The characterization of the EBV alkaline deoxyribonuclease cloned and expressed in *E.coli*

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Received July 19, 1989; Revised and Accepted September 4, 1989

ABSTRACT

Studies of nucleic acid homology suggest the BGLF5 open reading frame of Epstein-Barr virus (EBV) encodes an alkaline deoxyribonuclease (DNase) sharing some homology with that of herpes simplex virus. We report here the expression of the BGLF5 open reading frame in *E. coli* and the expression of high levels of a novel alkaline DNase activity in induced cells. This alkaline DNase has been purified to apparent homogeneity as a single protein species. This is the first report of the expression of a herpesvirus coded DNase in a prokaryotic system and of the purification of the EBV DNase to demonstrable purity. It has the biochemical characteristics of a typical herpesvirus alkaline exonuclease showing a high pH optimum, an absolute requirement for Mg^{2+} for activity and sensitivity to high salt concentrations and polyamines. The enzyme activity was neutralized by sera from patients with nasopharyngeal carcinoma and was reactive with these sera in Western blot analysis. Thus the prokaryotic expression system described here provides an economical and efficient source of the EBV DNase for biochemical and seroepidemiological analysis.

INTRODUCTION

Epstein-Barr virus (EBV) (1) is a human gammaherpesvirus (2) which has long been recognised as the aetiological agent of infectious mononucleosis (IM) (3) and has been associated with the development of a number of tumours: endemic Burkitt's lymphoma (BL) (1), undifferentiated nasopharyngeal carcinoma (NPC) (4), and B cell lymphomas in immunodeficient individuals (5).

Chemical treatment of Epstein-Barr virus producer cell lines with agents such as 12-O-tetradecanoylphorbol-13-acetate (TPA) results in the successive production of early antigens (EA), virus capsid antigens (VCA) and the synthesis of infectious virus (6). In the case of the non-producer cell line Raji only early antigens are synthesized, superinfection of such cells (with virus obtained from producer cell lines) leads to viral antigen expression and the production of infectious virus (7). A number of novel virus-specified enzymatic activities have been identified in chemically induced and superinfected cells, including a DNA polymerase (8,9,10), and a thymidine kinase (11). These enzymes together with other non-structural proteins are believed to make up the early antigen complex. However the lack of an effective lytic system for EBV has limited the quantity of proteins available for analysis. A viral DNase has been detected in EBV positive lymphoid cell lines expressing EA proteins (12,13,14,15). By analogy with the herpes simplex virus (HSV) enzyme we would expect that the EBV enzyme would be essential for DNA synthesis, repair and recombination. Biochemical studies of the HSV DNase show that the enzyme has endonuclease activity and both 5' and 3' exonuclease activities. Its precise role in viral replication is unclear. In the case of the EBV coded enzyme its biochemical properties

have yet to be determined on a preparation which has been shown to be demonstrably pure.

Sera from patients with NPC react strongly with EBV enzymes (16,17, M-Y. Liu, personal communication) and the use of antibodies to the EBV DNase as a marker for the early diagnosis and prognosis of NPC has been well documented (16,18,19,20,21). Sera obtained from patients with NPC have been shown to have elevated levels of neutralizing antibodies (IgG) to the EBV DNase compared to healthy seropositive and seronegative donors, and BL and IM patients. In such studies antibodies in sera are detected by an enzyme neutralization test using DNase extracts obtained from chemically induced EBV transformed cell lines (18).

Since the derivation of the entire DNA sequence of the B95-8 strain of EBV (22) more than eighty open reading frames have been identified. Comparative studies using amino acid sequence data of well characterized genes of HSV and open reading frames found in the EBV genomic DNA sequence has led to the identification of a number of EBV coded genes: for example a major capsid protein (23), potential glycoproteins with significant homology to the HSV gB and gH genes (24,25), a major DNA binding protein (26), and a number of viral enzymes including a ribonucleotide reductase (27), a DNA polymerase (26) and an alkaline DNase (28).

Here we describe the cloning and expression of the EBV BGLF5 open reading frame which is homologous to the alkaline DNase gene of HSV in a prokaryotic expression system. The biochemical properties of the purified enzyme are examined together with its use as an immunological target for antisera from NPC patients.

