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High-Level Expression of T4 Endonuclease V in Insect Cells as Biologically Active Form

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Abstract T4 endonuclease V (T4 endo V) [EC 3. 1. 25. 1], found in bacteriophage T4, is responsible for excision repair of damaged DNA. The enzyme possesses two activities: a cyclobutane pyrimidine dimer DNA glycosylase (CPD glycosylase) and an apyrimidic/apurinic endonuclease (AP lyase). T4 *denV* (414 bp cDNA) encoding T4 endo V (138 amino acid) was synthesized and expressed using either an expression vector, pTriEx-4, in *E. coli* or a baculovirus AcNPV vector, pBacPAK8, in insect cells. The recombinant His-Tag/T4 endo V (rHis-Tag/T4 endo V) protein expressed from bacteria was purified using one-step affinity chromatography with a HiTrap Chelating HP column and used to make rabbit anti-His-Tag/T4 endo V polyclonal antibody for detection of recombinant T4 endo V (rT4 endo V) expressed in insect cells. In the meantime, the recombinant baculovirus was obtained by cotransfection of BacPAK6 viral DNA and pBP/T4 endo V in *Spodoptera frugiperda* (Sf21) insect cells, and used to infect Sf21 cells to overexpress T4 endo V protein. The level of rT4 endo V protein expressed in Sf21 cells was optimized by varying the virus titers and time course of infection. The optimal expression condition was set as follows; infection of the cells at a MOI of 10 and harvest at 96 h post-infection. Under these conditions, we estimated the amount of rT4 endo V produced in the baculovirus expression vector system to be 125 mg/l. The rT4 endo V was purified to homogeneity by a rapid procedure, consisting of ion-exchange, affinity, and reversed-phase chromatographies, based on FPLC. The rT4 endo V positively reacted to an antiserum made against rHis-Tag/T4 endo V and showed a residual nicking activity against CPD-containing DNA caused by UV. This is the first report to have T4 endo V expressed in an insect system to exclude the toxic effect of a bacterial expression system, retaining enzymatic activity.

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The harmful effects of ultraviolet (UV) irradiation to normal human skin are immune suppression, photoaging, and, above all, skin carcinogenesis [9]. UV irradiation from 245 to 290 nm is absorbed maximally by DNA [26]. UV irradiation is able to induce mutagenic photoproducts or lesions in DNA between adjacent pyrimidine residues in the form of dimers: either cyclobutane pyrimidine dimers (CPDs) or (6-4) photoproducts [13, 19]. Unlike the repair of (6-4) photoproducts, which can be initiated by the nucleotide excision repair system or a (6-4) photolyase, CPD repair can be initiated by either the base excision or nucleotide excision repair pathways as well as by a dimer photolyase [12, 28].

The base excision repair pathway is initiated by DNA glycosylases, a class of lesion-specific enzymes that removes the damaged base. These enzymes may or may not have concomitant abasic (AP) lyase activity [18]. The most well characterized of the pyrimidine dimer glycosylases is T4 endonuclease V (T4 endo V) [16]. Upon recognizing and binding to DNA containing a cyclobutane pyrimidine dimer, T4 endo V cleaves the N1-C1' glycosyl bond of the dimer's 5'-pyrimidine and then cleaves the phosphodiester bond between the two dimerized pyrimidines. This is accomplished by formation of a covalent enzyme-DNA imino intermediate, which results in glycosyl bond scission, followed by β-elimination, generating phosphodiester bond cleavage [5, 6, 23].

T4 endo V, encoded by the bacteriophage T4 *denV* gene, has been characterized extensively since its discovery over 40 years ago, and its structure and mechanism of catalysis have been recently reviewed [16, 17]. T4 endo V is also

used as a reagent to facilitate studies in DNA repair mechanisms, including *in vivo* complementation assays as well as studies on immunological and signal transduction pathways associated with UV-induced immunosuppression. The ultimate application of these basic researches is to provide treatment in clinical trials to patients with high degrees of genetic susceptibility to UV-induced skin cancer [17]. Recently, an encouraging randomized study in patients with xeroderma pigmentosum, treated for 1 year with topically applied recombinant T4 endo V, showed that it lowered the rate of development of new cases of actinic keratoses and basal-cell carcinomas significantly [29].

