JOURNAL OF VIROLOGY, Aug. 1985, p. 374–378
0022-538X/85/080374-05$02.00/0

Vol. 55, No. 2

Neuraminidase Associated with Coliphage E That Specifically Depolymerizes the *Escherichia coli* K1 Capsular Polysaccharide

STEPHEN TOMLINSON[1] AND PETER W. TAYLOR[1,2]*

Molecular Diagnostics, Inc., West Haven, Connecticut 06516,[1] and Institut für Chemotherapie, Pharma-Forschungszentrum, Bayer AG, 5600 Wuppertal 1, Federal Republic of Germany[2]

Received 2 April 1985/Accepted 5 May 1985

Plaque morphology indicated that the five *Escherichia coli* K1-specific bacteriophages (A to E) described by Gross et al. (R. J. Gross, T. Cheasty, and B. Rowe, J. Clin. Microbiol. 6:548-550, 1977) encode K1 depolymerase activity that is present in both the bound and free forms. The free form of the enzyme from bacteriophage E was purified 238-fold to apparent homogeneity and in a high yield from ammonium sulfate precipitates of cell lysates by a combination of CsCl density gradient ultracentrifugation, gel filtration, and anion-exchange chromatography. The enzyme complex had an apparent molecular weight of 208,000, as judged from its behavior on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and was dissociated by sodium dodecyl sulfate at 100°C to yield two polypeptides with apparent molecular weights of 74,000 and 38,500. Optimum hydrolytic activity was observed at pH 5.5, and activity was strongly inhibited by Ca^{2+}; the K_m was 7.41×10^{-3} M. Rapid hydrolysis of both the *O*-acetylated and non-*O*-acetylated forms of the K1 antigen, an α2→8-linked homopolymer of *N*-acetylneuraminic acid, and of the meningococcus B antigen was observed. Limited hydrolysis of the *E. coli* K92 antigen, an *N*-acetylneuraminic acid homopolymer containing alternating α2→8 and α2→9 linkages, occurred, but the enzyme failed to release α2→3-, α2→6-, or α2→9-linked sialic residues from a variety of other substrates.

Escherichia coli is one of the most common causative agents of neonatal meningitis and septicemia; although 73 acidic polysaccharide or protein K antigens are currently recognized by the World Health Organization Escherichia Reference Center (26), one K type, K1, is associated with approximately 80% of all *E. coli* isolates from the cerebrospinal fluid of neonates with meningitis (27). Despite prompt diagnosis and antimicrobial chemotherapy, morbidity and mortality rates for *E. coli* K1 neonatal meningitis remain high; mortality ranges from 30 to 60%, and there appear to be few normal survivors (21, 22). Furthermore, the clinical outcome of the infection is directly related to the amount of K1 antigen present in serum and cerebrospinal fluid (22).

We examined a number of K1-specific bacteriophages for the presence of enzymes able to depolymerize the K1 capsular antigen, a homopolymer of α2→8-linked *N*-acetylneuraminic acid (NeuAc; 29). Bacteriophages infecting encapsulated bacteria frequently carry tail spikes that possess capsule-degrading enzymatic functions (4, 10, 31); these enzymes facilitate penetration by bacteriophage particles of the outermost layer of the host cell (3).

Because of the limited availability of antisera raised against the K1 antigen, a set of five K1-specific coliphages (14) has been widely used for the identification of isolates carrying the K1 antigen. Plaques of these coliphages are surrounded by large halos in which bacterial cells are decapsulated because of the overproduction of depolymerizing enzyme, which diffuses through the agar layer. This report describes the purification and characterization of an enzyme complex from one of these coliphages.

MATERIALS AND METHODS

Materials. Coliphages A to E were kindly provided by B. Rowe (Control Public Health Laboratory, Colindale, United Kingdom). *E. coli* LP1674 (O7:K1), isolated from a patient with a urinary tract infection (28), was used for bacteriophage propagation.

