Deoxyribonuclease Activity Found in Epstein–Barr Virus Producing Lymphoblastoid Cells†

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ABSTRACT: A deoxyribonuclease activity from Epstein–Barr (EB) virus producer lymphocyte cell lines which is correlated with viral production and which is not present in virus nonproducer or negative lymphocyte cell lines has been purified 220-fold with 20% recovery and characterized. This nuclease copurifies through diethylaminoethylcellulose column chromatography with the EB virus induced deoxyribonucleic acid (DNA) polymerase in EB virus producer cells which was recently reported by this laboratory, but elutes as a separate peak of activity upon phosphocellulose chromatography. This nuclease activity has a sedimentation coefficient of 4.0 S, a strong divalent cation requirement, an alkaline pH optimum, and the ability to utilize both native and denatured lymphocyte DNA as substrate, reducing both to monophosphonucleosides.

Epstein–Barr (EB) virus is a human herpes virus which is associated with infectious mononucleosis, African Burkitt's lymphoma, and nasopharyngeal carcinoma (Henle et al., 1968; Wolf et al., 1973; de Thé et al., 1978). Certain strains of EB virus also act as tumor viruses, transforming human or new world primate B lymphocytes into permanently dividing B cell lines (Miller et al., 1974). However, a full lytic cycle of infection has not been reported for EB virus; an abortive lytic cycle has been reported in which substantial viral DNA synthesis occurs without significant viral particle production (Nonoyama & Pagano, 1972; Shaw et al., 1977). Other herpes viruses such as herpes simplex are biologically different in that they readily infect cells lytically under laboratory conditions.

The lytic cycles of herpes simplex and other lytic herpes viruses have been extensively studied. Many virally induced enzymes have been reported to appear in infected cells and have been characterized; most notable among these are DNA polymerases, deoxyribonucleases (DNases), and pyrimidine deoxynucleotide kinases (Morrison & Keir, 1968; Weissbach et al., 1973; Jamieson & Subak-Sharpe, 1974; Huang, 1975; Allen et al., 1977; Hoffman & Cheng, 1978). The nonlytic nature of EB virus has complicated the search for virus-induced enzymes within EB virally transformed B lymphocytes. However, we report here that EB virus producer cell lines contain a DNase activity which is not present in B cell lines which have lost or never had the ability to undergo EB viral production and which is not present in virus-negative lymphocyte cell lines. This DNase copurifies through preliminary steps with the EB virus induced DNA polymerase activity which has recently been reported by this laboratory (Goodman et al., 1978). We have therefore demonstrated the appearance in nonlytically infected transformed cells of enzymes which may play an active role in viral replication.

Materials and Methods

Materials. [*methyl*-^{3}H]Thymidine and [*methyl*-^{3}H]thymidine 5′-triphosphate were from Schwarz/Mann. Nonradioactive deoxynucleotides, bovine serum albumin, ovalbumin, spermidine, and Nonidet P-40 (NP-40) were from Sigma. Spermine and pronase were obtained from Calbiochem. DEAE-52 cellulose and phosphocellulose (P11) were from Whatman, and the cellulose used for making DNA–cellulose was the gift of the Brown Paper Co., Berlin, NH. Calf thymus DNA for the DNA–cellulose was from Worthington. Cell culture media and sera were obtained from Gibco.

Cells. Almost all lymphocyte cell lines used were previously described by Goodman et al. (1978). These included B95-8 (a marmoset B cell producer line), Raji (a Burkitt's tumor nonproducer B cell line), Ramos (an EB virus negative human B cell line), and CCRF-CEM (a EB virus negative human T cell line). The one exception was the HR-1 cell line, derived from and used instead of P3HR-1; this was obtained from Dr. M. Nonoyama and is described elsewhere (Thorley-Lawson, 1979). Cell culture was prepared in a manner similar to that previously described (Goodman et al., 1978) except that the temperature was 34 °C, the medium was supplemented with 5% fetal calf serum, and the producer cell lines (HR-1 and B95-8) were fed by splitting the cells 1:1 once a week to maintain adequate levels of virus production (Thorley-Lawson, 1979).