These results were presented in a preliminary form at the Joint Meeting of the Sektion Virologie der Deutschen Gesellschaft für Hygiene und Microbiologie and the Virus Group of the Society for General Microbiology in September 1988.

MATERIALS AND METHODS

Materials

T4 DNA ligase and restriction enzymes were purchased from New England Biolabs or Bethesda Research Laboratories, [α - 32 P] dCTP and [3 H] thymidine were obtained from Amersham International plc. Unless otherwise stated, chemicals were purchased from BDH Chemicals Limited, Poole, England.

Bacterial strains

Escherichia coli JM105 cells were used for plasmid propagation. The *E. coli* strain BL21 (DE3) lysogenic for the T7 RNA polymerase gene was used in the expression studies (29).

Plasmids We initially tried to express the EBV DNase by cloning the gene into the plasmid pEVvrf1, which contains the pL promoter of bacteriophage lambda in front of a consensus Shine-Dalgarno sequence (30). Low levels of a novel alkaline nuclease activity were observed after temperature shift induction of cultures harbouring the recombinant plasmid. In order to increase levels of expression, the EBV DNase gene was inserted into an expression plasmid pET3a (31) regulated by T7 RNA polymerase. This vector was a gift from W. Studier. The *Bam*HI-B fragment of EBV (B95-8 strain) cloned in pBR322 has been previously described (32). A *Bam*HI to *Bgl*II fragment (nucleotides 122313-120345) was cloned into the *Bam*HI site of the plasmid vector pET3a. The recombinant plasmid has lost the first 8 amino acids of the BGLF5 open reading frame, these have been replaced by the first 12 amino acids of the T7 gene 10 protein. The resulting plasmid pETnuc was transformed into the *E. coli* strain BL21 (DE3) and used in all expression studies.

Fig. 1. Kinetics of induction of EBV-associated DNase in the *E. coli* strain BL21 (DE3). At the indicated times cells were processed as described in the text, and 10 μ l of the high salt extracts were assayed for 10 minutes for DNase activity. Extracts were from cells containing either pET3a induced with IPTG (●); or pETnuc non-induced (Δ); or pETnuc induced with IPTG (○).

Recombinant DNA methodology

Plasmid DNA purification and transformation of *E. coli* was achieved by standard procedures (33). Recombinant colonies were selected by colony hybridization. Radiolabelled probes were prepared by nick translation of the appropriate DNA fragment using a commercially available kit (Bethesda Research Laboratories). All ligations were carried out by using T4 DNA ligase overnight at room temperature.

Protein analysis

The recombinant cultures were screened for the production of the 52.7 kDa EBV alkaline DNase by analysis of enzyme extracts and column fractions using 10% polyacrylamide gel by the procedure described by Powell and Courtney (1975)(34). Coomassie Blue staining was used to visualise the protein bands after electrophoresis. Protein determinations were performed by using a Coomassie Blue protein assay kit (BioRad).

Induction of EBV DNase

E. coli cells transformed with the pETnuc plasmid were grown in minimal medium supplemented with 0.5% casaminoacids (Difco) and 0.5% glucose in the presence of carbenicillin (Sigma Chemical Co.), until the OD₆₀₀ reached 1.0, isopropylthiogalactoside (IPTG) was added to a final concentration of 1mM. The cells were harvested 7 hours post-induction, collected by centrifugation and stored at -70°C as cell pellets.

Enzyme extraction and purification procedures

All steps were performed at 0 to 4°C . The *E. coli* frozen cell pellets were thawed, suspended in 500 ml of extraction buffer (50 mM Tris-HCl pH 7.8, 1mM EDTA, 5mM 2-mercaptoethanol, 0.1 mM phenyl methyl sulphonyl fluoride (PMSF)) containing 5 mg/ml lysozyme. The cells were allowed to stand on ice for 15 minutes and then pelleted at 11 000 g for 10 minutes. The pellet was resuspended in 250 ml of high salt buffer (50 mM Tris-HCl pH 7.8, 1 mM EDTA, 5 mM 2-mercaptoethanol (MCE), 0.1 mM PMSF, 1

M KCl), left on ice for 5 minutes and sonicated briefly before being pelleted. The supernatant was retained and dialysed against several changes of DEA buffer (50 mM diethylamine pH 8.6, 10% glycerol). After dialysis the extract was clarified by sedimentation at 25 000 g for 20 minutes: the supernatant fluid containing the DNase activity was used for purification.