The *O*-acetylated form of K1 antigen was purified (13) from *E. coli* D698 (25); this strain was kindly supplied by I. Ørskov, International Escherichia and Klebsiella Center, Copenhagen, Denmark. The K92 antigen (12) was prepared from *E. coli* 6181-66 (O73:K92:H34). Capsular polysaccharides from *Neisseria meningitidis* groups B, C, Y, and W-135 were the gifts of H. J. Jennings, Canadian National Research Council, Ottawa, Ontario, Canada, and were used as supplied. The sodium salt of colominic acid, type 1 mucin from bovine submaxillary glands, type II and III bovine brain gangliosides, type IV fetuin from fetal calf serum, and *N*-acetylneuramin-lactose from bovine colostrum were purchased from Sigma Chemical Co., St. Louis, Mo. Disialoganglioside was obtained from Supelco SA, Crans, Switzerland.

Clostridium perfringens neuraminidase was purchased from Sigma; *Vibrio cholerae* neuraminidase was from Behringwerke AG, Marburg, Federal Republic of Germany.

Determination of capsule depolymerase activity. The assay was based on the release of free NeuAc from K1 antigen. For substrate production, *E. coli* LP1674 was grown in the succinic acid medium described by Barker et al. (2), and the K1 polymer was purified essentially according to Gotschlich and co-workers (13); this polymer contained no *O*-acetyl as determined by the method of Hestrin (15), gave a single precipitin arc in immunodiffusion assays with horse antimeningococcus B antiserum, contained no protein detectable by the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Richmond, Calif.), and was free of RNA as judged by no absorption maximum at 260 nm. K1 polymer (67 μl) in 0.05 M sodium acetate (pH 6.5) (4 mg/ml) was incubated at 37°C with 33 μl of enzyme preparation, and release of NeuAc was determined at regular intervals by the thiobarbituric acid assay procedure of Warren (30).

* Corresponding author.

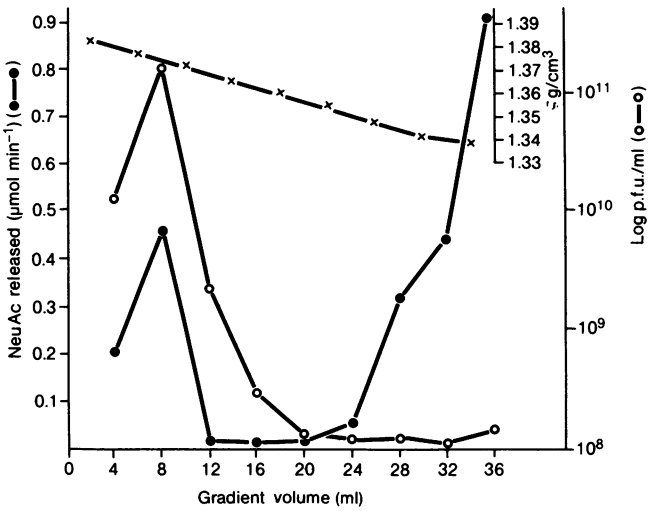

FIG. 1. CsCl density gradient ultracentrifugation of ammonium sulfate precipitate obtained from *E. coli* LP1674: bacteriophage E lysate.

Propagation and purification of bacteriophage particles. Batches (15 liters) of Mueller-Hinton medium were inoculated with *E. coli* LP1674 and incubated at 37°C with vigorous stirring and aeration until the optical density at 578 nm reached 0.8. At this stage bacteriophage was added to the cultures to give a multiplicity of infection of 0.25. After about 20 min of further incubation, the optical density began to fall; 60 min later, when the optical density had begun to rise again after dropping to 0.2 to 0.3, DNase 1 (Sigma) and $MgCl_2$ (to give a final concentration of 0.005 M) were added, the lysate was allowed to cool to 4°C, and bacteria and debris were removed by centrifugation (4°C, 20 min, 10,000 × *g*). Ammonium sulfate was added to the supernatant to a final concentration of 50% [wt/vol]; after centrifugation the precipitate was dissolved in 400 ml of phosphate-buffered saline (pH 7.4), and enough CsCl was added to give a density of 1.37 g/ml. The suspension was centrifuged to equilibrium in a fixed-angle rotor (Kontron TFT 65.38; 4°C, 24 h, 32,000 rpm), and fractions were collected by puncturing the bottom of the centrifuge tube. Infective bacteriophage particles were quantified by standard techniques (1); particles were further purified by two more centrifugations over a CsCl gradient.