Purification of the EB Virus Induced Nuclease Activity. Procedures for harvesting the cells, for making crude cell extracts, for performing DEAE-cellulose and phosphocellulose column chromatography, and for protein determination have been previously described by Goodman et al. (1978). The only modification was that column buffers A, B, C, and D had 5 mM mercaptoethanol substituted for the dithiothreitol, and 2 mM $MgCl_2$ and 10 μg of phenylmethanesulfonyl fluoride per mL were added to all column buffers.

DNA–Cellulose Column Chromatography. Native DNA–cellulose was prepared according to the method of Litman (1968). A 0.9 × 12.0 cm column of material in buffer C was poured and washed with buffer C. Several column volumes of buffer C containing 250 μg of bovine serum albumin (BSA) per mL were passed slowly through the DNA–cellulose, which was then washed with 2 to 3 column volumes of buffer C containing 600 mM KCl, followed by 4 to 5 column volumes of buffer C. The nuclease sample from the phosphocellulose column was dialyzed for 2 h against buffer C. This sample (3–6 mL total volume) was layered onto the washed DNA–cellulose column and allowed to pass slowly into the bed. The flow was then stopped for 15 min. The flow was resumed at a rate of 10–12 mL/h with a 10-mL wash of buffer C and with collection of 1-mL fractions. A 50-mL

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gradient of 0–600 mM KCl in buffer C was passed through the column followed by a further 15-mL wash with 600 mM KCl in buffer C. All steps were performed at 4 °C. Fractions were assayed for nuclease activity as described below; the active fractions were made 50% in glycerol and stored at −80 °C.

Glycerol Gradient Sedimentation. Five-milliliter (5–20% w/v) linear glycerol gradients in gradient buffer (500 mM KCl, 50 mM Tris buffer, pH 8.5, 2 mM $MgCl_2$, and 5 mM mercaptoethanol) were prepared and chilled. Each was layered with 0.2–0.4 mL of phosphocellulose-purified nuclease which had been dialyzed for 3 to 4 h against gradient buffer. Centrifugation was performed with a Beckman SW50.1 rotor in a Sorvall OTD-2 ultracentrifuge at 49 000 rpm for 18 h at 4 °C. BSA and ovalbumin markers made up as 1 mg/mL solutions in gradient buffer were centrifuged in parallel tubes. Gradients were subsequently collected in 0.2-mL fractions from the bottom of the tubes and assayed for nuclease activity and refractive index, or for A_{280} in the case of the marker tubes.

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis. This procedure was performed according to Goodman et al. (1978).

Enzyme Analysis. DNA polymerase assays were performed according to the method of Goodman et al. (1978). Deoxyribonuclease activity was assayed by a modification of the methods of Weissbach et al. (1973), Citarella et al. (1972), and Hoffman & Cheng (1978). Two hundred microliter final assay volumes consisted of 25 μL of enzyme sample and final concentrations of 5 mM $MgCl_2$, 5 μg/mL native lymphocyte DNA radioactively labeled in vivo with ^3H, 10 mM mercaptoethanol, and 50 mM Tris buffer, pH 8.3. All assays were incubated at 37 °C for 1 h. Subsequently, 50 μg of BSA (0.2 mL of a 250 μg/mL stock solution) and 0.15 mL of 6% perchloric acid were added to each assay with vortexing; the assays were then chilled for 20 min in an ice–water bath. Assays were centrifuged in a Sorvall RC-3 low-speed refrigerated centrifuge for 15 min at 2500 rpm, and the supernatant was poured into scintillation vials. Five milliliters of Triton X-100 scintillation fluid (Citarella et al., 1972) was added to each vial, and the samples were counted in a Beckman scintillation counter. One unit is defined as that amount of enzyme which digests 1 μg of native DNA to acid-soluble nucleotides during 1 h at 37 °C.

