was susceptible to cleavage by mung bean nuclease and DNA chains equal to the unit length of M13 DNA and downward remained intact, demonstrating that the displaced tail was single stranded.

ATP Hydrolysis Is Not Required for Strand Displacement DNA Replication by the EBV DNA Pol Holoenzyme

ATP hydrolysis is involved in primer recognition by, and increases in the processivities of some prokaryotic, bacteriophage, and eukaryotic DNA polymerase holoenzymes (19). ATP requirement for strand displacement by the EBV DNA Pol holoenzyme was examined (Fig. 3. lanes 4∼6). Neither the nucleotide polymerizing activity nor the strand displacement activity of the EBV DNA Pol holoenzyme was dependent on the presence of ATP. Further, ATPγ-S, a nonhydrolyzable analog of ATP and an inhibitor of ATPase, did not affect these activities. These results shows that strand displacement DNA synthesis catalyzed by the EBV DNA Pol holoenzyme does not require ATP hydrolysis and, furthermore, confirmed that there is no contamination of DNA helicases, which couple the energy liberated in the hydrolysis of ATP to strand displacement, in the preparation of the EBV DNA Pol holoenzyme. These results clearly indicate that the EBV DNA Pol holoen-

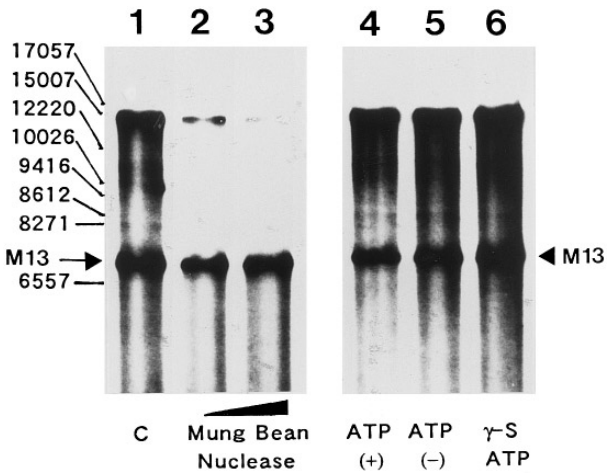

FIG. 3. ATP-independent strand displacement DNA replication by the EBV DNA Pol holoenzyme and susceptibility of the replication products to mung bean nuclease. Reaction mixtures containing primed M13ssDNA were incubated for 40 min with 0.5 mM ATP (lane 4); without ATP (lane 5); or with 1 mM ATPγ-S (lane 6). Reaction mixtures were incubated for 40 min at 35 °C and the reactions were sampled, processed, and analysed by alkaline 0.7 % agarose gel electrophoresis (lane 4∼6). Reaction mixtures for susceptibility to mung bean nuclease (lane 1∼3) were also incubated for 30 min at 35 °C and stopped by an equal volume of 0.5 % sodium dodecyl sulfate, 40 mM EDTA and 0.1 mg of proteinase K per ml, followed by further incubation for 15 min at 35 °C. The DNA was extracted with phenol/chloroform, precipitated with ethanol, and suspended in mung bean nuclease buffer. Mung bean nuclease was purchased from Takara Shuzo, Ltd.. The isolated product (lane 1) was digested with 10 units (lane 2) and 20 units (lane 3) of mung bean nuclease for 3 h at 37 °C. The reaction products were processed and analysed by alkaline 0.7 % agarose gel electrophoresis. The positions of marker fragments of the sizes (bases) were indicated at the left of the panel, as well as unit-size of M13 ssDNA.

zyme by itself is able to produce strand displacement coupled to polymerization process without aid of any other protein and that strand-displacement must be concomitant with the replication process.

Reproduction of the Strand Displacement DNA Synthesis by the Reconstituted EBV Pol Holoenzyme Consisting of the BALF5 Pol Catalytic Subunit and the BMRF1 Pol Accessory Subunit

To determine whether the strand displacement DNA synthesis is reproduced by the reconstitution of the EBV DNA Pol holoenzyme, the BALF5 protein and the BMRF1 protein which were purified from recombinant baculovirus infected insect cells were preincubated and then the Pol reaction was performed for 20 min. The replication products were analysed by alkaline 0.7% agarose gel electrophoresis (Fig.4).

As was previously reported (9), low levels of DNA synthesis were detected with the BALF5 Pol catalytic subunit alone (data not shown). Since the EBV Pol catalytic subunit alone extends primer slightly (~ 50 nucleotides) (9), we could not detect bands of the replication products on the gel (lane 3). Most of the replication products were passed through the gel.

