Glycoconjugate Journal 18, 751–758, 2001

Mutant bacteriophage with non-catalytic endosialidase binds to both bacterial and eukaryotic polysialic acid and can be used as probe for its detection

Juha Aalto[1], Sinikka Pelkonen[2], Hannu Kalimo[3] and Jukka Finne[1]*

[1] *Department of Medical Biochemistry and Molecular Biology, University of Turku, Finland,* [2] *Kuopio Department, National Veterinary and Food Research Institute, Finland,* [3] *Department of Pathology, University of Turku, Finland*

There is a molecular mimicry between the polysialic acid polysaccharide of bacterial pathogens causing sepsis and meningitis, and the carbohydrate units of the neural cell adhesion molecule NCAM. We investigated whether bacteriophage mutants with catalytically disabled endosialidase, which bind but do not cleave polysialic acid, could recognise and bind to bacterial and eukaryotic polysialic acid. In nitrocellulose dot blot assay the mutant bacteriophages, but not the wild-type phages, remained specifically bound to polysialic acid–containing bacteria including *Escherichia coli* K1 and K92, group B meningococci, *Mannheimia (Pasteurella) haemolytica* A2, and *Moraxella nonliquefaciens*. A minimum binding requirement was determined to be 10 sialyl residues in the polysialic acid chain. In Western blots the mutant phages specifically bound to the embryonic polysialylated form of NCAM, but not to the adult less sialylated form of the molecule. The mutant phages together with secondary anti-phage antibodies were subsequently successfully used in fluorescence microscopy of cultured cells and light microscopy of paraffin-embedded tissue sections as a probe for the eukaryotic polysialic acid. Thus, mutant bacteriophages of meningitis causing bacteria bind to and detect the molecularly mimicked polysialic acid of the neural cell adhesion molecule in host tissues.

Keywords: **bacteriophage, endosialidase, NCAM, polysialic acid**

Introduction

Polysialic acid occurs as a capsular polysaccharide of the bacterial pathogens *Neisseria meningitidis* group B, *Escherichia coli* K1, *Mannheimia (Pasteurella) haemolytica* A2, and *Moraxella nonliquefaciens* [1–3], and as a structural unit of carbohydrate side chains of the neural cell adhesion molecule NCAM [4,5]. In NCAM, polysialic acid is developmentally regulated and appears to modulate the cell adhesion activity of the molecule [6,7]. It has been ascribed important roles in development, differentiation, plasticity, regeneration and malignancy [8,9]. In bacteria polysialic acid participates in the pathogenesis of sepsis and meningitis by conferring serum resistance and escape from immune defence due to poor immunogenicity of the host-mimicking capsule [10]. The role and mechanism of polysialic acid in the determination of the tissue tropism of the infection remains, however, to be shown. Initially, the poor immunogenicity of polysialic acid was an obstacle in the production of reagents for its study [1,11], but antibodies are now available [12–14].

In *E. coli*, polysaccharide K1-specific bacteriophages specifically recognise and bind to the polysialic acid, and subsequently degrade it [15]. Both activities are thought to reside in the same molecule, i.e. in the endosialidase enzyme [16]. We have previously developed mutant bacteriophages which have lost the catalytic activity to degrade polysialic acid [17]. In the present investigation, we have investigated whether the mutant bacteriophages recognise and bind bacterial and eukaryotic polysialic acid. The results indicate that the bacteriophages with the catalytically disabled enzyme specifically bind to both the bacterial polysialic acid and the polysialic acid of the neural cell adhesion molecule, and can be used as a specific probe for their detection.

*To whom correspondence should be addressed: J. Finne, Department of Medical Biochemistry, University of Turku, Kiinamyllynkatu 10, FIN-20520 Turku, Finland. Tel.: +358 2 333 7240; Fax: +358 2 333 7229; E-mail: finne@utu.fi