Preparation of [^3H]Lymphocyte DNA for Use in Nuclease Assays. [^3H]Thymidine (diluted to a specific activity of 5 μCi/nmol with nonradioactively labeled thymidine) was added to 1.2 L of log phase Raji cells to a final concentration of 2.5 μCi/mL. After 36–40 h in culture at 34 °C, the radioactively labeled cells were harvested, washed twice with PBS (Goodman et al., 1978), resuspended in Tris–EDTA (10 mM Tris, pH 7.5, and 1 mM EDTA) to a final volume of 1/50 of the starting cell culture volume, and lysed by addition of Sarkosyl to a final concentration of 0.5%. Pronase was added to a final concentration of 100 μg/mL 3 times at 1-h intervals during incubation at 50 °C. The material was dialyzed overnight at 4 °C against Tris–EDTA and subsequently centrifuged to equilibrium in CsCl density gradients in which the starting refractive index had been adjusted to 1.3990–1.4000. The centrifugation was performed in a Beckman titanium 50 rotor in a Sorvall OTD-2 ultracentrifuge at 32 000 rpm, 20 °C, for 60–70 h. The band of DNA was collected from each gradient, dialyzed extensively against Tris–EDTA, and shown to have an A_{260}/A_{280} ratio of 1.9–2.0. The DNA was diluted to 500 μg/mL, passed multiple times through a 23 gauge needle, and stored at −80 °C. Specific activity of a typical preparation was (2.5–3) × 10^4 cpm/μg of DNA.

Thin-Layer Chromatography. Fifty-microliter amounts of nuclease assay mixtures which had been incubated for 1 h at 37 °C were mixed with markers of mono-, di-, and triphosphothymidine and chromatographed on CEL 300 PEI thin-layer chromatography plates (Brinkman) in 1.0 M LiCl at room temperature. The position of the markers was determined by examination of the plates with a shortwave ultraviolet lamp. Subsequently, the plate was cut into 2-cm squares and each square was placed in a scintillation vial. Each vial was incubated with 1 mL of 0.55 N HCl for 1 h at 37 °C with gentle shaking and counted after addition of 10 mL of Triton X-100 scintillation fluid.

Results

DEAE-cellulose Column Chromatography of Nuclease Activity from Crude Extracts of Various Lymphoblastoid Cell Lines. Crude cell extracts of various producer, nonproducer, and virus-negative lymphoblastoid cell lines were prepared as described under Materials and Methods and chromatographed on a preliminary DEAE-cellulose column, and the high-salt (buffer A) eluant was collected as a single fraction. This material was then dialyzed against low-salt buffer B and fractionated on a second DEAE-cellulose column with a shallow salt gradient of 20–400 mM K_2PO_4 buffer at pH 7.5 (a gradient of buffers B and A). When the resultant fractions were tested for nuclease activity, profiles such as those depicted in Figure 1 were obtained for various cell lines. The graphs in both parts A and C of Figure 1 show a peak of nuclease activity which elutes from the second DEAE-cellulose column at 200 mM K_2PO_4 and in fact coelutes with the EB virus induced DNA polymerase activity. As in the case of the polymerase (Goodman et al., 1978), the nuclease that elutes from DEAE-cellulose at high salt is present only in cell extracts from viral producer lines (HR-1 and B95-8, parts A and C of Figure 1) and not in cell extracts from Raji, an EB virus positive nonproducer cell line (Figure 1D), or cell extracts from the EB virus negative lines CCRF-CEM and Ramos (parts E and F of Figure 1). This species of nuclease activity is present only in extracts from cell cultures which actively produce viral particles and therefore have significant levels of viral capsid antigen (VCA). However, in producer cells in which the ability to produce virus has greatly decreased (HR-1, 1% of the cell population is VCA positive; Figure 1B), the relative amount of this nuclease activity has decreased proportionately. Furthermore, this enzymatic activity has the unique ability to adhere strongly to DEAE-cellulose and to coelute with the EB virus induced DNA polymerase in a manner similar to the herpes simplex virus induced enzymes (Weissbach et al., 1973). This fact, in addition to its strong correlation with viral production, has led us to refer to it as an EB virus induced nuclease.

Further Purification of the EB Virus Induced Nuclease. Since the EB virus induced nuclease activity coeluted with the polymerase from DEAE-cellulose, it was important to determine whether these EB virus induced enzymes eluted as separate peaks of activity from phosphocellulose as is the case for the herpes simplex enzymes. Figure 2 shows the results of phosphocellulose column chromatography of the nuclease and polymerase mixture from HR-1 cells that eluted from DEAE-cellulose at ≥200 mM K_2PO_4. Upon phosphocellulose chromatography the two enzymatic activities elute as two separate peaks, with nuclease activity eluting at 150 mM K_2PO_4 buffer, pH 8, and the polymerase, as previously reported (Goodman et al., 1978), eluting at 200 mM phosphate buffer. Those portions of the peak of nuclease activity which were free of polymerase activity were pooled, dialyzed, and