This prokaryotic DNA repair enzyme has been produced in a high-level expression system in *E. coli* [7, 20]. However, *E. coli* expression system produces compounds that are pathogenic or toxic to humans, such as endotoxins [1, 10]. In order to overcome these problems, we decided to utilize the baculovirus expression vector system (BEVS). The BEVS is one of the most popular systems for the production of large quantities of recombinant protein required for structural and functional study of therapeutically relevant biomolecules [14, 21, 22]. In addition, insect cells present several comparative advantages to mammalian cells, such as ease of culture, higher tolerance to osmolarity and by-product concentration, and higher expression levels when infected with a recombinant baculovirus [2, 4, 11]. We report in this work the cloning and the expression of biologically active rT4 endo V from the BEVS to be used in basic studies as well as in clinical applications.

MATREIALS AND METHODS

Designs of Gene Coding for T4 Endo V

The BamHI-NotI fragment (431 bp) containing T4 endo V (GenBank Accession No. M23414) cDNA (417 bp) was synthesized, annealed, and then ligated into a cloning vector. Briefly, 431 nucleotides of the gene were synthesized using a DNA synthesizer (Applied Biosystems, Inc., model 380 A) and purified by HPLC using a reversed-phase (TSK gel 80TM, Tosoh) and/or anion-exchange (TSK gel DEAE-ZSW, Tosoh) column. The purified BamHI-T 4 endo V-NotI fragment was cloned into pGEM-T Easy (Promega, U.S.A.) according to the procedures described by the manufacturer. This vector was named as pGEM-T4 endo V. The sequence of the cloned gene was confirmed by the dideoxy-chain termination method using a nucleotide sequencer (3100 Genetic analyzer, ABI).

Expression and Purification of Recombinant His-Tag/T4 Endo V (rHis-Tag/T4 Endo V) in the Bacterial System

To construct a bacterial expression vector having T4 endo V cDNA under the control of the T7 lac promoter, 424 bp of BamHI- and NotI-digested fragment from pGEM-T4 endo V was cloned into the BamHI and NotI sites of the expression vector pTriEx-4 (Novagen, U.S.A.). This vector was named as pTri-His-Tag/T4 endo V and was expected to express a His-tag protein at the N-terminus. The sequence of the cloned His-Tag/T4 endo V was verified by sequencing, as described above.

E. coli BL21 (DE3) was transformed with the pTri-His-Tag/T4 endo V, and desired clones were selected. The transformant was inoculated into 1 l of medium supplemented with a final concentration of 50 μg/ml ampicillin and grown at 37°C with shaking for 9 to 12 h. The culture was then diluted to 1:100 in fresh LB medium supplemented with a final concentration of 50 μg/ml ampicillin and grown at 37°C. At OD_{600}=0.6, IPTG was added to a final concentration of 0.4 mM to induce protein production for 3 h at 37°C. The cells were harvested by centrifugation at 8,000 rpm for 10 min at 4°C, dissolved in lysis buffer (25 mM Tris-HCl, pH 7.4, containing 5 mM $MgCl_2$, 1% Triton-X-100, 1% N-lauroyl-sarcosine, and 10 mM imidazole), and incubated on ice for 1 h. The suspension was sonicated on ice and centrifuged at 10,000 rpm at 4°C for 30 min to remove insoluble materials. Expression of the protein was detected by 15% SDS-PAGE and Western blotting using anti-His antibody (Amersharm Pharmacia Biotech, Sweden), as described below.

