JOURNAL OF BIOSCIENCE AND BIOENGINEERING
Vol. 90, No. 1, 62–68. 2000

Molecular Cloning and Characterization of a Novel Bacteriophage-Associated Sialidase

YUICHI MACHIDA,[1] KOUJI HATTORI,[1] KATSUHIDE MIYAKE,[1]* YUJI KAWASE,[2] MITSUO KAWASE,[2] AND SHINJI IIJIMA[1]

Department of Biotechnology, Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603[1] and NGK Insulators Ltd., Handa, Aichi 475-0825,[2] Japan

Received 18 February 2000/Accepted 6 April 2000

Bacteriophage 63D, previously isolated from sewage, is associated with α-2,8-linked polysialic acid degrading activity. We cloned a DNA fragment containing the sialidase gene from a 63D phage genomic library and the enzyme was functionally expressed in *Escherichia coli*. Determination of the nucleotide sequence of the fragment revealed that it contained one open reading frame (ORF) coding for a 108-kDa polypeptide consisting of 984 amino acid residues. The fragment had promoter sequences similar to the *E. coli* consensus promoters for σ^{70}. The deduced amino acid sequence of the central region of the ORF showed homology to those of phages K1F (51.6% identity) and PK1E (51.7% identity) endosialidases. Two Asp-box motifs that are widely found in sialidases were conserved. Purification of the soluble enzyme from lysed culture broth of infected *E. coli* yielded a 90-kDa protein upon SDS polyacrylamide gel electrophoresis, suggesting that the primary translational product is processed to the mature 90-kDa protein. The molecular mass of the enzyme was determined as 360 kDa by gel filtration, indicating that the native enzyme was probably a tetramer of identical 90-kDa subunits.

[**Key words:** polysialic acid, sialidase, bacteriophage, *Escherichia coli* K1]

Polysialic acid (PSA) is an α-2,8 linked sialic acid homopolymer and a main component of the capsular sugar moiety of the K1 strain of *Escherichia coli* (1). This sugar is also widely distributed in mammals. For instance, the polymer is an important component of the neural cell adhesion molecule and regulates cell adhesion between neurons (2, 3). In certain types of cancer, PSA is expressed on the cell surface and may have an important role in metastasis (4, 5).

Exosialidases, which remove terminal sialic acids from nonreducing ends of carbohydrate chains, have been found in many organisms, including viruses, bacteria, trypanosomes, and mammalian cells (6). In contrast, endosialidases, which catalyze a random degradation of PSA, have been reported only in bacteriophages that infect *E. coli* K1 strains having PSA as a capsular polysaccharide (7–11). The endosialidases associated with coliphages K1F and PK1E have been purified and analyzed (12–14), and their genes have been cloned and studied (15–17).

We also have isolated coliphages that specifically infect K1 strains of *E. coli* (18). Among them, phages 63a and 63D, referred to as No. a and No. D in a previous paper (18), seem to be unique since they showed activities to degrade sialyl trimer and tetramer. In contrast, the minimum substrates size for coliphage-associated endosialidases reported so far is a pentamer or longer oligosaccharides (13, 14). Bacteriophages 63a and 63D were also unique in that they had long tails and belonged to Bradley's morphology group B (19). As far as we know, no other group B coliphage with sialidase activity has been reported. In the present paper, we report the purification, characterization, and molecular cloning of the novel sialidase associated with bacteriophage 63D.

* Corresponding author.

MATERIALS AND METHODS

Purification of phage particles Bacteriophage 63D was propagated in liquid culture; *E. coli* strain 63 (18) was infected with phages at a multiplicity of infection of 0.1. Following complete lysis of the bacterial cells, cell debris was removed by centrifugation at $4000 \times g$ for 10 min, and phage particles in the supernatant were concentrated by adding polyethylene glycol 6000 and NaCl to a final concentration of 10% and 1 M, respectively. Phage particles were collected by centrifugation at $4700 \times g$ for 20 min, and dissolved in 10 mM Tris-HCl (pH 7.5) containing 10 mM $MgSO_4$. For isopycnic centrifugation, the phage suspension was placed on cesium chloride stepwise in three solutions whose densities were 1.45, 1.50, and 1.70, respectively. After centrifugation for 60 min at $150,000 \times g$, the phage band was withdrawn and dialyzed against 10 mM Tris-HCl (pH 7.5) containing 10 mM $MgSO_4$. From 1200 ml of culture, 8×10^{13} phage particles were obtained.

Cloning of the sialidase gene Phage particles were disrupted by incubation with 1% SDS containing 10 mM EDTA. After phenol-chloroform extraction, DNA was precipitated by adding ethanol. Following treatment of the DNA with RNase A (1 mg/ml) and proteinase K (50 μg/ml) in 20 mM Tris-HCl (pH 7.5) containing 0.5 mM EDTA, the sample was extracted again with phenol-chloroform and precipitated with ethanol. Isolated phage DNA was digested with *Sma*I and then inserted into a *Sma*I-digested plasmid Charomid 9-36 (Nippon Gene Co., Toyama). The ligated DNA was packaged by the standard method and *E. coli* HB101 was infected with the packaged product. A genomic library was screened by determining the sialidase activity of crude extracts of the transformants. One clone showing sialidase activity was selected and the recombinant plasmid was designated pSD1. The *Sma*I DNA insert of pSD1 was

subcloned to a high-copy-number plasmid, pUC19, and the recombinant plasmid was designated pSD2.