Q Sepharose Chromatography The supernatant was loaded onto a Q Sepharose column (2.5×18 cm) (Pharmacia) pre-equilibrated with DEA buffer and the load was cycled overnight. The DNase was eluted with a 150 ml gradient of 0 to 1M KCl in DEA buffer. The column fractions were assayed for alkaline exonuclease activity and those containing the enzyme pooled for further use.

Phosphocellulose chromatography The peak of DNase from the Q Sepharose column was dialysed against a buffer of 20 mM potassium phosphate pH 8.0, 20% glycerol, 0.5 mM dithiothreitol, 2 mM magnesium chloride and loaded onto a phosphocellulose column (2×14 cm) (P11 cellulose: Whatman). To prevent non-specific adsorption, the column was washed with bovine serum albumin (BSA) (nuclease free, Sigma Chemical Co.) at 500 µg/ml in the phosphate buffer and further washed with phosphate buffer before loading the enzyme preparation. After loading the column was washed with phosphate buffer and the nuclease was eluted with a 150 ml gradient of 0 to 0.4 M KCl in the phosphate buffer. Assays on the column fractions were done as described for Q Sepharose column fractions.

DNA agarose chromatography

Fractions containing the nuclease from the phosphocellulose column were pooled, adjusted to 500 µg/ml BSA (nuclease free, Sigma Chemical Co.), dialysed against two changes of a low salt buffer (20 mM Tris-HCl pH 7.8, 1 mM EDTA, 20% glycerol, 2 mM DTT, 50 mM KCl) and applied to a DNA agarose column (1.5×7.5 cm) (Pharmacia). Elution was achieved by step wise applications of a loading buffer containing successively higher concentrations of KCl. The column fractions were assayed as described for Q Sepharose column fractions.

Mono Q Fast Protein Liquid Chromatography The peak fractions from the DNA agarose column were pooled, diluted in loading buffer (50 mM DEA pH 8.6, 20% glycerol, 0.5 mM DTT) and applied to a Mono Q HR 5/5 column (Pharmacia) after pre-equilibration. The nuclease was eluted with a gradient of 0 to 1M KCl in the loading buffer. The column fractions were assayed for nuclease activity as described for the Q Sepharose column fractions. The purified protein was obtained from the eluate. This preparation was used for enzyme characterization.

Assays for exonuclease activity

The exonuclease activity of the alkaline DNase was measured by the release of acid soluble nucleotides from double stranded [³H] thymidine-labelled HEp-2 cell DNA. The labelling and DNA extraction procedure is a modification of the Morrison and Kier method (35) previously reported by Purifoy and Powell (36) and Banks *et al* (37). The assay mixture routinely contained 50 mM Tris-HCl pH 9.0, 2 mM magnesium chloride, 10 mM 2-mercaptoethanol, 10 µg/ml [³H] thymidine labelled native HEp-2 cell DNA (10 000 ct/min/µg) and appropriate amounts of enzyme extracts in a total volume of 0.2 ml. All assays were incubated at 37°C for 5 minutes unless indicated otherwise. The reaction was

Fig. 2. Chromatography of extracts obtained from pETnuc transformed BL21 (DE3) cells 6 hours after induction with IPTG. Details of the procedures for (a) Q Sepharose, (b) phosphocellulose, (c) DNA agarose chromatography and Mono Q Fast Protein Liquid Chromatography (d) are described in the text. DNase activity in each fraction (---); absorbance 280nm(——).