Enzyme purification. Fractions from CsCl gradient centrifugations representing free enzyme were pooled and dialyzed against 0.02 M Na_3PO_4 (pH 7.5) for 24 h with frequent buffer changes. The dialysate was concentrated to a volume of 8 ml by membrane ultrafiltration (YM5 membrane; Amicon Corp., Danvers, Mass.; molecular weight, >5,000), mixed with an equal volume of 10 M urea containing 0.1% (vol/vol) mercaptoethanol, and incubated at 37°C for 1 h, and 3-ml aliquots were applied to a column (1.6 by 80 cm) of Sephacryl S300 (Pharmacia, Uppsala, Sweden) equilibrated with 0.02 M Na_3PO_4 (pH 7.5). The column was eluted with this buffer at a flow rate of 20 ml cm^{-2} h^{-1}. Fractions displaying enzyme activity were pooled and applied to a column (1.6 by 40 cm) of DEAE-Sephadex (Pharmacia) previously equilibrated with 0.02 M Na_3PO_4 (pH 7.5). Active fractions were pooled, dialyzed in phosphate buffer to remove KCl, and concentrated by using the YM5 membrane.

Analytical methods. Protein was determined by using the protein assay reagent (Bio-Rad). Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted at pH 8.3 on gels containing 5% acrylamide and on exponential gradient gels containing 7 to 20% acrylamide by the buffer system of Laemmli (19).

Electron microscopy. Samples were applied to Formvar-coated specimen grids and negatively stained with 2% sodium phosphotungstate (pH 6.8). Specimens were examined with a Philips 400 T^R electron microscope at a magnification of ×100,000.

RESULTS

When grown on *E. coli* LP1674, all five bacteriophages produced plaques surrounded by large (>8 mm in diameter) halos, indicating overproduction of free, enzymatically active viral organelles capable of diffusion into the surrounding bacterial lawn. Bacteriophage E consistently gave rise to plaques surrounded by halos of uniform diameter and was therefore selected for further study. Electron microscopy indicated that bacteriophage E belonged to Bradley morphology group C (5).

Neuraminidase purification. With the addition of bacteriophage E to a midlogarithmic-phase culture of *E. coli* LP1674, rapid lysis occurred after 15 to 20 min; lysates were harvested when regrowth started to occur. The material obtained by precipitation of lysis supernatants with ammonium sulfate was subjected to CsCl density gradient ultracentrifugation (Fig. 1). Bacteriophage E equilibrated as a single band at $\rho = 1.373$ g/cm³. Neuraminidase activity could be resolved into two fractions; active enzyme was associated with bacteriophage E particles, although most of the enzyme activity was found in the fractions at the top of the gradient (Fig. 1). These less-dense fractions were pooled, dialyzed, concentrated, and used for gel filtration on Sephacryl S300 (Fig. 2). Enzyme activity was associated with a limited number of fractions; these were pooled and applied to a DEAE-Sephadex column (Fig. 3), and fractions were eluted with a linear 0 to 0.5 M KCl gradient. Neuraminidase activity was seen as a sharp peak eluting at a KCl concentration of 0.2 to 0.25 M. Active fractions were pooled and concentrated; SDS-PAGE of this material