Large-scale expression revealed that the rHis-Tag/T4 endo V was produced as a soluble protein. The soluble fraction of the protein was extensively dialyzed in loading buffer (25 mM Tris-HCl, pH 7.4, 500 mM NaCl and 10 mM imidazole) overnight. The protein was purified by using a HiTrap Chelating HP column (Amersham Pharmacia Biotech). The column was equilibrated with 0.1 M $NiSO_4 \cdot 6H_2O$ in loading buffer, loaded with solubilized lysate, and washed. The recombinant protein was then eluted with elution buffer in various concentrations of imidazole (100, 200, 300, 500 mM, and 1 M). The purified protein was resolved on a 15% SDS-PAGE and analyzed by Western blotting analysis. The eluted protein fractions of rHis-Tag/T4 endo V (positive by Western blotting analysis) were pooled and concentrated by using Biomax-10 Ultrafree-10 concentrators (Millipore, U.S.A.). This rHis-Tag/T4 endo V fusion protein was dialyzed overnight in phosphate-buffered saline (PBS) and sterilized by filtration to be used as an immunogen.

Expression and Purification of Recombinant T4 Endo V (rT4 Endo V) in the Baculovirus System

For expression in the BEVS, 424 bp of BamHI- and NotI-digested fragment containing the T4 endo V cDNA from pGEM-T4 endo V was cloned into the BamHI and NotI sites of the pBacPAK8 baculovirus transfer vector (Clontech, U.S.A.) that contained the polyhedrin promoter for expression of foreign proteins. This transfer vector was named as pBP8-T4 endo V (data not shown). The sequence of the cloned T4 endo V was verified by sequencing, as described above.

Spodoptera frugiperda (Sf21) insect cells were used for the production and amplification of recombinant baculovirus. The cells were cultured at 27°C in Grace's medium containing 10% fetal bovine serum (Clontech). Procedures for cultivating Sf21 cells, production of high-titer virus stock, infection of insect cells, and plaque purification were performed as described by Summers and Smith [25]. Sf21 cells were cotransfected with 5 μl (500 ng) of the transfer vector and 5 μl of linearized BacPAK6 viral DNA (Clontech), under conditions recommended by the supplier. Two rounds of plaque purification were used to generate a homogeneous viral stock, which was designated as AcBac-T4 endo V. A time-course analysis of expression was performed with AcBac-T4 endo V-infected Sf21 cells at a multiplicity of infection (MOI) of 10. For large-scale production of rT4 endo V, 1×10^6 cells/ml of Sf21 cells were seeded in 150 ml of culture medium and then infected with the recombinant baculovirus AcBac-T4 endo V at a MOI of 10. The cells in suspension culture were stirred at 80 rpm in a 500-ml Erlenmayer flask. After 72 h of infection at 27°C, the culture media were collected and clarified by centrifugation at 3,000 rpm for 10 min. Identity of samples was analyzed by Western blotting with anti-rHis-Tag/T4 endo V antibody, as described below.

The supernatants of Sf21 cells that were transfected with the recombinant AcBac-T4 endo V were diluted by addition of an equal volume of 25 mM sodium phosphate, pH 6.8, containing 0.1 M KCl. The sample was applied to a SP Sepharose XL column (Amersham Pharmacia Biotech) equilibrated with the same buffer. After the sample had been loaded onto this column and thoroughly washed with the same buffer, the bound proteins were eluted with a linear gradient of 0.1–1.0 M KCl. In all subsequent purification steps, the rT4 endo V was monitored by Western blotting analysis using anti-rHis-Tag/T4 endo V antibody, as described below. The fractions that contained the rT4 endo V proteins were pooled, dialyzed, and loaded onto a heparin Sepharose column (Amersham Pharmacia Biotech) that had been previously equilibrated with 25 mM sodium phosphate, pH 6.8, containing 0.1 M KCl. After washing the column with the same buffer, the adsorbed proteins were eluted with a linear gradient of 0.1–0.8 M KCl. The eluant was further purified by a Resource RPC column (Amersham Pharmacia biotech) using a linear gradient of acetonitrile containing 0.05% trifluroacetic acid (TFA). The TFA in the eluant was neutralized with Tris base, and the acetonitrile was removed in a SpeedVac evaporator at 4°C. The purity of rT4 endo V was checked on SDS-PAGE and the concentration was determined by the method of Bradford [4] using bovine serum albumin (Sigma, U.S.A.) as a standard.