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Table 1. Purification of the EBV alkaline DNase

Stage of Purification	Volume (ml)	Total protein (mg)	Enzyme* (specific activity)	Fold Purification	Recovery (%)
Whole cells	500	2825	6.5×10^4	1	100
High salt extract	250	1140	1.9×10^5	2.9	118
Dialysate	170	370.6	2.1×10^5	3.3	42.6
Peak from Q Sepharose	48	174.7	2.8×10^5	4.4	26
Peak from phosphocellulose	46	25.3	1.3×10^6	20.5	18
Peak from DNA agarose	8	2.3	1.1×10^7	171.8	13.9
Peak from Mono Q	8	0.0028 ⁺	1.4×10^8	2187.5	2.2

* Ct/min released per mg protein per 5 minutes at 37°C.

⁺ Protein concentration determined by SDS-PAGE by comparison to known standards.

stopped by the addition of 1.0ml of 5% trichloroacetic acid and 50 μ l of a 5mg/ml BSA solution with vortexing. The assays were then chilled for 5 minutes in an ice water-bath. Assays were centrifuged at 15 000 g for 5 minutes and 200 μ l of the supernatant were dispensed into scintillation vials, 10 mls of Optiphase Safe scintillation fluid (LKB) was added to each vial, and then samples counted in a Beckman scintillation counter. For the biochemical studies components of the assay mixture were varied accordingly.

Serological tests

Neutralization tests Neutralization of DNase activity by antibodies from a variety of patients with EBV associated diseases and malignancies was measured using dilutions of sera incubated at 20°C for 30 minutes with purified enzyme prior to a standard enzyme assay. (Sera dilutions were made in a 100 mg/ml solution of nuclease free BSA (Sigma Chemical Co.) in PBS). The remaining DNase activity was then assayed and expressed as a percentage of a control preparation of enzyme incubated with BSA alone.

Western blot analysis The technique used was described by Towbin *et al* (38). The purified protein was analysed by SDS-PAGE and transferred to Immobilon P (Millipore). Blots were then incubated with sera obtained from asymptomatic EBV seropositive (n = 7) and seronegative (n = 7, a gift from L. Young) individuals and from patients with NPC (n = 10), BL (n = 15, a gift from L. Young) and a chronic infectious mononucleosis donor (a gift from A. Rickinson). Protein reactivity was detected using a rabbit anti-human alkaline phosphatase linked antibody (DAKO, Copenhagen) and a naphthol AS-MX/Fast Blue BBN substrate described by Cordell *et al* (39). Dilutions of primary antisera were 1:40, dilution of the anti-human alkaline phosphatase was 1:100. The amount of protein loaded in each track was 0.2 μ g.

RESULTS

Induction of a novel alkaline DNase in *E. coli* cells

Previous characterizations of the EBV coded DNase have used proteins extracted from EBV infected lymphoblastoid cells and as a result have not studied the properties of pure

preparations of the enzyme. In order to obtain relatively large amounts of pure EBV DNase we cloned the BGLF5 open reading frame of EBV (which has been shown previously to have amino acid homology to the HSV coded enzyme) into the prokaryotic expression vector pET3a. The resulting plasmid pETnuc was transformed into the *E. coli* strain BL21 (DE3). Expression was induced by the addition of IPTG. Extracts were prepared as described in the materials and methods, at times from 0 to 18 hours post-induction and assayed for exonuclease activity. Fig. 1 shows the kinetics of induction of the alkaline DNase activity in extracts from *E. coli* cells. A novel alkaline DNase activity was observed in induced cells transformed with the pETnuc plasmid but not in non-induced cells nor in IPTG treated cells transformed with the pET3a vector. Optimal alkaline DNase expression was observed 6 to 7 hours post-induction. These results directly confirm the observations of McGeoch *et al* (1986)(28) who predicted that the BGLF5 open reading frame of EBV should code for an alkaline DNase. It should be noted however, that the recombinant DNase molecule will be a hybrid protein (Materials and Methods) and this may affect the enzymatic activity of the product.

Purification of the EBV alkaline DNase from E. coli (DE3)

E. coli BL21 (DE3) cells transformed with pETnuc and induced with IPTG were harvested 6 hours post-induction and used for enzyme purification. A high salt extract was prepared from the cells and successively chromatographed by Q Sepharose, phosphocellulose, DNA agarose chromatography and Mono Q Fast Protein Liquid Chromatography. The purification obtained from a 5l volume of cultured cells is shown in Fig. 2 and Table 1.