Sequence analyses The nucleotide sequence of the phage sialidase was determined with both strands by the dideoxy chain termination method (20) using dye primer and dye termination cycle sequencing core kits (Perkin Elmer, Applied Biosystems Division, Foster City, CA, USA).

Primer extension experiments Total cellular RNA was isolated from either phage-infected cells or cells carrying plasmid pSD2. *E. coli* strain 63 cells grown to 10^8 cells/ml in nutrient broth were infected with the phage 63D at a multiplicity of infection of 0.4 and cultured at 37°C for 30 min. *E. coli* DH5α cells harboring the plasmid pSD2 were grown to 10^8 cells/ml in L-broth containing ampicillin (100 μg/ml). Bacterial cells were harvested by centrifugation (4000 × *g*, 10 min) and suspended in AC/SDS (20 mM sodium acetate/NaOH (pH 5.5) containing 0.5% SDS, 1 mM EDTA). RNA was extracted two times by adding 2 volumes of phenol equilibrated with AC/SDS and vigorous shaking at 60°C for 10 min. RNA was precipitated by adding ethanol, and treated with 50 μg/ml of RNase-free DNase I (Sigma Chemical Co., St Louis, MO, USA).

Primer extension analysis was performed as described previously (21), using a synthetic primer (5′-CCATCGCT GAATCCGTC-3′, complementary to nucleotides 98 to 114) labeled with [γ-^{32}P]ATP (~6000 Ci/mmol, Amersham Pharmacia Biotech, Tokyo) by T4 polynucleotide kinase (Takara Shuzo, Kyoto) and RNA of phage-infected or plasmid-bearing cells. The DNA synthesized by reverse transcriptase (RAV-2, Takara Shuzo) was analyzed by electrophoresis on an 8% polyacrylamide gel containing 8 M urea. As a standard, a sequence ladder was prepared with the plasmid pSD2 by using a *Bca*BEST™ dideoxy sequencing kit (Takara Shuzo) with [α-^{32}P]dCTP (~3000 Ci/mmol, Amersham Pharmacia Biotech) and the above primer.

Enzyme assays Sialidase activity was assayed by measuring the release of sialic acid from PSA (Sigma Chemical Co.). The reaction mixture contained 10 mg/ml of PSA in 20 mM Tris-HCl (pH 7.5) containing 0.5 mM EDTA and the enzyme solution in a total volume of 20 μl. After the enzyme reaction at 37°C, the free sialic acid ends generated were quantified by using thiobarbituric acid (22). One unit of sialidase activity was defined as the amount of enzyme that released 1 μmol of sialic acid equivalent ends per min under the assay conditions.

In some experiments, 5 μl of the reaction mixture was spotted on a silica gel plate (Merck, Darmstadt, Germany) and developed with 1-propanol/25% NH_4OH/H_2O (6 : 1 : 2.5 by volume). Degradation products were detected by spraying with 5% sulfuric acid in ethanol followed by heating at 140°C for 10 min.

Purification of soluble sialidase Protein in lysed culture broth (1200 ml) was precipitated with 50% saturated ammonium sulfate. The pellet was dissolved in 20 ml of 50 mM Tris-HCl (pH 7.5) containing 0.5 mM EDTA. After dialysis against the same buffer, the sample was centrifuged at 130,000 × *g* for 90 min to remove the phage particles. The sample was then heated at 60°C for 30 min. After removal of the precipitate by centrifugation (27,000 × *g* for 20 min), the sample was fractionated by ammonium sulfate (25-40% saturation). The fractionated proteins were dissolved in a small volume of 20 mM Tris-HCl (pH 7.5) containing 0.5 mM EDTA and dialyzed against the same buffer. The sample was then loaded on a Sepharose CL-6B column (2.5 × 96 cm, Amersham Pharmacia Biotech) equilibrated with the same buffer. Fractions were collected and loaded on a Mono Q column (0.7 × 5.3 cm, Amersham Pharmacia Biotech) equilibrated with 20 mM Tris-HCl (pH 7.5). Proteins were eluted with a 0 to 1 M NaCl linear gradient (20 ml). Active fractions were collected and dialyzed against 20 mM Tris-HCl (pH 7.5) containing 0.5 mM EDTA.

Antiserum and Western blot analyses An antiserum was raised against the purified cloned sialidase by immunizing 8-week-old BALB/c female mice. For Western blot analysis, protein samples were fractionated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech). The membranes were then blocked with 5% skim milk in phosphate buffered saline (8.0 g NaCl, 0.2 g KCl, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, and 2.0 g KH_2PO_4 per liter), containing 0.05% Tween-20, and incubated with 1 : 1000-diluted anti-sialidase antiserum. Immunoreactivity was determined with horseradish peroxidase-conjugated anti-mouse IgG antibody, and detected by using enhanced chemiluminescence reagents (Amersham Pharmacia Biotech).