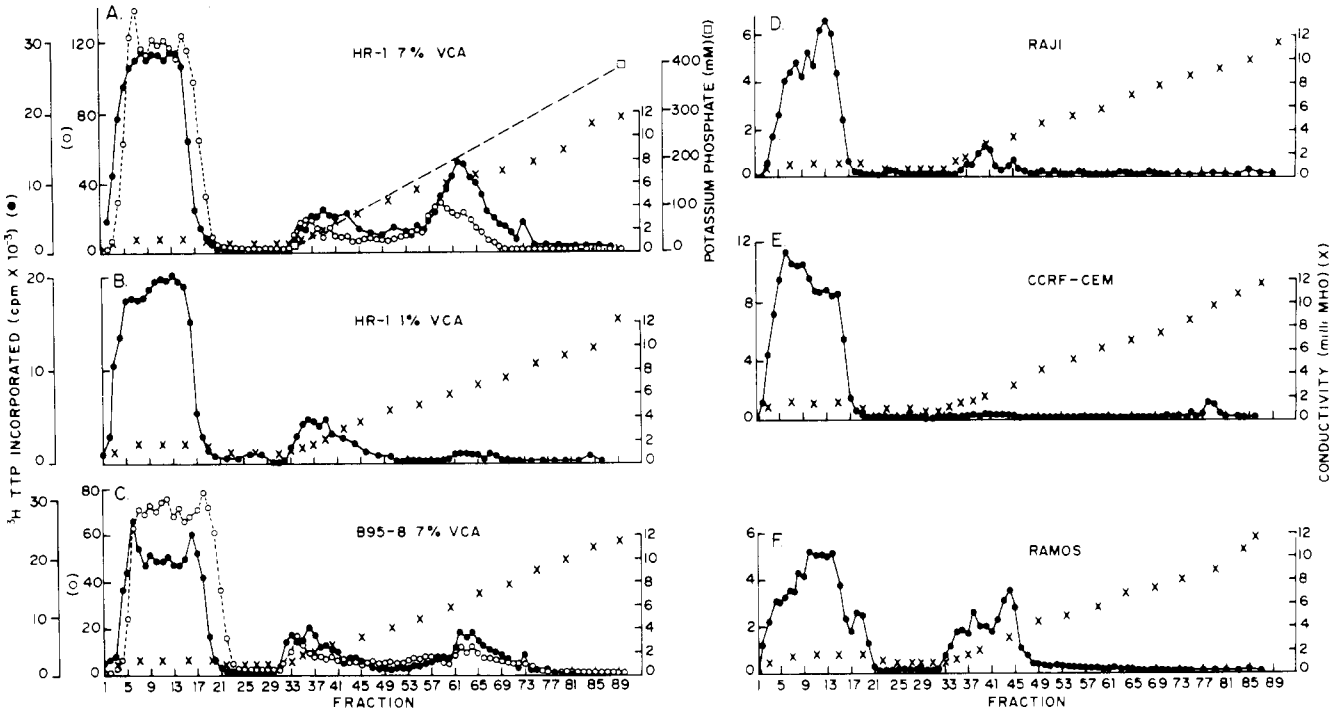

FIGURE 1: DEAE-cellulose column chromatography of extracts from various cell lines. Extracts from various lymphocyte cell lines were prepared as described in the text and by Goodman et al. (1978), and the fractions from the second DEAE-cellulose column were assayed for nuclease activity (●) and in some cases for DNA polymerase activity (○). (A) Cell extract from 6.5 L of HR-1 cell culture (7% VCA positive); (B) cell extract from 6.0 L of HR-1 (1% VCA positive); (C) cell extract from 3 L of B95-8 (7% VCA positive); (D) cell extract from 3 L of Raji; (E) cell extract from 3 L of CCRF-CEM; and (F) cell extract from 3.5 L of Ramos.