The strand displacement DNA synthesis was reproduced by the reconstituted EBV Pol holoenzyme which was formed by preincubation of the BALF5 Pol catalytic subunit and the BMRF1 Pol accessory subunit in vitro. This observation confirmed that the EBV DNA Pol holoenzyme by itself is able to carry out strand displacement DNA synthesis without aid of any other protein factor.

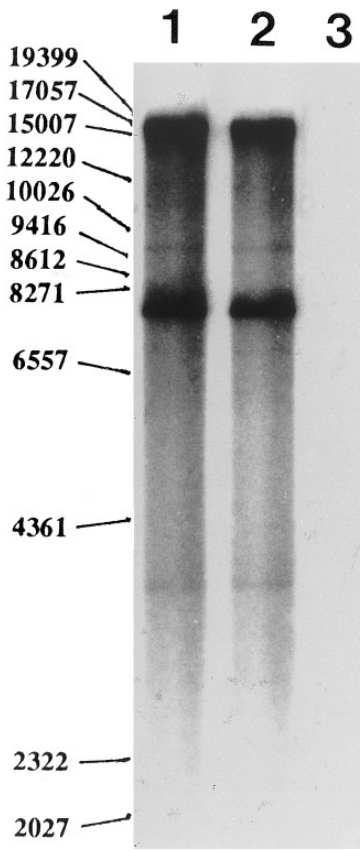

FIG. 4. Strand displacement DNA synthesis catalyzed by the reconstituted EBV Pol holoenzyme. The BALF5 Pol catalytic subunit and the BMRF1 Pol accessory subunit (0.5 ng of each) were preincubated at 0 °C for 10 min. The same amount of the BALF5 protein alone or 0.8 ng of the EBV Pol holoenzyme was also preincubated. Series of reaction mixture (30 μl) contained the EBV Pol holoenzyme (lane 1), the reconstituted Pol complex (lane 2), or the BALF5 Pol catalytic subunit (lane 3) and 24 ng of singly primed M13 single-stranded DNA (10 fmol as circle). Replication assays were performed under the conditions described in Materials and Methods. Reactions were incubated at 35 °C for 30 min. After quenching by addition of 5 μl of 200 mM EDTA, the samples were used for analysis of the replication products by electrophoresis in an alkaline 0.7 % agarose gel. The positions of the DNA size standards (bases) are indicated at the left of panel.

DISCUSSION

The EBV DNA Pol holoenzyme is a highly processive enzyme and had been thought to produce DNA chains equal to unit length of M13 DNA when singly primed M13 ssDNA circle was used as template-primer (8, 9). The replication products had been analyzed by neutral agarose gel or alkaline 1.2 % agarose gel electrophoresis (120 mA, 4.5 h), which is not suitable for resolution of the high molecular weight DNAs. In this report we have employed low concentration of alkaline agarose gel (0.6 ~ 0.7 %) electrophoresis (80 mA, 15 ~ 20 hrs) using vertical type electrophoresis apparatus to determine the molecular size of the high molecular weight replication products synthesized by the EBV DNA Pol holoenzyme. As a result, it turned out that DNA chains greater than unit length of M13 DNA in addition to full length products were produced.

Evidently the ability to catalyze strand displacement DNA synthesis without any other protein factor has been a common intrinsic property of several protein-primed DNA polymerases, as was described for the Bacillus phage f29 DNA polymerase (20) or PRD1 DNA polymerase (21). ϕ29 SSB enhances the strand displacement DNA synthesis catalyzed by the ϕ29 DNA polymerase (22). Other DNA polymerases which are known to be able to catalyze strand displacement DNA synthesis without the aid of other proteins on nicked, duplex DNA templates are E.coli Pol I (23) and T5 DNA polymerase (24). E.coli Pol I catalyzes strand displacement DNA synthesis at nicks, but only after its 5′-to-3′ exonuclease activity has removed 20 to 50 nucleotides. The 5′-to-3′ hydrolytic activity is not, however, a prerequisite for strand displacement since elimination of the exonuclease activity does not prevent this reaction. In eukaryotic DNA polymerases, Pol δ holoenzyme, in contrast to Pols α and ϵ, has been shown to

carry out strand displacement DNA synthesis on double-stranded gapped circular DNA (25). However, strand displacement DNA synthesis catalyzed by Pol δ holoenzyme was slow and limited. No products longer 190 nucleotides were observed even after 30 min of incubation. However, some prokaryotic DNA polymerases are known to catalyze strand displacement DNA synthesis only in the presence of the cognate SSB. Strand displacement DNA polymerization can also be carried out to a limited extent by T7 DNA polymerase in the presence of T7 gene 2.5 protein or E.coli SSB (26), or by T4 DNA Pol holoenzyme in the presence of T4 gp32 protein (27). It remains to be determined whether specific protein-protein interaction between the EBV Pol holoenzyme and the EBV SSB facilitates strand displacement DNA synthesis. The EBV SSB and the EBV helicase-primase would help EBV DNA Pol holoenzyme at replication fork to synthesize more efficiently both leading and lagging strands of the EBV genome.