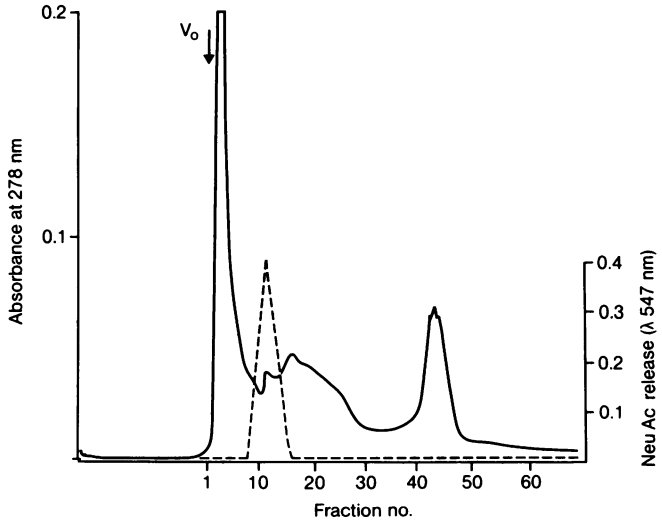

FIG. 2. Gel filtration of enzymatically active fractions on Sephacryl S300. Details are given in the text. Neuraminidase activity measured by release of NeuAc from K1 antigen is represented by the broken line.

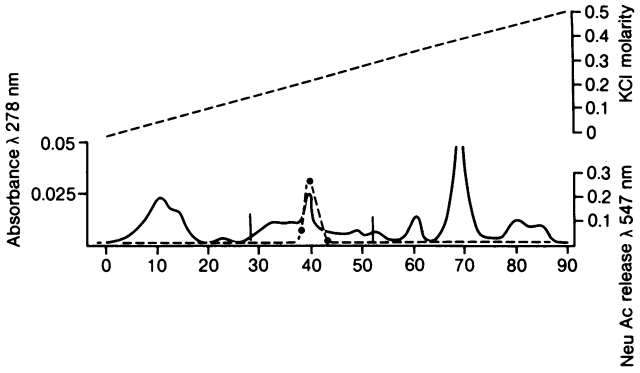

FIG. 3. DEAE-Sephadex chromatography of neuraminidase-containing material. The column was eluted with a linear gradient of 0 to 0.5 M KCl, and 4-ml fractions were collected. Enzyme activity is represented by the lower broken line.

solubilized in SDS-mercaptoethanol-containing sample buffer at 37°C for 30 min revealed a single band with a molecular weight of 208,000 (Fig. 4). This complex could be dissociated at 100°C (2 min) to yield two polypeptides of 74,000 and 38,500 molecular weight that corresponded to two polypeptides associated with complete bacteriophage particles (Fig. 5).

The purification procedure is summarized in Table 1; the enzyme complex was purified 238-fold to homogeneity, as judged by SDS-PAGE, in a high yield. The apparent reduction in unit activity recovered from the CsCl gradient is a reflection of the fact that only fractions containing free enzyme were used for further purification.

Initial characterization of purified enzyme. The effect of pH on neuraminidase activity was investigated by using 0.1 M

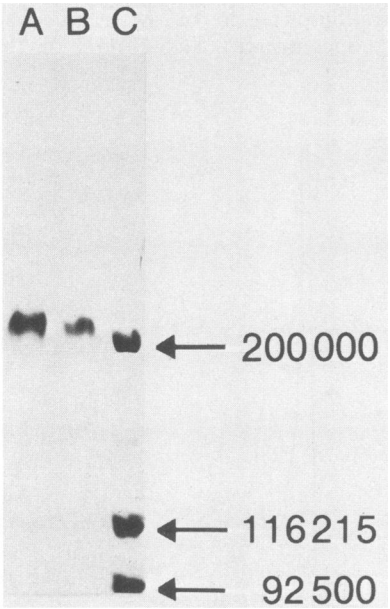

FIG. 4. SDS-PAGE of pooled neuraminidase-containing fractions eluted from a DEAE-Sephadex column. The separating gel contained 5% acrylamide. Samples were pretreated in sample buffer at 37°C for 30 min. Lanes: A, enzyme (25 μg of protein); B, enzyme (15 μg of protein); C, molecular weight standards. Gel was stained with Coomassie blue.