Polyclonal Antibody Preparation

New Zealand white adult rabbits were simultaneously immunized with 200 μg of rHis-Tag/T4 endo V fusion protein, emulsified in the same volume of complete Freund's adjuvant. Three weeks later, the rabbits were immunized again with a mixture of 100 μg rHis-Tag/T4 endo V fusion protein and the same volume of incomplete Freund's adjuvant. Two weeks later, the rabbits were immunized again with the same mixture. Five days later, the blood serum was harvested from the blood of the rabbit's carotid artery. The antibody titer of the harvested serum was measured by indirect ELISA using the method of Engvall and Pelmann [8] and the serum was stored at −20°C following the addition of 0.1% sodium azide as a preservative. Control nonimmune serum was obtained before the first injection.

SDS-PAGE and Western Blotting Analysis

SDS-PAGE and Western blotting were performed essentially by the methods of Laemmli [15] and Towbin *et al.* [27], respectively. Briefly, protein samples were separated on a 15% SDS-PAGE under reducing conditions and then transferred onto a PVDF membrane by electroblotting. To confirm that rHis-Tag/T4 endo V is a His-tagged fusion protein, anti-His antibody (Amersham Pharmacia Biotech) was used in the Western blot. To detect expression of rT4 endo V, the anti-rHis-Tag/T4 endo V antibody produced in our laboratory was used as a primary antibody. The secondary antibody used was an anti-rabbit IgG alkaline phosphatase conjugate (Bio-Rad, U.S.A.), diluted to 1:15,000, and the alkaline phosphatase activity was detected according to the manufacturer's instructions. Densitometric analyses of Western blotting were performed by using a FluroS-Multiimager and Quantity One analysis software (Bio-Rad).

T4 Endo V Assay

UV endonuclease activity was determined by a pyrimidine dimer-specific nicking test according to Schrock and Lloyd [24], with pUC18 of supercoiled form as a substrate. Briefly, supercoiled pUC18 DNA (25 ng/μl) was irradiated with 254-nm UV light at 100 pW/cm^2 for 245 s. The DNA was diluted to 0.1 mg/ml in 25 mM sodium phosphate buffer (pH 6.8), containing 10 mM EDTA, 20 mM NaCl, 10% (v/v) ethylene glycol, and 0.1 mg/ml bovine serum albumin. To 250 ng of this irradiated or nonirradiated pUC18 as a negative control in the described buffer, varying concentrations of rT4 endo V were added and incubated at 37°C for 30 min. Activity was assessed on a 0.8% agarose gel by determining the conversion of supercoiled irradiated pUC18 into a relaxed form, while unirradiated plasmid remains unchanged. The Image Quant analysis system (Molecular Dynamics, U.K.) was employed to measure the percentage of pUC18 in the relaxed form. One unit of rT4 endo V activity was defined as the amount of rT4 endo V used to completely relax 250 ng of UV-irradiated plasmid DNA in 30 min at 37°C. The commercial T4 endo V (Trevigene, U.S.A.) was used to compare the relative UV endonuclease activity of the purified rT4 endo V from our insect system.

RESULTS AND DISCUSSION

Cloning of T4 Endo V cDNA

The 431 bp BamHI-NotI fragment containing T4 endo V cDNA was synthesized, purified, and cloned into the pGEM-T vector, and its sequence was confirmed by DNA sequencing, as described in Materials and Methods. The DNA fragment was cloned into pTriEx-4 and pBacPAK8, both at the BamHI and NotI restriction sites. Resulting recombinant vectors, pTri-His-Tag/T4 endo V and pBP8-T4 endo V, expressed the recombinant proteins, rHis-Tag/T4 endo V and rT4 endo V, in the bacteria and insect expression systems, respectively.

Bacterial Expression and Purification

The production of the rHis-Tag/T4 endo V in bacteria was to make specific antibody for the detection and the quantification of the rT4 endo V expressed in the insect system. The rTag-His/T4 eno V was expressed as a soluble form by the *E. coli* expression vector and purified by using one-step affinity chromatography with a HiTrap Chelating HP column, quickly and efficiently. Under the culture condition, the rHis-Tag/T4 endo V was expressed with a relative molecular weight of approximately 22.59 kDa (Fig. 1). To obtain the purified rHis-Tag/T4 endo V, the soluble extract from *E. coli* was directly loaded and purified onto the 6×His-tagged-based nickel HiTrap Chelating HP column. Following extensive washing, the bound proteins were eluted with 100, 200, 300, and 500 mM, and 1 M imidazole, and the fraction containing pure protein was found in elution buffer with 200 mM imidazole. The purity of the recombinant protein was confirmed by a homogeneous banding of 22.59 kDa when analyzed by 15% SDS-PAGE stained with Coomassie brilliant blue R250 (Fig. 2). The purified rHis-Tag/T4 endo V was used for the production of anti-rHis-Tag/T4 endo V antibody.