Materials and methods

Bacteriophages and bacterial strains

The bacteriophages PK1A and PK1E were provided by B. Rowen, Central Public Health Laboratory, Colindale, London, England [15]. The mutant bacteriophages PK1A2, PK1A5, PK1A8 and PK1E3 were isolated on the basis of their capability of infecting *E. coli* mutants resistant to wild-type phages due to a reduced amount of surface polysialic acid [17]. *E. coli* strains IH3088 (K1) and IH3083 (K2) were provided by P. H. Mäkelä, National Public Health Institute, Helsinki, Finland [18]. The mutant K1-defective *E. coli* strain EH1008 has been described elsewhere [17,19]. *E. coli* strain Bi8337-41 (K5) has been described before [20]. *E. coli* strain Bos 12 (K92) was obtained from S. Stirm, Biochemisches Institut, Giessen, Germany. *E. coli* K-12 (K12), *Neisseria meningitidis* serotypes A, B, C and Y, and *Moraxella nonliquefaciens* EF 10057 were obtained from E. Eerola, Department of Medical Microbiology, University of Turku, Finland. *M. nonliquefaciens* strain KK 987/84 was supplied by H. Järvinen, National Institute of Health, Turku, Finland. *Mannheimia (Pasteurella) haemolytica* KU 201/83 (A2), KU 363/84 and KU 606/83 were our own isolates.

Detection of endosialidase activity

An oligomer containing 12 sialyl residues was purified from colominic acid and labelled in its reducing end by tritium [17,21]. After incubation of the substrate with the wild type and mutant bacteriophages the digestion products were analysed by polyacrylamide gel electrophoresis and fluorography as described before [22,23].

Production of bacteriophage antisera

New Zealand White rabbits were immunised subcutaneously with 250 μg (as protein) of PK1A2 bacteriophage in 0.5 ml of PBS emulsified with 0.5 ml of Freund's complete adjuvant. The rabbits were boosted every 4 weeks with the same antigen using Freund's incomplete adjuvant. The sera were stored frozen in aliquots. The production of bacteriophage antibodies was followed by dot blot analysis. The bacteriophage suspensions (5 μg protein per ml) were spotted (1 μl) on nitrocellulose membranes which were blocked by incubation with 3% bovine serum albumin and 0.5% Tween 20 in PBS for 1 h at room temperature [24]. The membranes were incubated with the sera diluted in the blocking solution for 15 min at room temperature, and the bound antibodies were detected as described below. The phage antibodies crossreacted readily with both the PK1A and the PK1E phages and their mutants.

Bacteriophage binding to bacteria on nitrocellulose membranes

Bacterial suspensions were adjusted to an A_{540} of 0.2 and 1:2 dilution series of the suspensions were spotted (1 μl) onto nitrocellulose membranes. After drying the membranes were treated by incubation with 3% bovine serum albumin and 0.5% Tween 20 in PBS for 1 h at room temperature. The membranes were incubated with the mutant PK1A2 bacteriophages (50 μg/ml) in blocking solution for 1 h at room temperature, washed three times with 0.5% Tween 20 in PBS for 10 min, and incubated with rabbit anti-bacteriophage antibodies (1:1000) in blocking solution for 20 min. The membranes were washed three times as above, and incubated with 1:1000 diluted peroxidase-conjugated swine anti-rabbit immunoglobulins (DAKO, Glostrup, Denmark) in blocking solution for 1 h. After washing the colour was developed by incubation with 0.5 mg/ml 3,3′-diaminobenzidine, 0.015% hydrogen peroxide in PBS.

Sialyloligomer binding to phages

Colominic acid and sialyl oligosaccharides of defined length were prepared and labelled in their reducing ends by tritium as described before [17,21]. Freshly isolated PK1A2 phages (12.5 μg in 50 μl of PBS) were coated onto wells of microtiter plates (Rigid Pate 1450–410, Wallac Turku, Finland) by incubation overnight at 4°C. After washing the plates three times with PBS, 25 pmol of the sialyloligomers were applied in 100 μl of PBS and the plates incubated for 1 h at 4°C. In control samples 5 mg/ml of colominic acid was included with the sialyl oligomers. The plates were washed three times with PBS, 100 μl of Hi-Load scintillation liquid (Wallac) was added, and the plates were counted in a microplate liquid scintillation counter (Microbeta 1450, Wallac).