The DNase activity in the fractions obtained from the Q Sepharose column is shown in Fig. 2(a), the peak of DNase activity was eluted with 0.75 to 1.0M KCl. Fig. 2(b) shows that the DNase activity eluted with 0.26 to 0.38M KCl from the phosphocellulose column. The DNase activity eluted as a single peak from the DNA agarose column (Fig. 2(c)) with 0.4M KCl. Finally the DNase activity was eluted from the Mono Q column with 0.27 to 0.33M KCl. Table 1 summarises the enzyme purification. The net-purification was greater than 2000-fold, however this is possibly an over-estimate since the protein concentration of the final enzyme preparation was determined from Coomassie stained SDS-polyacrylamide gels by comparison to known standards to determine the quantity of the EBV alkaline DNase. Approximately 30 μ g of the purified enzyme was recovered. The specific activity of the final product was estimated to be 8.8×10^4 units/mg protein (where 1 unit of enzyme activity is defined as the amount of enzyme required to catalyze the conversion of 1 μ g of double-stranded DNA to acid soluble nucleotides in 10 minutes at 37°C). This activity is considerably higher than those reported previously for the EBV DNase which had been partially purified from lymphoblastoid cells (12,14) of 28.5 and 480.0 units/mg respectively. The overall recovery of the enzyme (2.2%) may be increased using improved methods of purification. However, it must be noted that the expression of the EBV DNase itself is toxic to *E. coli* and hence limits the amount of enzyme expressed (data not shown).

SDS-polyacrylamide gel electrophoresis was used to monitor the purification of the nuclease activity; the results are shown in Figure 3. Lane (A) shows the whole cell extract which was finally purified to a single polypeptide species (lane (F)) corresponding to the peak of nuclease activity from the Mono Q column. The protein was observed to have a molecular weight of 52.5 kDa corresponding well to the size of 52666 Da predicted from the open reading frame parameters (22). Occasionally it was observed that a second polypeptide with a molecular weight of 50kDa was present in the final enzyme preparations

Fig. 3. SDS-PAGE analysis of the polypeptide components of the nuclease preparation throughout the purification procedure. Coomassie Brilliant Blue staining of (A) Whole cell extract, (B) high salt extract following dialysis, (C) Q Sepharose peak, (D) phosphocellulose peak, (E) DNA-agarose peak, (F) and (G)—adjacent fractions from the Mono Q column showing nuclease activity. (M) Molecular weight markers (66 kDa, 45 kDa, 36 kDa, 29 kDa).

(Lane G)). This may represent a breakdown product of the enzyme since it was also reactive in Western blots of the purified EBV DNase (data not shown).

Biochemical characterization of the recombinant EBV alkaline DNase

In order to confirm that the enzyme purified from the prokaryotic expression system was an authentic EBV coded enzyme the biochemical properties of the purified recombinant alkaline DNase were characterized. These results are shown in Fig 4. The properties of the recombinant EBV enzyme produced in *E.coli*, the DNase observed in chemically induced lymphoblastoid cell lines carrying the EBV genome (12,40) and the herpes simplex virus enzyme observed in lytically infected cells (37,41) are compared in Table 2.

The recombinant alkaline DNase showed the typical biochemical characteristics of

Fig. 4. Properties of the recombinant EBV alkaline DNase. The exonuclease activity was determined under varying conditions. (a) The effect of pH on enzyme activity was measured. (b) The concentration of divalent cation was varied and the activity of the enzyme with each was determined: (●), Mg^{2+} ; (○), Mn^{2+} ; (□), Ca^{2+} . (c) The effects of reducing agents on the enzyme: (○), dithiothreitol; (●), 2-mercaptoethanol. (d) The effect of pOHMB on enzyme activity. (e) The effect of salts on the enzyme was measured: (○), NaCl; (●), KCl. (f) The effect of the polyamine spermidine on enzyme (Fig. 4) activity was determined.