Sialidase digestion of ^{3}H-labeled PSA PSA was labeled at its nonreducing ends by periodate oxidation and NaB^3H_4 (5 Ci/mmol; NEN Life Science Products, Boston, MA, USA) reduction as described previously (23). Labeled PSA was dialyzed against 50 mM Tris-HCl (pH 7.5) containing 0.15 M NaCl with a dialysis membrane which cuts off molecules under 10,000 Da in size. This substrate was digested by sialidase, and analyzed by TLC followed by exposure to X-ray films.

Nucleotide sequence accession number The DNA

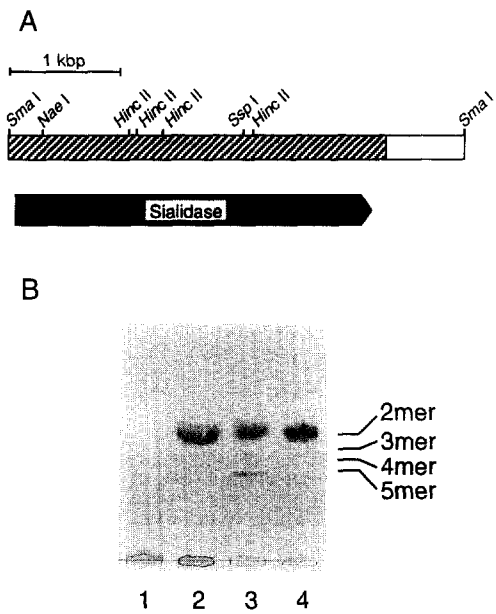

FIG. 1. Molecular cloning of the sialidase gene and its functional expression in *E. coli*. (A) Restriction map of the DNA fragment containing the sialidase gene in pSD1 and pSD2. The position of the ORF of sialidase is indicated by the large arrow. The shaded bar indicates the region of the DNA sequence determined. (B) Limit digestion of PSA by cloned sialidase. PSA (20 μg) was digested for 72 h with bacteriophage 63D (10^{11} plaque forming units, lane 2) and cloned sialidase (pSD1 and pSD2; lanes 4 and 3, respectively). Lane 1, untreated PSA. The number of polymerized sialyl residues is indicated on the right.

sequence in this paper has been submitted to the DDBJ (DNA Data Bank of Japan) database and is available under accession number AB015437.

RESULTS AND DISCUSSION

Molecular cloning and functional expression in *E. coli* of the sialidase gene To isolate the sialidase gene, a phage 63D genomic library was constructed using Charomid 9-36 as a vector and the sialidase activity of crude extracts prepared from each transformant was assayed. The clone containing a plasmid designated as pSD1 showed sialidase activity. The restriction map of the 4-kb *Sma*I DNA fragment is given in Fig. 1A.

To characterize the enzyme activity of the cloned sialidase, PSA was digested extensively with each enzyme for 72 h (limit digestion). Completely digested products of PSA were analyzed by TLC (Fig. 1B). Cloned sialidases from transformants carrying pSD1 and pSD2 could degrade PSA to the sialyl dimer, as was the case with phage particles.

DNA sequence determination and derived amino acid sequence of sialidase We partially determined the nucleotide sequence of the *Sma*I fragment of the plasmid pSD2 using the dideoxy chain termination method. It contained an open reading frame (ORF) of 2952 nucleotides (nucleotides 137 to 3088), which encoded a polypeptide consisting of 984 amino acid residues with an estimated molecular mass of 108 kDa. In front of the ORF, a Shine-Dalgarno ribosomal binding site (AAGAG G, nucleotides 124 to 129) and an ATG translation start codon were identified. A potential Rho-independent transcription terminator (AAGGCCCCTAACGGGGCCTT at nucleotides 3096 to 3115, $\Delta G = -20.9$ kcal/mol) was found downstream of the ORF. In this plasmid, the transcriptional direction of the sialidase gene was opposite to that of the *lac* promoter of pUC19.

The deduced amino acid sequence of the cloned sialidase was compared with that of other sialidases through the DDBJ database. The result of the alignment indicated that bacteriophage 63D sialidase had similarity to previously reported coliphage-associated endosialidases.