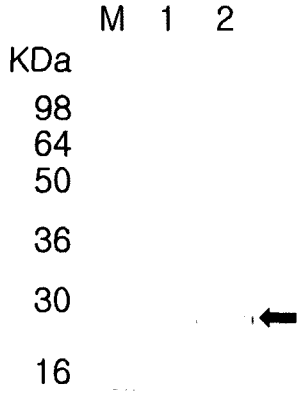

Fig. 1. Expression of the rHis-Tag/T4 endo V in *E. coli*. rHis-Tag/T4 endo V was separated on a 15% SDS-PAGE and the gel was visualized by Western blotting using anti-His antibody.
Lane 1, normal cell lysate; lane 2, infected cell lysate; M, protein size marker (SeeBlue plus prestained standard; Invitrogen, U.S.A.).

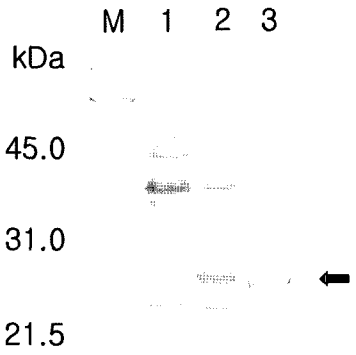

Fig. 2. One-step purification of rHis-Tag/T4 endo V.
The expressed protein was purified by using a HiTrap Chelating HP column. The purified rHis-Tag/T4 endo V was visualized by 15% SDS-PAGE following Coomassie blue staining. Lane 1, normal cell lysate; lane 2, infected cell lysate; lane 3, 3 μg of purified rHis-Tag/T4 endo V; M, protein size marker (SDS-PAGE molecular weight standard, broad range; Bio-Rad).

Baculovirus Expression and Purification

The cDNA corresponding to the 138 amino acids of T4 endo V from pGEM-T4 endo V was cloned under the control of the polyhedrin promoter present in the transfer vector pBacPAK8 to yield pBP8-T4 endo V. Sf21 cells were cotransfected with DNA of recombinant transfer vector pBP8-T4 endo V and DNA of BacPAK6 baculovirus. The recombinant virus AcBac-T4 endo V produced by pBP8-T4 endo V was purified by the plaque assay. The strong signals corresponding to T4 endo V during the viral purification process were indicative of a high level of expression. To confirm the introduction of the T4 endo V gene under the control of the polyhedrin gene promoter, genomic DNA extracted from AcBac-T4 endo V was analyzed by PCR (data not shown). The 424-bp band expected for the T4 endo V gene was observed in AcBac-T4 endo V. The recombinant viral stocks were produced (3×10^9 PFU/ml) and used for the production of rT4 endo V.

To verify the expression level of T4 endo V in AcBac-T4 endo V-infected Sf21 cells, the samples were separated on a 15% SDS-PAGE and subjected to Western blotting analysis. A protein band appeared with the size expected for the T4 endo V protein in infected culture media, but was not detected in noninfected culture media (Fig. 3A). A band at the same location was also detected by Western blotting analysis using the rabbit anti-rHis-Tag/T4 endo V polyclonal antibodies (Fig. 3B). These results indicated that the rT4 endo V was secreted into the cell culture media.