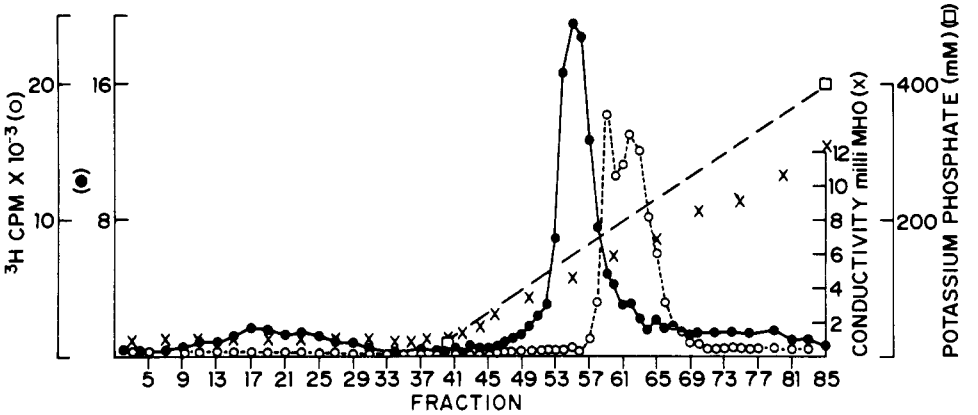

FIGURE 2: Phosphocellulose column chromatography of nuclease activity from HR-1 which elutes from DEAE-cellulose at high salt. Nuclease activity eluting from a DEAE-cellulose column such as that depicted in Figure 1A at ≥200 mM K_2PO_4 buffer was dialyzed against low-salt buffer C and applied to a phosphocellulose column as described in the text and by Goodman et al. (1978). Fractions were assayed for both nuclease (●) and DNA polymerase (○) activity.

further chromatographed on a column of native DNA–cellulose using a gradient of 0–600 mM KCl in buffer C. Figure 3 shows that the the nuclease activity elutes as a distinct peak at ~300 mM KCl. No detectable polymerase activity was present in any DNA–cellulose column fractions. The nuclease activity was found to be very unstable after passage through DNA–cellulose, and even the presence of BSA or additional glycerol in the sample could not render it stable at −80 °C for more than 2 to 3 weeks. DNA–cellulose-purified enzymatic activity without added BSA was used for all characterization studies reported below. In the absence of BSA, the enzyme was active for less than 1 week at −80 °C or in liquid nitrogen.

Table I shows the purification of the EB virus induced nuclease through DNA–cellulose chromatography. After DEAE-cellulose, phosphocellulose, and finally DNA–cellulose chromatography, the nuclease showed a net purification of 220-fold. The final yield after DNA–cellulose chromatography was 18% of the EB virus induced enzyme in the crude extract.

Table I: Purification of EB Virus Induced Deoxyribonuclease Activity[a]

	vol (mL)	total protein (mg)	total act. (units)	sp act. (units/mg)	yield (%)	purification
10K supernatant	7.5	435	293	0.7	100	1
DEAE-cellulose 1 and dialysis in buffer B	45.0	224	270	1.2	92	1.7
DEAE-cellulose 2	32.0	29	162	5.6	55	8.0
phosphocellulose	6.0	2.0	141	71	48	101
DNA-cellulose	5.0	0.34	52	153	18	219

[a] Methods are as described under Results and Materials and Methods and by Goodman et al. (1978). 1 unit of activity is defined as that amount of activity which digests 1 μg of native DNA to acid-soluble material during 1 h at 37 °C.

Because of the great degree of instability of the purified enzyme, all characterization studies were performed within

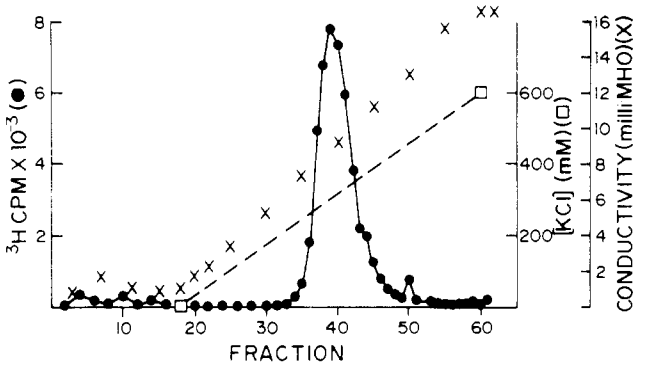

FIGURE 3: DNA–cellulose column chromatography of nuclease-active fractions eluted from a phosphocellulose column. Those nuclease-active fractions which did not contain demonstrable DNA polymerase activity were obtained from a phosphocellulose column such as that depicted in Figure 2, pooled, dialyzed against buffer C, and applied to a DNA–cellulose column as described under Materials and Methods. Fractions were assayed for nuclease activity.