Polyacrylamide gel electrophoresis and phage blotting

Brain, liver, kidney and spleen samples of adult (37 days) and developing (8 days) Wistar male rats were homogenized with a ground-glass homogenizer on ice in two volumes of PBS containing 0.228 TIU/ml aprotinin, 5 mM iodoacetamide and 5 μg/ml α_2-macroglobulin. Equal amounts of tissue homogenates (corresponding to 0.35 mg wet weight of tissues) were subjected to SDS polyacrylamide gel electrophoresis as described before [25,26], and blotted to nitrocellulose membranes [27]. The membranes were blocked and treated for polysialic acid detection as described above, using a 1:10 000 dilution of bacteriophage antiserum and an incubation time of 30 min.

Polysialic acid detection in fluorescence microscopy

Baby-hamster kidney fibroblasts (BHK-21 C-13) were grown on Permanox slides (Nunc) at 37°C in 5% CO_2 in Dulbecco's modified Eagle medium (Gibco) supplemented with 10% fetal calf serum (Gibco, E.C. approved), 100 IU/ml penicillin and 50 μg/ml streptomycin. The cells were fixed for 15 min with 2% paraformaldehyde in PBS, washed three times with 0.1% BSA in PBS, and incubated with 10 μg/ml PK1A2 phages in 3% BSA in PBS for 30 min at room temperature. After washing three

times the slides were incubated with rabbit anti-bacteriophage antibodies (1:500) in 3% BSA in PBS for 30 min at room temperature, washed three times, and incubated with swine rhodamine conjugated anti-rabbit immunoglobulins (1:50, DAKO) in 3% BSA in PBS for 30 min. The slides were briefly rinsed with distilled water and mounted with Glysergel (DAKO) containing 2.5 mg/ml 1,4-diazabicyclo[2.2.2]octane (Sigma). The slides were examined and photographed in a Zeiss IM 35 microscope with epifluorescence set.

Polysialic acid detection in light microscopy

Paraffin sections (4 μm) of tissue samples fixed with 4% phosphate-buffered paraformaldehyde were mounted on polylysine-coated glass slides, kept for 10 min at 56°C, and rehydrated by incubation in xylene, descending alcohol series, and two washes with 0.05 mM Tris-buffered saline, pH 7.6. Endogenous peroxidase activity was blocked by incubation after dehydration with ascending alcohol series in 0.3% hydrogen peroxide in methanol for 30 min at room temperature. After treatment with 3% swine serum (Vientiteurastamo Oy, Turku, Finland) in Tris-buffered saline for 30 min at room temperature the slides were incubated with 4 μg/ml PK1A2 phages in 3% BSA in PBS for 30 min at room temperature. After washing three times for 5 min with Tris-buffered saline the slides were incubated with rabbit anti-bacteriophage antibodies (1:10000) in 3% BSA in Tris-buffered saline for 30 min at room temperature, washed three times, incubated with swine anti-rabbit immunoglobulins (1:100, DAKO) in 3% BSA in Tris-buffered saline for 30 min at room temperature, washed, and incubated with a complex of horseradish peroxidase and rabbit antibody to horseradish peroxidase [28] (1:100, DAKO) in 3% BSA in Tris-buffered saline for 30 min at room temperature. After washing the colour was developed by incubation with 0.015% hydrogen peroxide as the substrate and 0.5 mg/ml 3,3′-diaminobenzidine as the chromogen in Tris-buffered saline. The slides were washed with distilled water, 70% ethanol, and distilled water, stained with 0.1% hematoxylin, washed with distilled water, dehydrated in an ascending alcohol series, washed twice with xylene, and mounted with Permount (Fisher Scientific). In control experiments polysialic acid was digested in the sections before the addition of the mutant phages by incubation with the wild-type PK1A phages (22.5 μg/ml) in PBS for 1 h at 37°C followed by three washes with Tris-buffered saline. The slides were examined and photographed in a Leitz Dialux light microscope.

Results

Binding of bacteriophages to bacteria on nitrocellulose membranes

Incubation of polysialic acid with bacteriophage containing endosialidase activity resulted in its complete cleavage into small-size fragments, whereas no apparent cleavage was observed with mutant bacteriophage, even after incubation for 2 h at 37°C (Figure 1). Gel electrophoresis of the total phage proteins did not show any apparent differences in the band patterns between the wild-type and corresponding mutant phages. The mobility of the endosialidase band was unchanged in the mutant phages with preserved polysialic acid binding activity, which suggests that the loss of enzymatic activity is due to minor changes in the enzyme molecule (Figure 2).