The level of protein expression was optimized by evaluating the expression levels with various virus titers and time course of infection. The effect of recombinant baculovirus loading on rT4 endo V production was investigated by densitometric scanning of the Western blotting analysis using the rabbit anti-rHis-Tag/T4 endo V

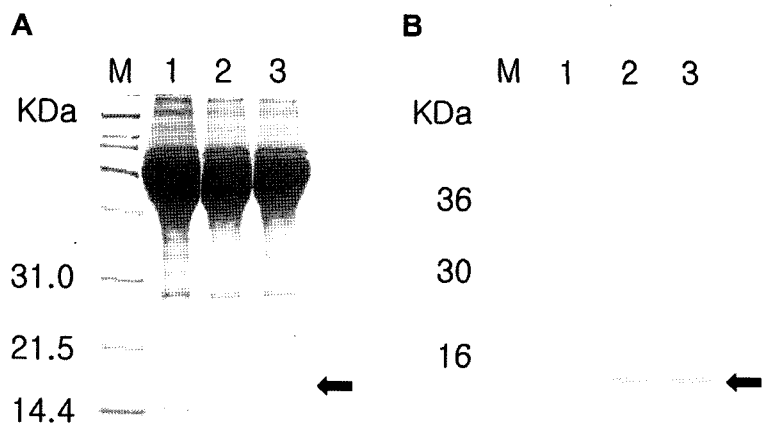

Fig. 3. Expression of the rT4 endo V in Sf21 cells.
Rcombinant virus AcBac-T4 endo V-infected cell culture media were separated on a 15% SDS-PAGE. The gels were visualized by Coomassie blue staining (**A**) and subjected to immunoblot (**B**) with anti-rHis-Tag/T4 endo V antibody. Lane 1, normal cell media; lanes 2 and 3, infected cell media; M, protein size marker.

polyclonal antibodies, as shown in Figs. 4A and 4B. The highest yield was detected at a MOI of 10. We also monitored the quantification of rT4 endo V mass during the time course of infection by Western blotting analysis. The rT4 endo V mass reached a maximum between 84 and 108 h post-infection (Figs. 5A and 5B), whereas no yield could be detected in wild-type AcMNPV virus-infected cells (data not shown). Thus, the protein expression condition was set as follows; infection of the cells at a MOI of 10 and harvest at 96 h post-infection. Under these conditions, we estimated the amount of rT4 endo V produced in the baculovirus expression system to be 125 mg/l, based on the intensity of the bands.

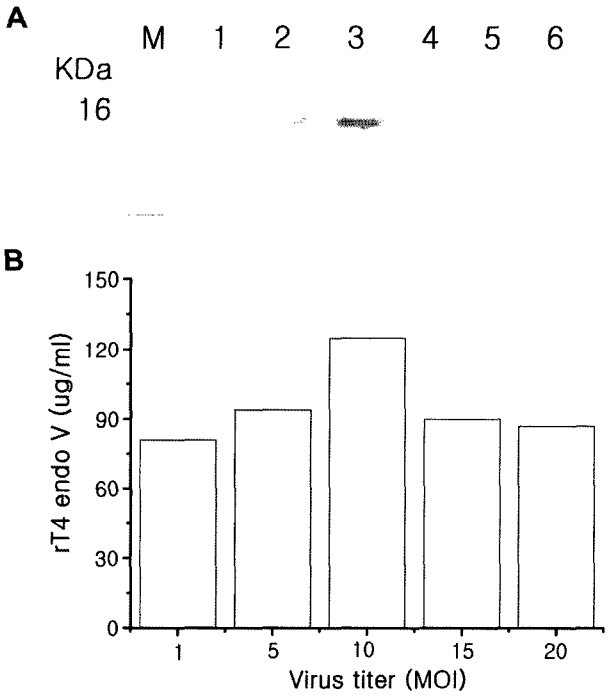

Fig. 4. Effects of AcBac-T4 endo V loading on rT4 endo V production.
A. Quantification of rT4 endo V in the culture medium of AcBac-T4 endo V-infected Sf21 cells at 96 h post-infection. **B**. The corresponding densitometric scanning results after Western blotting. Lanes 1–5, MOI 1, 5, 10, 15, and 20, respectively; lane 6, 200 ng of commercial T4 endo V; M, protein size marker.