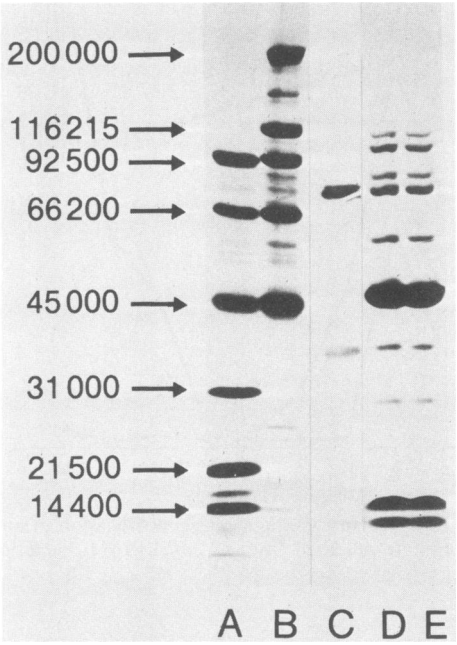

FIG. 5. SDS-PAGE of pooled neuraminidase-containing fractions eluted from a DEAE-Sephadex column. The separating gel consisted of an exponential 7 to 20% acrylamide gradient. Samples were boiled in sample buffer for 2 min. Lanes: A, low-molecular-weight standards; B, high-molecular-weight standards; C, enzyme (20 μg of protein); D and E, bacteriophage E (20 and 35 μg of protein, respectively).

sodium acetate and 0.1 M sodium phosphate buffers. With the acetate buffer, maximal activity was found in the pH range of 5.2 to 5.5. Similar maxima were encountered with the phosphate buffer, although the rate of hydrolysis of K1 substrate in this buffer was significantly lower than that with acetate. All subsequent experiments were therefore performed at pH 5.5. The effect of buffer molarity was examined; with both phosphate and acetate buffers, the maximum rate of K1 hydrolysis by neuraminidase was achieved at 0.05 M. Addition of 9 mM $CaCl_2$ to the acetate buffer sharply reduced enzyme activity. Inhibition was even more pronounced in the presence of 9 mM $CaCl_2$ plus 155 mM NaCl. Rates of hydrolysis lower than those found with the acetate buffer were obtained with a Tris hydrochloride system over a range of pH and molarity. The value for K_m in 0.05 M acetate buffer (pH 5.5) was 7.41×10^{-3} M.

Spectrum of bacteriophage E neuraminidase activity. The specificity of purified bacteriophage E neuraminidase was examined by using the substrates listed in Table 2; enzyme E

TABLE 1. Purification of bacteriophage E neuraminidase

Step	Vol (ml)	Total protein (mg)	Enzyme (U)[a]	Sp act (U/mg)	Purity (fold)
Ammonium sulfate	400	998	4.44	0.004	1
CsCl gradient	70	196	2.33	0.012	3
Sephacryl S300	80	17.6	2.18	0.12	30
DEAE-Sephadex	30	2.1	2.00	0.952	238

[a] One unit was defined as that amount of enzyme that released 1 μmol of NeuAc from LP1674 K1 polymer in 1 min at 37°C (pH 6.5).

TABLE 2. Release of sialic acids from various substrates by neuraminidases

Substrate[a]	Type of sialic acid linkage	*O* substitution	Reference	Enzyme (λ 547 nm)[b]		
				E	*C. perfringens*	*V. cholerae*
E. coli K1 antigen	→8)-α-Neu 5Ac-(2→	± 7-OAc[c] ± 9-OAc	25	0.20	0.04	0
E. coli K1 antigen	→8)-α-Neu 5Ac-(2→	None	23, 25	0.25	0.26	0.38
Meningococcus B antigen	→8)-α-Neu 5Ac-(2→	None	17	0.24	0.28	0.52
Meningococcus C antigen	→9)-α-Neu 5Ac-(2→	± 7-OAc ± 8-OAc	17	0	0.15	0.16
Meningococcus Y antigen	→4)-α-D-Glu-α(2→6)Neu5Ac-(1→	OAc, position unknown	17	0	0	0
Meningococcus W135 antigen	→4)-α-D-Gal-α(2→6)Neu5Ac-(1→	None	17	0	0	0
E. coli K92 antigen	→9)-α-Neu5Ac-α(2→9)Neu5Ac-α(2→8) Neu5Ac-α(2→8) Neu5Ac- (2→	None	12, 20	0.16	0.32	0.31
N-acetylneuraminyl-lactose (bovine colostrum)	Neu5Ac-α(2→3)-β-D-Gal(1→4)-D-Glu Neu5Ac-α(2→6)-β-D-Gal(1→4)-D-Glu	None		0	1.2	1.3
Bovine brain ganglioside II	Galβ(1→3)-GalNAcβ(1→4)-Galβ(1→4)-GlcCer | α(2→3) | α(2→3) Neu5Ac Neu5Gc	None	11	0	0.30	0.32
Bovine brain ganglioside III	Galβ (1→3)-GalNAcβ(1→4)-Galβ(1→4)-GlcCer | (2→3) | (2→3) Neu5Gc Neu5Ac	None	11	0	0.30	0.41
Bovine submaxillary mucin I	Neu5Ac / Neu5Gc } -α(2→6)-GalNac	± 7-OAc ± 8-OAc ± 9-OAc	6	0	0.16	0.10
Fetal calf serum fetuin	Neu5Ac-α(2→3)-Galβ(1→3)-GalNAcα- | α(2→6) Neu5Ac	None	24	0	0.20	0.19
Disialoganglioside				0	0.39	0