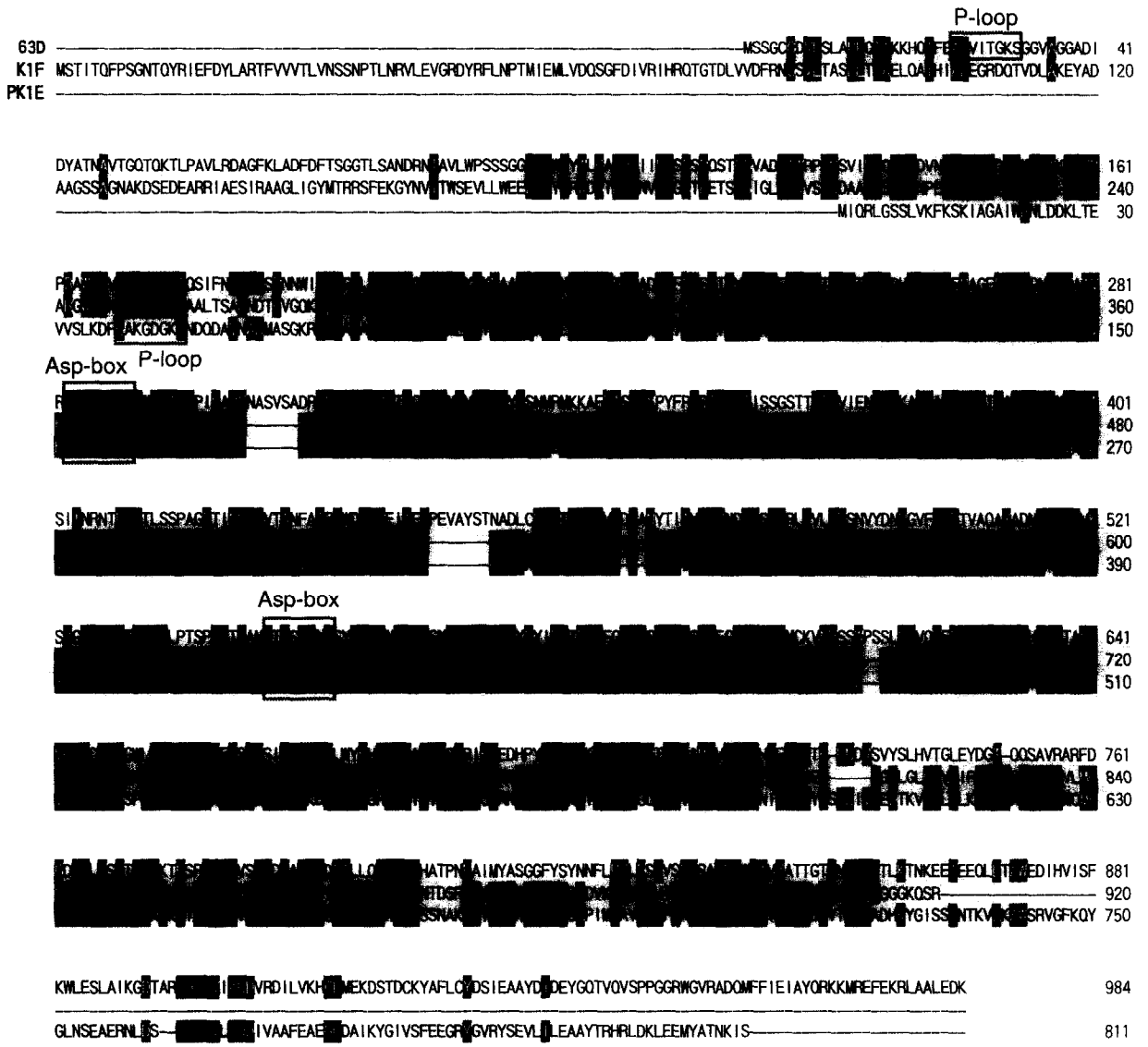

FIG. 2. Alignment of the amino acid sequence of bacteriophage 63D sialidase with those of the K1F and PK1E endosialidase. Conserved residues are shown by white letters in black columns. P-loops and Asp-boxes are boxed.

The amino acid sequence of the central domain (amino acids 192 to 856) of the sialidase showed homology to K1F (51.6% identity) and PK1E (51.7% identity) endosialidases (Fig. 2). Homology between the K1F and PK1E sialidases is higher than 80%, but that between the 63D sialidase and these sialidases was relatively low. This may reflect the fact that the enzyme of 63D is derived from a different type of bacteriophage.

K1F sialidase has an extra sequence in the N-terminus, whereas in the PK1E enzyme there is an extra sequence in the C-terminus. The enzyme from bacteriophage 63D had extra sequences in both the C- and N-termini but the N-terminal one was shorter than that of K1F sialidase. A certain part of the N-terminal extra sequence of K1F endosialidase has been reported to be homologous to the N-terminal domains of phage T7 and T3 tail proteins, which appear to mediate the binding of these proteins to the virion head (15). However, we could detect no such sequence in the N-terminal region of bacteriophage 63D sialidase. Instead, a P-loop motif ([A or G]XXXXGK[T or S]) (24), which is present in ATP/GTP binding proteins, was found in its N-terminal region (AEVITGKS), as is the case with PK1E endosialidase (GAKGDGKT) (17). The function of this motif in sialidase is so far unknown.

Two Asp-box (SXDXGXTW) sequences were found in phage 63D sialidase. This motif is reported to be conserved among exo- and endosialidases (25, 26). The distance between these boxes was also conserved among the three phage-derived enzymes: 451 amino acids for 63D, 440 for K1F, and 460 for PK1E (15–17).

Determination of transcriptional initiation site To investigate the transcriptional initiation of the sialidase gene in phage infected cells, a primer extension experiment was performed using a primer specific for the 5′ end region of the phage 63D sialidase gene. When total cellular RNA isolated from cells 30 min after phage infection was used as a template for reverse transcription, a major band and several minor bands were observed (Fig. 3A). Comparison of the major band with the standard DNA sequence ladder showed that the transcriptional initiation site was located 85 bp upstream of the translational start codon. Upstream of the transcriptional initiation site, the consensus bacterial promoter sequences TATGAT for the −10 region and CGGTCA for the −35 region were found at nucleotides 41 to 46 and at 18 to 23, respectively, and the spacing between the two sequences was 17 bp (Fig. 3B). These features were similar to the −10 and −35 control regions of *E. coli* promoters for σ^{70} (27). On the other hand, no such regulatory sequences were found for the minor bands.