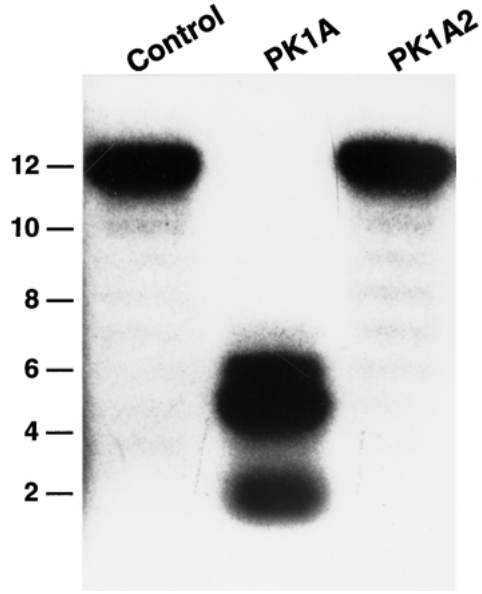

Figure 1. Endosialidase activity of wild-type and mutant bacteriophages. A ^{3}H-labelled sialyl oligomer consisting of 12 sialyl residues purified from colominic acid was incubated with wild-type PK1A and mutant PK1A2 phage particles for 2 h at 37°C and subjected to gel electrophoresis and fluorography. The positions of oligomers with the indicated numbers of residues are shown on the left.

A binding assay was developed in order to investigate whether the mutant bacteriophages bound to bacteria spotted onto nitrocellulose membranes. The detection reaction consisted of incubation of the nitrocellulose membrane-bound bacteria with bacteriophages, followed by anti-bacteriophage antibody and peroxidase-conjugated secondary antibody. Bacteriophage mutants defective in endosialidase activity revealed clear reactions with the polysialic acid containing *E. coli* K1 bacteria, whereas no reactions were observed using the wild-type parent phages with active endosialidase (Figure 3).

Specificity of the phage binding

Bacteria containing different types and amounts of polysialic acid (Table 1) were used to investigate the specificity of the phage-binding. *E. coli* K1 containing a polysialic acid capsule with α2–8 linkages as well as *E. coli* K92 containing α2–8 and α2–9 linkages both reacted with the mutant phages (Figure 4), in accordance with the substrate specificity of active endosialidase enzyme [29]. A capsular mutant of *E. coli* K1 with a low

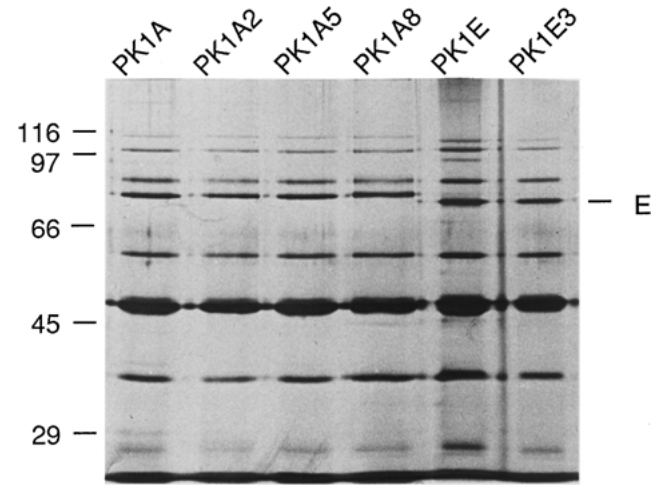

Figure 2. Polyacrylamide gel electrophoresis of phage proteins. The purified bacteriophages (1 μg protein) were subjected to electrophoresis in a 10% polyacrylamide gel in the presence of SDS, and the proteins were visualized by silver stain. The mobilities of the molecular weight standards are indicated on the left, end the band corresponding to endosialidase on the right (E).