[a] All substrates were dissolved in sodium acetate buffer (pH 5.5) to a concentration of 4 mg/ml.

[b] Substrate (75 μl) was incubated for 75min at 37°C with 25 μl of enzyme, and sialic acid release was determined by using the thiobarbituric acid reaction. Values represent formation of λ 547-nm chromogen in comparison with substrate alone. Incubation for longer periods (up to 5 h) did not result in the release of sialic acids from those substrates not hydrolyzed after 75 min.

[c] OAc, *O*-acetyl.

was compared with commercially available neuraminidase preparations from *C. perfringens* and *V. cholerae*. Compared with the bacterial enzymes, neuraminidase E exhibited a narrow substrate specificity, hydrolyzing only *E. coli* K1 antigen, the identical non-*O*-acetylated meningococcus B antigen, and the *E. coli* K92 antigen. These three bacterial homopolymers all contain sialic acid residues linked α2→8. In contrast to the bacterial enzymes, the activity of neuraminidase E was not inhibited by *O*-acetylation of the K1 polymer. Interestingly, the bacteriophage enzyme did not release sialic acid from *N*-acetylneuramin-lactose, a widely used substrate for the detection of neuraminidase activity (7).

DISCUSSION

The five bacteriophages described by Gross and co-workers infected virtually all *E. coli* strains carrying the K1 capsular polysaccharide but were unable to produce plaques on any of the standard *E. coli* test strains carrying K antigens 2 to 99 (14). Single-step isogenic K1-negative mutants have been derived from *E. coli* K1 cultures by selecting for resistance to these bacteriophages (9), indicating that the bacteriophage specifically recognizes the K1 polymer.

In the present study, an enzyme complex from bacteriophage E-induced bacterial lysates was purified 238-fold to homogeneity and found to have a molecular weight of 208,000; the dissociated complex yielded two polypeptides of 74,000 and 38,500 molecular weight. The higher-molecular-weight band appeared to stain with approximately twice the intensity of the smaller band, and it is therefore likely that each complex consists of two larger and one smaller polypeptides. The size of the complex is similar to that reported for neuraminidases from various myxoviruses; for example, the molecular weight of the neuraminidases isolated from influenza viruses is in the region of 200,000 (16). In contrast, bacterial neuraminidases appear to be smaller, with molecular weights in the 65,000 to 90,000 range (8). The pH optimum for purified E neuraminidase of 5.5 falls within a range typical of that of the majority of bacterial, viral, and mammalian neuraminidases (7). More unusually, E neuraminidase hydrolyzes the *O*-acetylated and non-*O*-acetylated forms of the K1 polymer equally well; the hydrolytic activity of the majority of neuraminidases characterized to date is strongly reduced by the presence of *O*-acetyl substitutions on sialic acid residues (7). In addition, enzyme E activity was reduced in the presence of 9 mM $CaCl_2$; many viral neuraminidases require Ca^{2+} as a cofactor, and the cofactor function can be inhibited by EDTA (7). The substrate specificity of E neuraminidase was investigated by using 13 different sialic acid-containing substrates (Table 2). Both forms of the K1 antigen and the meningococcus B polysaccharide, identical to the non-*O*-acetylated K1 polymer, were rapidly hydrolyzed. Sialic acid was also released from the K92 polysaccharide, a NeuAc homopolymer containing alternating α2→8 and α2→9 linkages. No hydrolysis of meningococcus C polysaccharide, a α2→9 linked homopolymer, could be detected, and no release of sialic acid was observed from substrates containing α2→3- or α2→6-linked Neu5Ac residues. The data appear, therefore, to suggest an absolute specificity for α2→8 linkages and possibly also a requirement for a minimum disialyl region within a potential substrate. In contrast, the two bacterial