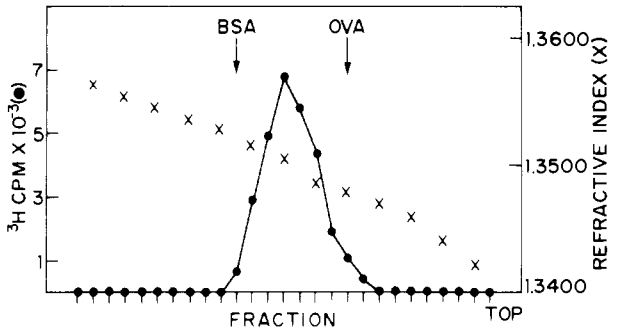

FIGURE 4: Glycerol gradient sedimentation analysis of phosphocellulose-purified nuclease activity. Nuclease purified through the phosphocellulose chromatography step was dialyzed against gradient buffer, and 0.2–0.4 mL was layered onto 5–20% (w/v) 5-mL glycerol gradients. BSA (4.4 S) and ovalbumin (OVA, 3.6 S) were used as markers and centrifuged in parallel tubes. Centrifugation was performed as described under Materials and Methods.

a few days after purification, and further purification steps were not possible.

Glycerol Gradients Sedimentation of Phosphocellulose-Purified EB Virus Induced Nuclease Activity. Phosphocellulose-purified nuclease was chosen for this experiment since DNA–cellulose-purified nuclease was too unstable to be used in this procedure. The enzymatic activity was sedimented in 5–20% (w/v) glycerol gradients, using BSA (4.4 S) and ovalbumin (3.6 S) as markers; 500 mM KCl was present in the gradient buffer to prevent aggregation. As shown in Figure 4, under these sedimentation conditions the EB virus induced nuclease has a sedimentation coefficient value of 4.0 S. A globular protein of this sedimentation coefficient value would have a molecular weight of ~58 000 (Martin & Ames, 1961). After DNA–cellulose column chromatography, enzymatic activity remained as depicted in Figure 3. However, the low concentration of protein in the eluant required that the volume be reduced prior to analysis by gel electrophoresis. Despite these efforts, the concentrated nuclease-active fractions from DNA–cellulose chromatography produced only faint peptide banding patterns in $NaDodSO_4$–polyacrylamide gels. Principal peptide bands of 60 000 and 120 000 were present, as well as several bands of lower molecular weight in the range of 30 000 (data not shown). The latter observation indicates either that breakdown of the larger molecular weight material had already occurred or that lower molecular weight peptides had co-purified with the 60 000 and 120 000 material. It cannot be ruled out at this time that other peptide bands were present in amounts below the level of visualization.

Table II: Mg^{2+} Requirement of DNA–Cellulose-Purified EB Virus Induced Deoxyribonuclease Activity[a]

$[MgCl_2]$ (mM)	^{3}H acid soluble (cpm)	% rel act.[b]
0	1000	22
0.1	1630	36
0.5	2460	54
1.0	2920	64
2.5	4540	100
5.0	4060	90
10.0	3540	78

[a] Nuclease assays were performed as described under Materials and Methods with the concentrations of $MgCl_2$ indicated. [b] Activity with 2.5 mM $MgCl_2$ is 100%.

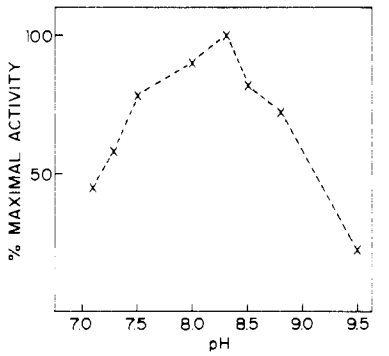

FIGURE 5: pH optimum of the EB virus induced nuclease activity. Nuclease assays were performed by using DNA–cellulose-purified enzyme as described under Materials and Methods except that the pH of the Tris buffer used was varied as indicated. Incubation was for 1 h at 37 °C. Maximal activity was that amount of enzymatic activity at the optimum of pH 8.3 and represents 5140 cpm of acid-solubilized material.