To examine whether the transcriptional initiation site of the cloned gene was identical to that observed in the phage sialidase gene, a primer extension experiment was carried out with total RNA isolated from cells harboring the plasmid pSD2 by using the same primer. As shown in Fig. 3A, the pattern of synthesized DNA was exactly the same as that observed with the phage-infected cells. Northern blotting of total RNA from phage-infected cells and cells carrying pSD2 detected sialidase gene transcripts of exactly the same size (3.0 kb) (data not shown). The endosialidase genes of the K1F and PK1E phages contain consensus sequences of phage promoters recognized by T7 or SP6 RNA polymerase (15, 17), and it is assumed that sialidase genes of these phages are transcribed by phage RNA polymerases. In contrast, no promoter sequences recognized by phage RNA polymerases, including the T7 and SP6 RNA polymerases, were found in the phage 63D sialidase gene. These results suggest that the sialidase gene was transcribed by *E. coli* RNA polymerase. However, we cannot rule out the possibility that phage 63D has its own RNA polymerase that catalyzes the formation of the other phage mRNAs. To elucidate this point, we are analyzing the entire genome structure of bacteriophage 63D.

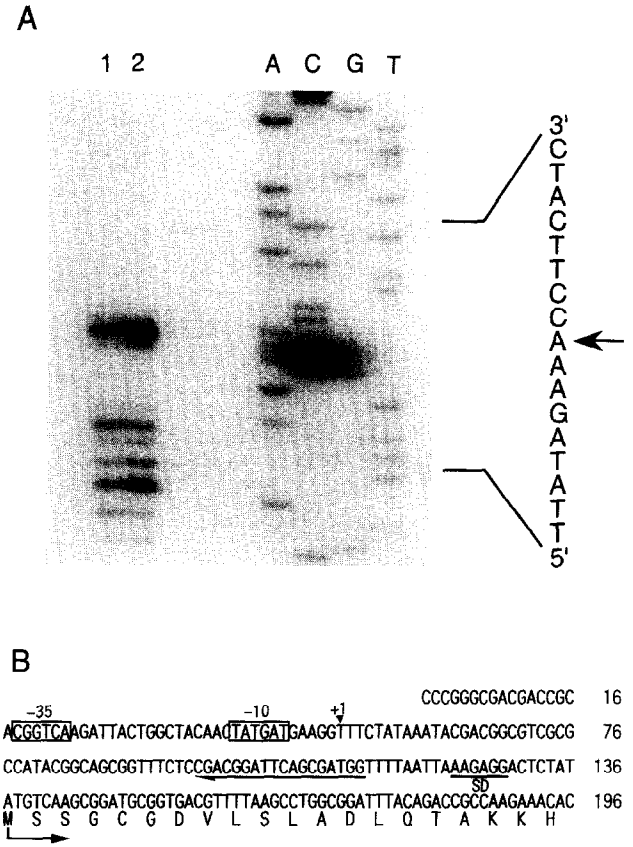

FIG. 3. Determination of the 5′ end of the sialidase gene transcripts. (A) Primer extension analysis of the transcripts for the sialidase gene. Cellular RNA was isolated from either *E. coli* DH5α cells carrying pSD2 (lane 1) or *E. coli* strain 63 infected with bacteriophage 63D (lane 2). The arrow indicates the estimated 5′ end of the sialidase gene mRNA. The DNA sequence ladders shown were synthesized using the same primer. (B) Promoter region of the sialidase gene. The possible −10 and −35 promoter regions are boxed and the transcriptional initiation site is indicated (+1). The position of the primer used for the primer extension experiment is indicated by a horizontal arrow.

Purification of soluble sialidase As reported with other phages, sialidase activities were detected not only in the phage particles but also in the phage lysate (12, 13). A part of the newly synthesized sialidase may not be incorporated in the phage particle and exist as a soluble form in the lysate. The soluble enzyme was purified from lysed culture broth by ammonium sulfate precipitation, ultracentrifugation, heat treatment, second ammonium sulfate fractionation, gel filtration, and mono Q column chromatography. By ultracentrifugation, about 70% of the enzyme activity was removed in the pellet of phage particles or insoluble aggregates. Two main peaks of enzyme activity were observed on gel filtration using a Sepharose CL-6B column (Fig. 4). The first peak, which corresponded to the void volume, presumably contained

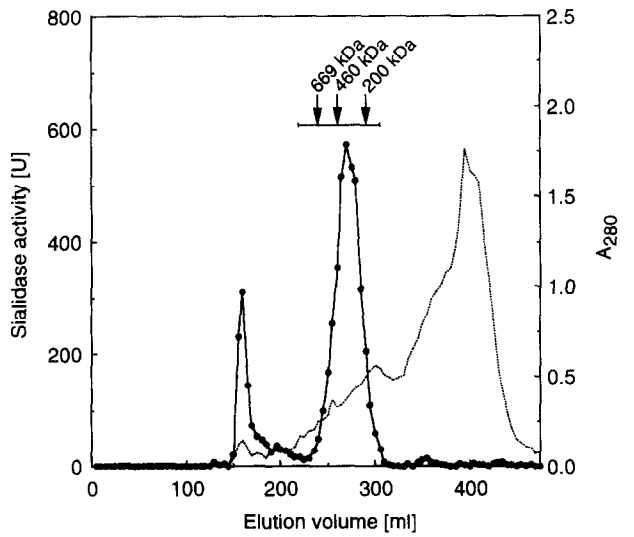

FIG. 4. Elution profile of soluble sialidase from a Sepharose CL-6B column. Thyroglobulin (669 kDa), ferritin (460 kDa), and β-amylase (200 kDa) were used as molecular mass standards. Sialidase activity is indicated by symbol (●), and A_{280}, absorbance at 280 nm, is indicated as broken line.