neuraminidases used in this study showed a very broad substrate specificity and released α2→3-, α2→6-, α2→8-, and α2→9-linked sialic acid residues from a variety of substrates (Table 2; 7).

Recently, Kwiatkowski and co-workers (18) identified and investigated endo-*N*-acetylneuraminidase activity associated with the bacteriophage ϕ1.2, which is also capable of infecting K1-carrying *E. coli* strains. This Bradley morphology group C bacteriophage also depolymerizes *O*-acetylated and non-*O*-acetylated K1 polymer as well as the K92 antigen by an endo mechanism, even though it cannot complete the lytic cycle on *E. coli* strains producing this latter antigen. The enzyme carried by ϕ1.2 appears, therefore, to be very similar to that described in this study.

LITERATURE CITED

1. **Adams, M. H.** 1950. Methods of study of bacterial viruses. p. 1–73. *In* J. H. Comroe (ed.), Methods in medical research, vol. 2. The Year Book Publishers, Inc., Chicago.
2. **Barker, S. A., R. G. Jones, and P. J. Somers.** 1967. Improvements in the production and isolation of colominic acid. Carbohydr. Res. **3**:369–376.
3. **Bayer, M. E., H. Thurow, and M. H. Bayer.** 1979. Penetration of the polysaccharide capsule of *Escherichia coli* (Bi161/42) by bacteriophage K29. Virology **94**:95–118.
4. **Bessler, W., F. Fehmel, E. Freund-Mölbert, H. Knüfermann, and S. Stirm.** 1975. *Escherichia coli* capsule bacteriophages. IV. Free capsule depolymerase 29. J. Virol. **15**:976–984.
5. **Bradley, D. E.** 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriol. Rev. **31**:230–314.
6. **Buscher, H.-P., J. Casals-Stengel, and R. Schauer.** 1974. New sialic acids. Identification of *N*-glycoloyl-*O*-acetylneuraminic acids and *N*-acetyl-*O*-glycoloylneuraminic acids by improved methods for detection of *N*-acyl and *O*-acyl groups and by gas-liquid chromatography. Eur. J. Biochem. **50**:71–82.
7. **Corfield, A. P., J. C. Michaelski, and R. Schauer.** 1981. The substrate specificity of sialidases from microorganisms and mammals, p. 3–70. *In* G. Tettamanti, P. Duraud, and S. DiDonato (ed.), Sialidases and sialidoses: perspectives in inherited metabolic diseases, vol. 4. Edi Ermes, Milan.
8. **Drzeniek, R.** 1972. Viral and bacterial neuraminidases. Curr. Top. Microbiol. Immunol. **59**:35–74.
9. **Gemski, P., A. S. Cross, and J. C. Sadoff.** 1980. K1 antigen-associated resistance to the bactericidal activity of serum. FEMS Microbiol. Lett. **9**:193–197.
10. **Geyer, H., K. Himmelspach, B. Kwiatkowski, S. Schlecht, and S. Stirm.** 1983. Degradation of bacterial surface carbohydrates by virus-associated enzymes. Pure Appl. Chem. **55**:637–653.
11. **Ghidoni, R., S. Sonnino, G. Tettamanti, H. Wiegandt, and V. Zambotti.** 1976. On the structure of two new gangliosides from beef brain. J. Neurochem. **27**:511–515.
12. **Glode, M. P., J. B. Robbins, T.-Y. Lui, E. C. Gotschlich, I. Ørskov, and F. Ørskov.** 1977. Cross-antigenicity and immunogenicity between capsular polysaccharides of group C *Neisseria meningitidis* and of *Escherichia coli* K92. J. Infect. Dis. **135**:94–102.
13. **Gotschlich, E. C., T.-Y. Lui, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. III. Preparation and immunochemical properties of the group A, group B, and group C meningococcal polysaccharides. J. Exp. Med. **129**:1349–1365.