Table 2. A comparison of the biochemical characteristics of the HSV and EBV alkaline DNases with the recombinant EBV nuclease produced in *E. coli*

	Recombinant	EBV DNase* obtained from chemically treated EBV genome positive lymphoblastoid cells	HSV DNase**
pH optimum	9	8.5	9
Optimum concentration Mg ²⁺ for activity	5 mM	2–5 mM	2–8 mM
50% inhibitory concentration of KCl	60 mM	75 mM	85 mM
Effect of reducing agents	No effect	ND*	No effect
50% inhibitory concentration of p-OHMB	0.125 mM	ND*	1.9 mM
50% inhibitory concentration of spermidine	10 mM	12 mM	1.5 mM

* Collation of results obtained by Clough (12) and Liu *et al* (40). ** Collation of results obtained by Hoffman and Cheng (41), and Banks *et al* (37)

ND* Not determined

herpesvirus nucleases. A high alkaline pH optimum together with a dependence on magnesium as a co-factor were required for optimal activity. High ionic strengths and polyamines were inhibitory to the alkaline exonuclease activity. Reducing agents had no apparent effect on the enzyme, suggesting a lack of requirement for sulphhydryl groups. Paradoxically, the compound p-hydroxymercuribenzoate (pOHMB) was inhibitory to enzyme activity.

Serological Studies

Enzyme neutralization The EBV DNase has previously been shown to be an important marker for the early diagnosis of nasopharyngeal carcinoma. In order to determine if the recombinant EBV DNase could be useful for such studies, a number of sera from human donors were examined for their ability to neutralize the activity of the purified EBV DNase obtained from *E. coli* cells. Fig. 5 shows the neutralization test results for the purified enzyme. A serum pool obtained from patients with NPC gave the highest levels of enzyme neutralization followed by a serum sample obtained from a patient with chronic infectious mononucleosis. A relatively low non-specific inactivation effect was observed in the serum samples from both the asymptomatic EBV seronegative and seropositive donors and the BL patients.

Western blot analysis In order to further determine the EBV origin of the DNase activity, purified enzyme was examined by Western blot analysis using a number of human sera from healthy donors and patients with a number of EBV related diseases and malignancies. The results are shown in Fig. 6. The polypeptide was found to be the most highly reactive with sera from patients with NPC. Little reaction was detected with the other sera.

Fig. 5. Neutralizing activity of patients serum on the purified EBV DNase. Enzyme was incubated with dilutions of a pooled NPC patients sera, (●); sera from a chronic IM patient (□); serum pools from asymptomatic seropositive (■) and seronegative donors, (○); and a pooled BL patients sera (△). Following incubation, exonuclease activity was determined as described in the text.

These results indicate the successful expression of an active EBV alkaline DNase in *E. coli* cells.

DISCUSSION

Evidence is presented in this report for the successful expression of the EBV alkaline deoxyribonuclease in *E. coli*. The BGLF5 open reading frame has been shown to be weakly homologous with the amino acid sequence of the HSV enzyme (24) and Zhang *et al* (42) reported a novel nuclease activity in *in vitro* transcription/translation studies of a cDNA clone from the P3HR-1 cell line mapping to the *Bam*HI B/G region of the B95-8 EBV genome. Here we have expressed the BGLF5 open reading frame in *E. coli* under the control of an inducible T7 promoter. Following induction, a novel alkaline exonuclease activity was observed. This is the first reported expression of a herpesvirus nuclease in a prokaryotic system.

The enzyme purified from the recombinants possesses all the biochemical characteristics of a typical herpesvirus alkaline DNase showing an absolute requirement for magnesium ions for activity, a high alkaline pH optimum and a sensitivity to high ionic strengths and polyamines. The reducing agents dithiothreitol and 2-mercaptoethanol have no effect on the EBV alkaline DNase which would indicate that sulphhydryl groups are not required in the enzyme. Paradoxically however the EBV DNase like the HSV enzyme is sensitive to ρ OHMB (37,41). Biochemically the HSV alkaline DNase, the recombinant EBV enzyme

Fig. 6. Western blot analysis of the EBV DNase. The purified enzyme was run on a 10% SDS-polyacrylamide gel transferred to Immobilon P as described in Methods. Blots were then incubated with human sera. Lanes were incubated with sera from: (A) a pool of NPC patients; (B) a pool of asymptomatic EBV seropositive donors; (C) a pool of BL patients; (D) a chronic infectious mononucleosis patient; (E) a pool of asymptomatic EBV seronegative donors; lane (G) shows a stained gel with the 52.5 kDa nuclease protein. Molecular weight markers are indicated in lane (M) 66 kDa, 45 kDa, 36 kDa and 29 kDa.

and the EBV DNase obtained from EBV transformed lymphoblastoid cells are extremely similar, suggesting functional conservation.