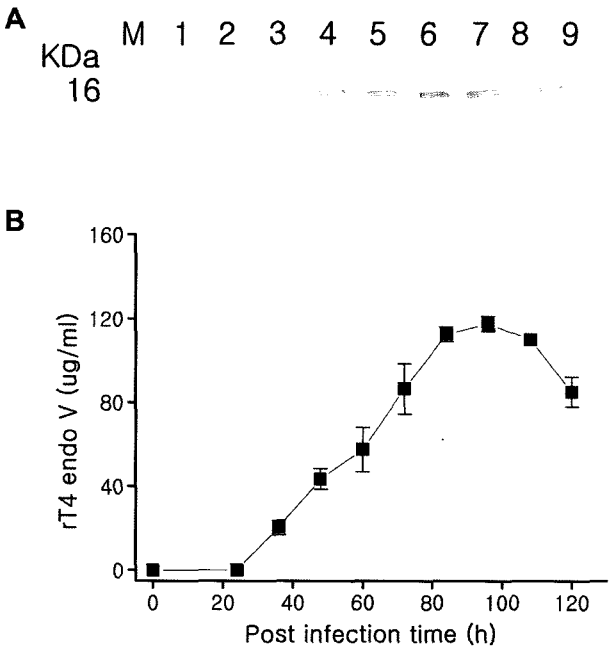

Fig. 5. Time course of rT4 endo V production.
A. Quantification of rT4 endo V in the culture medium of AcBac-T4 endo V-infected insect Sf21 cells at MOI 10. **B**. The corresponding densitometric scanning results after Western blotting. Lanes 1–9, culture medium for 24, 36, 48, 60, 72, 84, 96, 108, and 120 h, respectively; M, Protein size marker.

Table 1. Purification of rT4 endo V.

Purification step	Total protein (mg)[a]	Purity (%)[b]	rT4 endo V protein (mg)[c]	Specific activity (Units/mg)	Recovery yield (%)
Conditioned media[d]	2,500.00	5	125.00	N.M.	100.00
SP Sepharose	126.40	38.34	48.46	N.M.	38.77
Heparin affinity	42.94	86.52	37.15	N.M.	29.72
Resource RPC	26.25	95.24	25.00	20,000	20.00

[a]Total protein mass was estimated by a protein assay kit (Pierce) with BSA as a standard.
[b]Purity was determined by optical densitometry on SDS-PAGE.
[c]rT4 endo V protein mass was determined by densitometric analysis of Western blotting.
[d]One liter of culture media was used for the purification of rT4 endo V.
N.M.: not measured.

For the purification of rT4 endo V from Sf21 cell-conditioned media, we developed a three-step purification procedure consisting of ion-exchange, affinity, and reversed-phase chromatographies, based on FPLC. One liter of supernatant was loaded onto a SP Sepharose XL column and rT4 endo V was eluted with a linear gradient of 0.41–0.45 M KCl (data not shown). This ion-exchange chromatography was quite effective as a first purification step, because the bulk of non-T4 endo V media proteins was eluted during sample loading and column washing with 25 mM sodium phosphate at pH 6.8. Since the heparin affinity column separates DNA repair enzymes effectively [6], we used this column chromatography in our second purification step. Heparin affinity chromatography of the rT4 endo V-enriched ion-exchange sample yielded proteins of >86% purity (Table 1). The partially purified rT4 endo V was further purified by FPLC on a Resource RPC column using a linear gradient of acetonitrile containing 0.1% TFA. Fig. 6 shows the last stage of the purification of rT4 endo V eluted at 50% acetonitrile. The homogeneity of the purified rT4 endo V was more than 95% pure, based on Coomassie blue staining (Fig. 6 inset) and densitometric analysis. The recovery rate of the purification procedure consisting of three steps was about 20% (Table 1). Therefore, the yield of rT4 endo V protein was about 20 mg from 1,000 ml of the culture media.