ACKNOWLEDGMENTS

We thank T. Ikuta for technical assistance. This work was supported by grants-in-aid for Scientific Research on Priority Areas from the Ministry of Education, Science, and Culture of Japan (08277211 and 08670340 to T.T.).

REFERENCES

1. Baer, R., Bankier, A. T., Biggin, M. D., Deininger, P. L., Farrell, P. J., Gibson, T. J., Hatfull, G., Hudson, G. S., Satchwell, S. C., Seguin, C., Tuffnell, P. S., and Barrell, B. G. (1984) *Nature* **310,** 207–211.
2. Hammerschmidt, W., and Sugden, B. (1988) *Cell* **55,** 427–433.
3. Fixman, E. D., Hayward, G. S., and Hayward, S. D. (1995) *J. Virol.* **69,** 2998–3006.
4. Kallin, B., Sternas, L., Saemundssen, A. K., Luka, J., Jornvall, H., Eriksson, B., Tao, P-Z., Nilsson, M. T., and Klein, G. (1985) *J. Virol.* **54,** 561–568.
5. Li, J-S., Zhou, B-S., Dutschman, G. E., Grill, S-P., Tan, R-S., and Cheng, Y-C. (1987) *J. Virol.* **61,** 2947–2949.
6. Tsurumi, T. (1991a) *Virology* **182,** 376–381.
7. Tsurumi, T. (1991b) *Biochem. J.* **280,** 708–713.
8. Tsurumi, T., Kobayashi, A., Tamai, K., Yamada, H., Daikoku, T., Yamashita, Y., and Nishiyama, Y. (1996) *Virology* **222,** 352–364.
9. Tsurumi, T., Daikoku, T., Kurachi, R., and Nishiyama, Y. (1993a) *J. Virol.* **67,** 7648–7653.
10. Tsurumi, T., Kobayashi, A., Tamai, K., Daikoku, T., Kurachi, R., and Nishiyama, Y. (1993b) *J. Virol.* **67,** 4651–4658.
11. Tsurumi, T., Daikoku, T., and Nishiyama, Y. (1994) *J. Virol.* **88,** 3354–3363.
12. Crute, J. J., Tsurumi, T., Zhu, L., Weller, S. K., Olivo, P. D., Challberg, M. D., Mocarski, E. S., and Lehman, I. R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2186–2189.
13. Crute, J. J., Mocarski, E. S., and Lehman, I. R. (1988) *Nucleic Acids Res.* **16,** 6585–6596.
14. Tsurumi, T. (1993) *J. Virol.* **67,** 1681–1687.
15. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
16. O'Donnell, M. E., and Kornberg, A. (1985) *J. Biol. Chem.* **260,** 12875–12883.
17. O'Donnell, M. E., and Kornberg, A. (1985) *J. Biol. Chem.* **260,** 12884–12889.
18. Villani, G., Fay, P. J., Bambara, R. A., and Lehman, I. R. (1981) *J. Biol. Chem.* **256,** 8202–8207.
19. Hurwitz, J., Dean, F. B., Kwong, A. D., and Lee, S. H. (1990) *J. Biol. Chem.* **265,** 18043–18046.
20. Blanco, L., Bernad, A., Lazaro, J. M., Martin, G., Garmendia, C., and Salas, M. (1989) *J. Biol. Chem.* **264,** 8935–8940.
21. Zhu, W., and Ito, J. (1994) *Biochem. Biophys. Acta* **1219,** 267–276.
22. Soengas, M. S., Gutierrez, C., and Salas, M. (1995) *J. Mol. Biol.* **253,** 517–529.
23. Kornberg, A., and Baker, T. (1992) DNA Replication, 2nd ed., Freeman, New York.
24. Fujimura, R. K., and Roop, B. C. (1976) *J. Biol. Chem.* **251,** 2168–2175.
25. Podust, V. N., Podust, L. M., Muller, F., and Hubscher, U. (1995) *Biochemistry* **34,** 5003–5010.
26. Nakai, H., and Richardson, C. C. (1988) *J. Biol. Chem.* **263,** 9831–9839.
27. Cha, T-A., and Alberts, B. M. (1989) *J. Biol. Chem.* **264,** 12220–12225.