From the sequence of the BGLF5 open reading frame (22) the EBV DNase would be a polypeptide with a predicted molecular weight of 52666 Da. This corresponds well with the 52.5 kDa size of the polypeptide observed in the purified preparations containing the alkaline DNase activity. Recently however Littler *et al* (43) have shown that polyclonal rabbit sera to the purified HSV alkaline DNase are cross-reactive with a doublet of proteins of 64 kDa found in EBV producer cell lines (p3HR-1). In the case of HSV the predicted size for the alkaline DNase from HSV-1 and HSV-2 is 67400 Da and 66100 Da respectively. However SDS-PAGE analysis of purified preparations of the enzyme reveal a protein with a molecular weight of 85–90 kDa. The HSV enzyme has been shown to be highly phosphorylated (44). Moreover, from the sequence predictions, the HSV alkaline DNase has a high proline content. The discrepancies observed with the size of the B95-8 EBV alkaline DNase expressed in *E. coli* cells and the cross-reacting alkaline DNase observed in P3HR-1 cells may be partly explained by the lack of post-translational modification events in *E. coli* cells and variations between EBV strains. Certainly in terms of size the HSV alkaline DNase has been shown to be one of the most variable proteins between different strains of HSV (34). In the case of EBV, Williams *et al* (45) have recently reported strain differences in the electrophoretic profile of the alkaline DNase under non-denaturing gel electrophoresis.

Immunological characterization of the recombinant EBV DNase revealed that the purified DNase was reactive with a pooled NPC serum on Western blots and the enzyme activity

could be specifically neutralized by a serum pool obtained from NPC patients and by a serum sample from a patient with chronic infectious mononucleosis. Both types of condition show high levels of antibodies to the diffuse component of the early antigen complex of EBV and the use of antibodies against the EBV DNase have been used as a diagnostic and prognostic marker for NPC by the use of an enzyme neutralization test (17,18,19,20,21,22). The enzyme from recombinant *E. coli* cells appears to be neutralized equally as well as the enzyme preparations obtained from chemically induced EBV transformed lymphoblastoid cells. Moreover, the enzyme induction in the bacterial cells is easier to perform and is more routinely reproducible. The enzyme expressed in *E. coli* is far more stable than the alkaline DNase obtained from chemically induced P3HR-1 cells, for example, in 1979 Clough (12) reported the alkaline DNase to be stable for less than one week when stored in liquid nitrogen. Even frozen cell pellets of chemically induced P3HR-1 cells lose all DNase activity in less than 3 months at -70°C (M-Y. Liu, personal communication). The purified recombinant enzyme is stable at -70°C for more than three months and is resistant to heating at 37°C for more than 2 hours (data not shown). Furthermore the availability of the EBV DNase from the recombinant bacteria will enable more convenient tests, such as ELISA, to be used to assay for immunoreactivity.

In HSV infected cells the role of the virus-coded DNase is not completely clear; however it does appear to be important for a late stage in DNA replication (37,46,47,48,49). Like the HSV enzyme the EBV enzyme is likely to be involved in active virus production and replication, it may be involved in the resolution of concatemeric viral DNA replicative intermediates possibly utilizing the endonuclease activity of the enzyme which has so far been poorly characterized (50).

The system described here reproducibly yields relatively large amounts of the EBV DNase and is far more economical than the use of lymphoblastoid cells as a source of enzyme. The availability of such preparations of the alkaline DNase will allow further detailed characterization of the function of this enzyme and provides a more suitable source of the protein to be used for screening programmes for the early diagnosis of nasopharyngeal carcinoma.

ACKNOWLEDGEMENTS

We thank J.R. Arrand and K.L. Powell for critical review of the manuscript and W. Pelham for manuscript preparation. This work was supported by the Science and Engineering Research Council and the Cancer Research Campaign (UK).

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