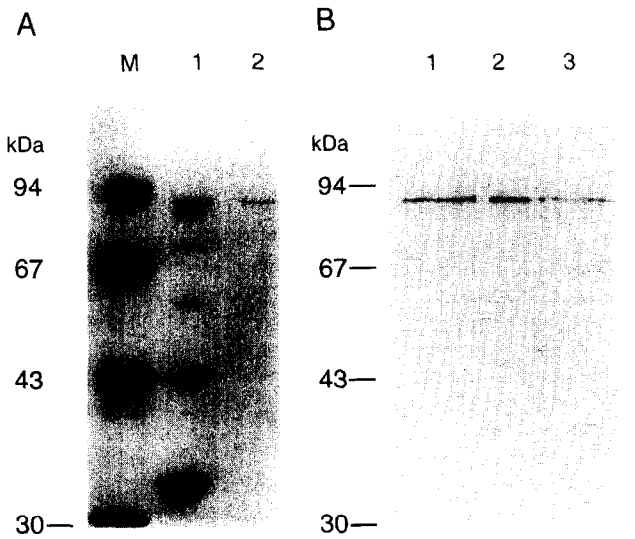

FIG. 5. Identification of the bacteriophage 63D sialidase. (A) SDS-PAGE (10% acrylamide, in the presence of 2-mercaptoethanol) of purified soluble sialidase and bacteriophage 63D proteins. Lane M, markers; lane 1, bacteriophage 63D proteins; lane 2, purified soluble sialidase. (B) Western blot analysis of sialidases. Lane 1, bacteriophage 63D; lane 2, soluble fraction of phage lysate; lane 3, *E. coli* harboring plasmid pSD2.

intact or destroyed phage particles, and the second peak, soluble sialidase. The molecular mass of the native soluble sialidase was estimated to be approximately 360 kDa. The fractions of this peak were pooled and applied to a Mono Q column. Through the steps shown in Table 1, soluble sialidase was purified 106-fold. The final preparation gave a single band on SDS-PAGE with an estimated molecular mass of 90 kDa (Fig. 5A). This result indicated that the enzyme was a tetramer and consisted of identical 90 kDa subunits.

We also purified the cloned enzyme from *E. coli* harboring plasmid pSD2 essentially by the same procedure as that used for the soluble enzyme (data not shown). From a 1-*l* culture, 0.382 mg of recombinant enzyme was obtained by the procedure. The molecular mass of the cloned sialidase was estimated to be approximately 360 kDa by gel filtration, and the subunit molecular mass was 90 kDa by SDS-PAGE, which are the same values as those obtained for soluble sialidase.

Western blotting using antibody raised against the cloned sialidase detected a 90-kDa protein with the samples from phage particles, the soluble fraction of the phage lysate, and *E. coli* harboring plasmid pSD2 (Fig. 5B). This result indicated that the phage-associated, soluble, and cloned sialidases were antigenically identical. The molecular mass of the subunit estimated by SDS-PAGE for the three sialidases (90 kDa) differs from that predicted from the DNA sequence. The reason for this difference is not yet clear, but it may be caused by post-translational modifications. In this respect, it is noteworthy that the C-terminal region of PK1E phage sialidase is cleaved from the precursor protein (17).

TABLE 1. Purification of bacteriophage 63D soluble sialidase

Step	Total protein (mg)	Total activity (U)	Yield (%)	Specific activity (U/mg)	Purity (fold)
50% Saturated ammonium sulfate	445	57113	—	128.3	—
High-speed centrifugation	339	17890	100	52.8	1
Heat treatment	338	11384	63.6	33.7	0.6
25–40% Saturated ammonium sulfate	66	2392	13.4	36.3	0.7
Sepharose CL-6B	1	507	2.8	507.0	9.6
Mono Q	0.036	201	1.1	5583.3	105.7

Time course of polysialic acid degradation To study the mode of PSA degradation by the enzyme, the initial time course of the degradation was analyzed. For this purpose, PSA was labeled with ^3H at the nonreducing ends. This labeled PSA substrate was incubated with bacteriophage 63D and with soluble and cloned sialidases, and the initial time course of the PSA degradation was analyzed by TLC. When incubated with bacteriophage 63D, the digestion products were observed as a ladder on TLC (Fig. 6A). Thus, it was considered that the enzyme randomly degraded PSA and had endo-type activity. After 5 min digestion, sialyl trimer was detected as a strong band compared to other oligomers. Trimers to heptamers were detected as strong bands after 60 min. Since the shorter oligomers, especially the trimer, were poor substrates, the latter might accumulate in the reaction mixture. However, we cannot rule out the possibility that this enzyme may also have exo-type activity, which releases sialyl trimer units from the nonreducing end of PSA, because the sialyl trimer appeared at an early stage of the reaction.