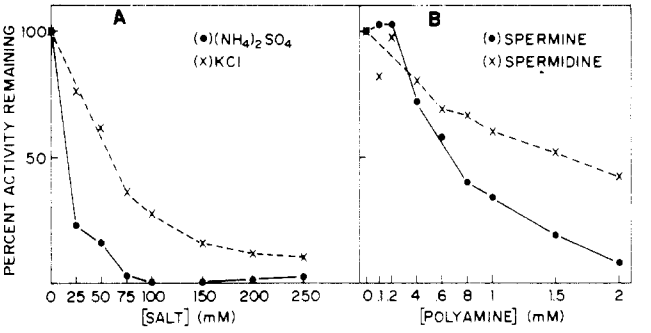

FIGURE 6: Inhibition of nuclease activity by salts and polyamines. Nuclease assays were performed by using DNA–cellulose-purified enzyme as described under Materials and Methods with the indicated concentrations of $(NH_4)_2SO_4$ or KCl (A) and spermine or spermidine (B). Incubations were for 1 h at 37 °C. 100% activity was that amount of enzymatic activity present when no salt or polyamine was added and was equivalent to 4250 (salt) or 4230 (polyamines) cpm.

Divalent Cation Requirement of the EB Virus Induced Nuclease. Table II shows that DNA–cellulose-purified EB virus induced nuclease has a strong requirement for magnesium, with a broad optimum at 2.5–5 mM. When manganese was substituted for magnesium, the optimum was around 0.2 mM manganese, but the maximum activity was only 25% of the maximum activity with magnesium (data not shown).

pH Optimum. The DNA–cellulose-purified nuclease was tested in standard assay mixtures with Tris buffers of varying pH. The data in Figure 5 show a broad pH optimum centering around 8.3. This is similar to observations made for the herpes

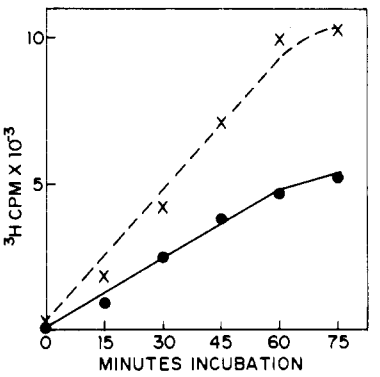

FIGURE 7: Comparison of denatured and native lymphocyte DNA as nuclease substrate. Lymphocyte DNA was radioactively labeled in vivo with [^{3}H]thymidine and isolated as described under Materials and Methods and was further sheared by multiple passages through a 27 gauge needle. A sample of such DNA was divided into two portions, and one portion was incubated in a boiling water bath for 15 min and quick-chilled in an ice–water bath. The other portion was untreated. Nuclease assays using DNA–cellulose-purified material were performed at 37 °C by using one or the other of these DNA preparations as substrate. Samples were taken at the times indicated. (×) Denaturated DNA; (●) native DNA.

simplex induced nucleases (Morrison & Keir, 1968; Hoffman & Cheng, 1978).

Inhibition of Nuclease Activity by Salt and Polyamines. Figure 6A shows the effects of KCl and $(NH_4)_2SO_4$ on EB virus induced nuclease activity which had been purified through DNA–cellulose chromatography. The enzyme is 50% inhibited at ~60 mM KCl. This activity is far more sensitive to $(NH_4)_2SO_4$, being almost 100% inhibited at 75 mM. As in the case of the herpes simplex induced nucleases (Hoffman & Cheng, 1978), the EB virus induced enzyme is inhibited by both spermine and spermidine, but more strongly by the former (Figure 6B).

Native vs. Denatured DNA as Substrate. Figure 7 depicts a time course of DNA–cellulose-purified nuclease activity utilizing both native and denatured lymphocyte DNA as substrate. There is a preference for denatured DNA, although both are effectively digested to acid-soluble material. Thin-layer chromatography of assay mixtures using mono-, di-, and triphosphonucleosides as markers revealed that both single- and double-stranded DNA substrates were digested to monophosphonucleosides (data not shown).