14. **Gross, R. J., T. Cheasty, and B. Rowe.** 1977. Isolation of bacteriophages specific for the K1 polysaccharide antigen of *Escherichia coli*. J. Clin. Microbiol. **6**:548–550.
15. **Hestrin, S.** 1949. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. J. Biol. Chem. **180**:249–261.
16. **Jackson, D. C., G. J. Howlett, A. Nestorowicz, and R. G. Webster.** 1983. The equilibrium constant for the interaction between monoclonal Fab fragment and an influenza virus neuraminidase. J. Immunol. **130**:1313–1316.
17. **Jennings, H. J., A. K. Bhattacharjee, D. R. Bundle, C. P. Kenny, A. Martin, and I. C. P. Smith.** 1977. Structures of the capsular polysaccharides of *Neisseria meningitidis* as determined by ^{13}C-nuclear magnetic resonance spectroscopy. J. Infect. Dis. **136**:S78–S83.
18. **Kwiatkowski, B., B. Boschek, H. Thiele, and S. Stirm.** 1982. Endo-*N*-acetylneuraminidase associated with bacteriophage particles. J. Virol. **43**:697–704.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Lui, T-Y., E. C. Gotschlich, W. Egan, and J. B. Robbins.** 1977. Sialic acid-containing polysaccharides of *Neisseria meningitidis* and *Escherichia coli* strain Bos-12: structure and immunology. J. Infect. Dis. **136**:S71–S77.
21. **McCracken, G. H., and L. D. Sarff.** 1974. Current status and therapy of neonatal *E. coli* meningitis. Hosp. Pract. **9**:57–64.
22. **McCracken, G. H., L. D. Sarff, M. P. Glode, S. G. Mize, M. S. Schiffer, J. B. Robbins, E. C. Gotschlich, I. Ørskov, and F. Ørskov.** 1974. Relation between *Escherichia coli* K1 capsular polysaccharide antigen and clinical outcome in neonatal meningitis. Lancet **ii**:246–250.
23. **McGuire, E. J., and S. B. Binkley.** 1964. The structure and chemistry of colominic acid. Biochemistry **3**:247–251.
24. **Montreuil, J.** 1980. Primary structure of glycoprotein glycans. Basis for the molecular biology of glycoproteins. Adv. Carbohydr. Chem. Biochem. **37**:157–223.
25. **Ørskov, F., I. Ørskov, A. Sutton, R. Schneerson, W. Lin, W. Egan, G. E. Hof, and J. B. Robbins.** 1979. Form variation in *Escherichia coli* K1: determined by *O*-acetylation of the capsular polysaccharide. J. Exp. Med. **149**:669–685.
26. **Ørskov, I., F. Ørskov, B. Jann, and K. Jann.** 1977. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. Bacteriol. Rev. **41**:667–710.
27. **Robbins, J. B., G. H. McCracken, E. C. Gotschlich, F. Ørskov, I. Ørskov, and L. A. Hanson.** 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. **290**:1216–1220.
28. **Taylor, P. W.** 1976. Immunochemical investigations on lipopolysaccharides and acidic polysaccharides from serum-sensitive and serum-resistant strains of *Escherichia coli* isolated from urinary-tract infections. J. Med. Microbiol. **9**:405–421.
29. **Troy, F. A.** 1979. The chemistry and biosynthesis of selected bacterial capsular polymers. Annu. Rev. Microbiol. **33**:519–560.
30. **Warren, L.** 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. **234**:1971–1975.
31. **Yurewicz, E. C., M. A. Ghalambor, D. H. Duckworth, and E. C. Heath.** 1971. Catalytic and molecular properties of a phage-induced capsular polysaccharide depolymerase. J. Biol. Chem. **246**:5607–5616.