To investigate the degradation patterns of soluble and cloned sialidases, the same experiments were performed with these enzyme preparations. When partially purified soluble and cloned sialidases were incubated with end-labeled PSA, patterns similar to that of bacteriophage 63D digestion were obtained (Fig. 6B, C).

Previously, we showed that the sialidase of phage particles was composed of four 90-kDa subunits and that each subunit was cross-linked by disulfide bonds (28). Electron microscopic observation suggested that this tetramer formed the phage tail plate. The results of the

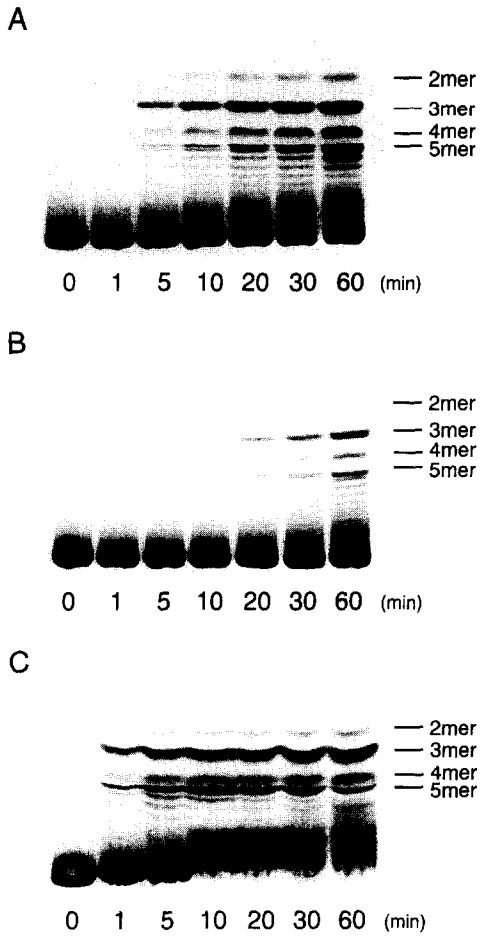

FIG. 6. Time course of PSA degradation. PSA labeled with ^3H at the nonreducing ends was digested with bacteriophage 63D (2×10^{10} plaque-forming units; (A), soluble sialidase (B), and cloned sialidase encoded by plasmid pSD1 (C), and the products were analyzed by TLC. The reaction time is shown under the figures and the number of polymerized sialyl residues is indicated on the right.

present work indicate the same tetrameric structure was formed with both soluble and cloned sialidases. Furthermore, as shown in Fig. 6, all three enzyme preparations exhibited essentially the same catalytic properties. These results suggest that phage-incorporated sialidase which appears to form the tale plate, may have the same catalytic properties and structure as the soluble and cloned enzymes.

ACKNOWLEDGMENTS

We are grateful to the Radioisotope Research Center, Nagoya University for performing experiments using radioisotopes. This work was supported in part by a Grant in Aid (No. 11555219) from the Ministry of Science, Education, Sports and Culture, Japan. One author (Y. Machida) was supported by a research fellowship for young scientists from the Japan Society for the Promotion of Science. The authors thank Ms. M. Goto for preparation of the manuscript.