Discussion

The data presented in this report show that EB virus producer cells have a DNase activity which is not present in nonproducer or virus-negative cells, is correlated with viral production, and copurifies through DEAE-cellulose chromatography with the EB virus induced DNA polymerase activity. The nuclease as reported here and the polymerase as reported by Goodman et al. (1978) have certain characteristics of similar enzyme activities found in cells lytically infected with herpes simplex (Morrison & Keir, 1968; Weissbach et al., 1973; Hoffman & Cheng, 1978) and other herpes viruses (Huang, 1975; Allen et al., 1977). The EB virus induced nuclease activity, like those induced by herpes simplex virus types 1 and 2, has an alkaline pH optimum, a sedimentation coefficient between that of BSA and ovalbumin, and a strong requirement for Mg^{2+} and is unstable when highly purified. The EB virus induced enzyme utilizes both single- and double-stranded DNA as template with a preference for the former, reducing both to monophosphonucleosides.

We have noted that the EB virus induced deoxyribonuclease and polymerase, as in the case of the herpes simplex induced enzymes, require different conditions for optimal function. Also, certain data reported here might explain why some herpes virus induced DNA polymerase activities are apparently stimulated by certain salts in the partially pure state (Weissbach et al., 1973; Allen et al., 1977; Miller et al., 1977). Nuclease activity that copurifies with the polymerase may act in opposition to the polymerizing activity in the assay system; however, since low concentrations of $(NH_4)_2SO_4$ markedly inhibit nuclease activity, the presence of certain salts might permit an increase in apparent polymerase activity. It is possible that the ratio of nuclease and polymerase activities varies as intracellular conditions change; such variations could have some biological regulatory or antimutator function in vivo.

The presence of these EB virus associated enzymatic activities in cells which are undergoing active EB virus DNA replication suggests that they play an important role in the viral DNA replicative process. A separate set of studies in our laboratory provide further evidence for this hypothesis. Based on the earlier observation of Nonoyama & Pagano (1972) that nonproducer Raji cells when superinfected with the P3HR-1 strain of EB virus undergo large amounts of viral DNA synthesis accompanied by the breakdown of host cell DNA, we have recently shown that, while the EB virus induced nuclease and polymerase are not present in nonproducer Raji cells alone (Goodman et al., 1978; and Figure 1D), both enzymes are present in P3HR-1 superinfected Raji cells which are actively undergoing viral DNA synthesis and host DNA breakdown (W. Clough and McMahon, unpublished experiments).

References

Allen, G. P., O'Callaghan, D. J., & Randall, C. C. (1977) *Virology 76*, 395–408.

Citarella, R. V., Miller, R., Schlabach, A., & Weissbach, A. (1972) *J. Virol. 10*, 721–729.

de Thé, G., Geser, A., Day, N. E., Tukei, P. M., Williams, E. H., Beri, D. P., Smith, P. G., Dean, A. G., Bornkamm, G. W., Feorino, P., & Henle, W. (1978) *Nature (London) 274*, 756–761.

Goodman, S. R., Prezyna, C., & Benz, W. C. (1978) *J. Biol. Chem. 253*, 8617–8628.

Henle, G., Henle, W., & Diehl, V. (1968) *Proc. Natl. Acad. Sci. U.S.A. 59*, 94–101.

Hoffman, P. J., & Cheng, Y-C. (1978) *J. Biol. Chem. 253*, 3557–3562.

Huang, E. S. (1975) *J. Virol. 16*, 298–310.

Jamieson, A. T., & Subak-Sharpe, J. H. (1974) *J. Gen. Virol. 24*, 481–492.

Litman, R. M. (1968) *J. Biol. Chem. 243*, 6222–6233.

Martin, R. G., & Ames, B. N. (1961) *J. Biol. Chem. 236*, 1372–1379.

Miller, G., Robinson, J., & Heston, L. (1974) *Cold Spring Harbor Symp. Quant. Biol. 39*, 773–781.

Miller, R. L., Glaser, R., & Rapp, F. (1977) *Virology 76*, 494–502.

Morrison, J. M., & Keir, H. M. (1968) *J. Gen. Virol. 3*, 337–347.

Nonoyama, M., & Pagano, J. S. (1972) *J. Virol. 9*, 714–716.

Shaw, J. E., Seebeck, T., Li, J.-H., & Pagano, J. S. (1977) *Virology 77*, 762–771.

Thorley-Lawson, D. (1979) *Cell 16*, 33–42.

Weissbach, A., Hong, S. L., Aucker, J., & Muller, R. (1973) *J. Biol. Chem. 248*, 6270–6277.

Wolf, H., zurHausen, H., & Becker, V. (1973) *Nature (London), New Biol. 244*, 254–247.