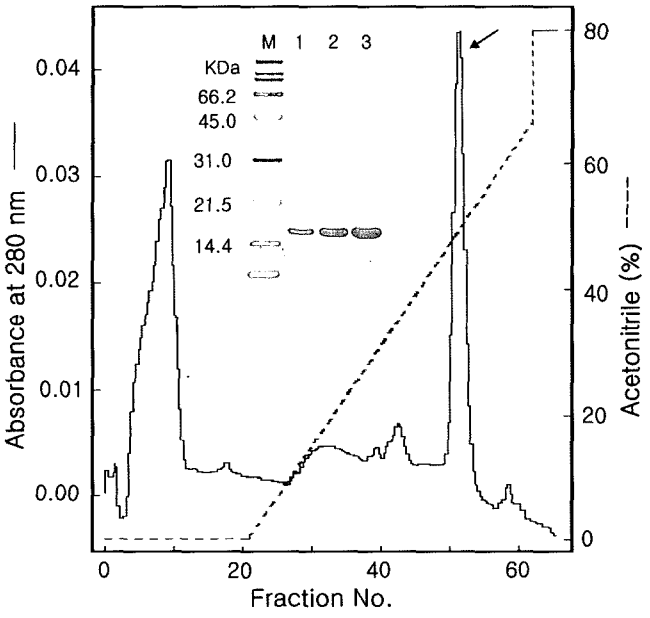

Fig. 6. Final purification of the rT4 endo V by a Resource RPC column.
The peak of rT4 endo V is indicated by an arrow. Inset: SDS-PAGE for purified rT4 endo V. Lanes 1–3, 1 μg, 3 μg, and 5 μg of finally purified rT4 endo V subjected to the gel, respectively; M, protein size marker.

Biological Activity

The UV-specific endonuclease activity of rT4 endo V based on pyrimidine dimer-specific nicking activity was analyzed on supercoiled UV-irradiated pUC18 DNA. As shown in Fig. 7, upon treatment of the rT4 endo V on the supercoiled pUC18 DNA (lanes 3 and 6) with (UV+) and without (UV–) exposure to a UV transilluminator, it converted the supercoiled DNA (closed circle) to the relaxed pUC18 DNA (open circle) (lane 6). Lane 6 also

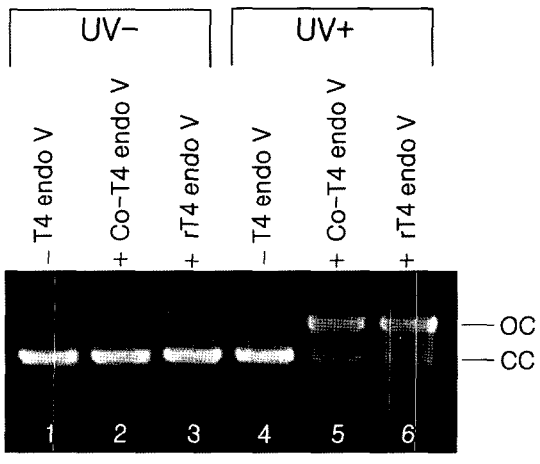

Fig. 7. UV endonuclease activity of rT4 endo V.
pUC18 (250 ng) with (UV+) or without (UV–) exposure was digested with rT4 endo V and Co-T4 endo V (commercial T4 endo V). Lanes 1 and 4, negative control without enzyme; lanes 2 and 5, 100 unit of commercial T4 endo V; lanes 3 and 6, 50 ng of rT4 endo V. CC, closed circles; OC, open circles.

shows some low molecular weight DNA molecules, which may be the degradation products of covalent closed circular or relaxed circular molecules [24]. Furthermore, 50 ng of rT4 endo V, purified to more than 95% purity, displayed similar UV endonuclease activity to 100 units of the commercial T4 endo V (lane 5), which was made in *E. coli*, indicating that our rT4 endo V could replace the commercial T4 endo V for safety reasons.

Although T4 endo V was originated from bacteriophage T4, its recombinant protein expressed in *E. coli* has been applied to patients with zeroderma pigmentosum as topical application for clinical prophylaxis and treatment [8, 14]. As described above, any product expressed in *E. coli* is endowed with potential toxic effect because of the endotoxin, which makes it difficult to be used in humans. We expressed rT4 endo V in insect cells via a eukaryotic baculovirus expression system, to avoid pathogenic or toxic effects of bacteria. Our results indicated that this system could be used for production of therapeutical agents by recombinant DNA technology.

Acknowledgments

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