REFERENCES

1. **Barry, G. T. and Goebel, W. F.:** Colominic acid, a substance of bacterial origin related to sialic acid. Nature (London), **179**, 206 (1957).
2. **Rutishauser, U., Watanabe, M., Silver, J., Troy, F. A., and Vimr, E. R.:** Specific alteration of NCAM-mediated cell adhesion by an endoneuraminidase. J. Cell Biol., **101**, 1842–1849 (1985).
3. **Rutishauser, U., Acheson, A., Hall, A. K., Mann, D. M., and Sunshine, J.:** The neural cell adhesion molecule (NCAM) as a regulator of cell–cell interactions. Science, **240**, 53–57 (1988).
4. **Livingston, B. D., Jacobs, J. L., Glick, M. C., and Tory, F. A.:** Extended polysialic acid chains ($n > 55$) in glycoproteins from human neuroblastoma cells. J. Biol. Chem., **263**, 9443–9448 (1988).
5. **Scheidegger, E. P., Lackie, P. M., Papay, J., and Roth, J.:** In vitro and in vivo growth of clonal sublines of human small cell lung carcinoma is modulated by polysialic acid of the neural cell adhesion molecule. Lab. Invest., **70**, 95–106 (1994).
6. **Roggentin, P., Schauer, R., Hoyer, L. L., and Vimr, E. R.:** The sialidase superfamily and its spread by horizontal gene transfer. Mol. Microbiol., **9**, 915–921 (1993).
7. **Stirm, S.:** *Escherichia coli* K bacteriophages. I. Isolation and introductory characterization of five *Escherichia coli* K bacteriophages. J. Virol., **2**, 1107–1114 (1968).
8. **Stirm, S., Bessler, W., Fehmel, F., and Freund-Mölbert, E.:** Bacteriophage particles with endo-glycosidase activity. J. Virol., **8**, 343–346 (1971).
9. **Gross, R. J., Cheasty, T., and Rowe, B.:** Isolation of bacteriophages specific for the K1 polysaccharide antigen of *Escherichia coli*. J. Clin. Microbiol., **6**, 548–550 (1977).
10. **Kwiatkowski, B., Boschek, B., Thiele, H., and Stirm, S.:** Endo-*N*-acetylneuraminidase associated with bacteriophage particles. J. Virol., **43**, 697–704 (1982).
11. **Kwiatkowski, B., Boschek, B., Thiele, H., and Stirm, S.:** Substrate specificity of two bacteriophage-associated endo-*N*-acetylneuraminidases. J. Virol., **45**, 367–374 (1983).
12. **Tomlison, S. and Taylor, P. W.:** Neuraminidase associated with coliphage E that specifically depolymerizes the *Escherichia coli* K1 capsular polysaccharide. J. Virol., **55**, 374–378 (1985).
13. **Hallenbeck, P. C., Vimr, E. R., Yu, F., Bassler, B., and Troy, F. A.:** Purification and properties of a bacteriophage-induced endo-*N*-acetylneuraminidase specific for poly-α-2,8-sialosyl carbohydrate units. J. Biol. Chem., **262**, 3553–3561 (1987).
14. **Pelkonen, S., Pelkonen, J., and Finne, J.:** Common cleavage pattern of polysialic acid by bacteriophage endosialidases of different properties and origins. J. Virol., **63**, 4409–4416 (1989).
15. **Petter, J. G. and Vimr, E. R.:** Complete nucleotide sequence of the bacteriophage K1F tail gene encoding endo-*N*-acetylneuraminidase (Endo-*N*) and comparison to an Endo-*N* homolog in bacteriophage PK1E. J. Bacteriol., **175**, 4354–4363 (1993).
16. **Gerardy-Schahn, R., Bethe, A., Brennecke, T., Mühlenhoff, M., Eckhardt, M., Ziesing, S., Lottspeich, F., and Frosch, M.:** Molecular cloning and functional expression of bacteriophage PK1E-encoded endoneuraminidase Endo NE. Mol. Microbiol., **16**, 441–450 (1995).
17. **Long, G. S., Bryant, J. M., Taylor, P. W., and Luzio, J. P.:** Complete nucleotide sequence of the gene encoding bacteriophage E endosialidase: implications for K1E endosialidase structure and function. Biochem. J., **309**, 543–550 (1995).
18. **Miyake, K., Muraki, T., Hattori, K., Machida, Y., Watanabe, M., Kawase, M., Yoshida, Y., and Iijima, S.:** Screening of bacteriophages producing endo-*N*-acetylneuraminidase. J. Ferment. Bioeng., **84**, 90–93 (1997).
19. **Bradley, D. E.:** Ultrastructure of bacteriophage and bacteriocins. Bacteriol. Rev., **31**, 230–314 (1967).
20. **Sanger, F., Nicklen, S., and Coulsen, A. R.:** DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA, **74**, 5463–5467 (1977).
21. **Nakayama, S., Kaneko, T., Ishimaru, H., Moriwaki, H., and Mizobuchi, K.:** Cloning, sequencing, and expression of bacteriophage BF23 late genes 24 and 25 encoding tail proteins. J. Bacteriol., **176**, 7280–7290 (1994).
22. **Skoza, L. and Mohos, S.:** Stable thiobarbituric acid chromophore with dimethyl sulphoxide. Application to sialic acid assay in analytical de-*O*-acetylation. Biochem. J., **159**, 457–462

(1976).
23. **Finne, J. and Mäkelä, P. H.:** Cleavage of the polysialosyl units of brain glycoproteins by a bacteriophage endosialidase. Involvement of a long oligosaccharide segment in molecular interactions of polysialic acid. J. Biol. Chem., **260,** 1265–1270 (1985).
24. **Saraste, M., Sibbald, P. R., and Wittinghofer, A.:** The P-loop–a common motif in ATP- and GTP-binding proteins. Trends Biochem. Sci., **15,** 430–434 (1990).
25. **Roggentin, P., Rothe, B., Kaper, J. B., Galen, J., Lawrisuk, L., Vimr, E. R., and Schauer, R.:** Conserved sequences in bacterial and viral sialidases. Glycoconjugate J., **6,** 349–353 (1989).
26. **Rothe, B., Rothe, B., Roggentin, P., and Schauer, R.:** The sialidase gene from *Clostridium septicum*: cloning, sequencing, expression in *Escherichia coli* and identification of conserved sequences in sialidases and other proteins. Mol. Gen. Genet., **226,** 190–197 (1991).
27. **Hawley, D. K. and McClure, W. R.:** Compilation and analysis of *Escherichia coli* promoter sequences. Nucl. Acid. Res., **11,** 2237–2255 (1983).
28. **Machida, Y., Miyake, K., Hattori, K., Yamamoto, S., Kawase, M., and Iijima, S.:** Structure and function of a novel coliphage-associated sialidase. FEMS Microbiol. Lett., **182,** 333–337 (2000).