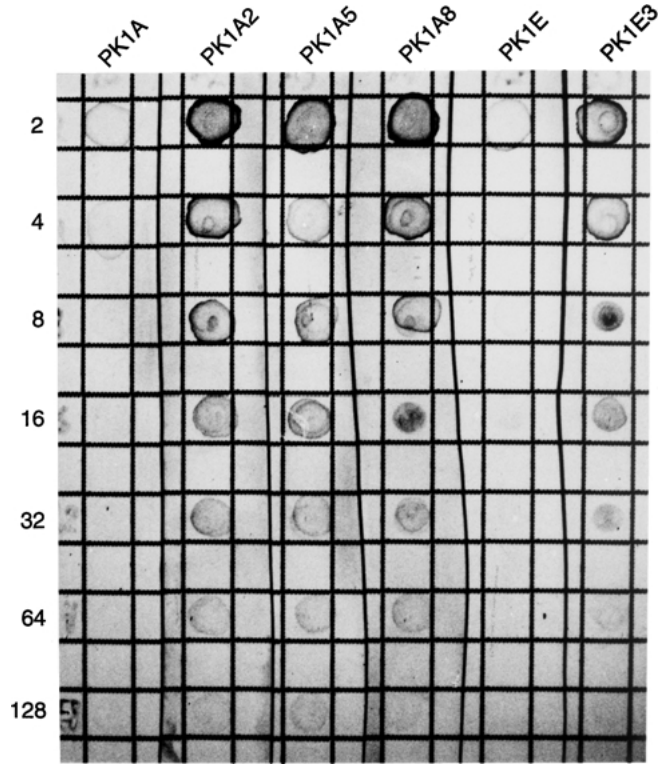

Figure 3. Binding of bacteriophages containing wild-type active or mutant inactive endosialidase to bacteria containing polysialic acid. Suspensions of the polysialic acid containing *E. coli* IH3088 were spotted (1 μl) onto the nitrocellulose membrane in dilutions 1:2 to 1:128. Each strip was incubated with the wild-type or mutant bacteriophages indicated, followed by detection with anti-bacteriophage antibody and peroxidase-conjugated secondary antibody.

Table 1. Type and amount of polysialic acid in bacterial strains studied

Bacterial strain	*Capsule type/group*	*Type of polysialic acid*
E. coli IH3088	K1	α2–8
E. coli 1008 (mutant)	K1	(α2–8)[1]
E. coli IH3083	K2	–[2]
E. coli Bi8337-41	K5	–
E. coli K-12	K12	–
E. coli Bos 12	K92	α2–8, α2–9
N. meningitidis	A	–
N. meningitidis	B	α2–8
N. meningitidis	C	α2–9
N. meningitidis	Y	–
M. haemolytica KU 201/83	A2	α2–8
M. haemolytica KU 363/84		–
M. haemolytica KU 606/83		–
M. nonliquefaciens KK 987/84		α2–8
M. nonliquefaciens EF 10057		–

[1]Low amount (1%) of cell surface polysialic acid, [2] –, no polysialic acid.

amount of polysialic acid gave, as expected, a weaker reaction. With meningococci, the mutant bacteriophage reacted strongly with type B bacteria containing a polysialic acid capsule with α2–8 linkages, whereas minimal reaction was observed with type C bacteria containing a polysialic acid capsule with α2–9 linkages and no reaction with the other unrelated capsular types. With *Mannheimia haemolytica* A2 and *Moraxella nonliquefaciens* the mutant phages reacted strongly with the bacteria containing an α2–8 polysialic acid capsule, and displayed little or no activity with the non-polysialylated strains.

Length-dependence of polysialic acid binding by phages

In order to determine the length of the polysialic acid required for binding to the phage, ^{3}H-labelled oligomers of α2–8 linked polysialic acid of defined length were incubated with the mutated bacteriophage immobilized to microtiter wells. A minimum oligomer size of 10 sialic acid residues (consisting of nine intact residues and one ^{3}H-reduced residue) were found to be required for the interaction (Figure 5).

Phage binding after polyacrylamide gel electrophoresis

One high-molecular-weight band of polysialic acid reactivity was detected in whole tissue extracts of rat brain using the mutant bacteriophage as probe in Western blots (Figure 6). The band corresponds to the embryonic form of the neural cell adhesion molecule N-CAM detected before using anti-polysialic acid antibodies [26]. This molecule is the major polysialic acid containing molecule in rat brain [5,30]. In accordance with previous results, the polysialic acid reactivity was higher in developing than adult brain, while liver, spleen

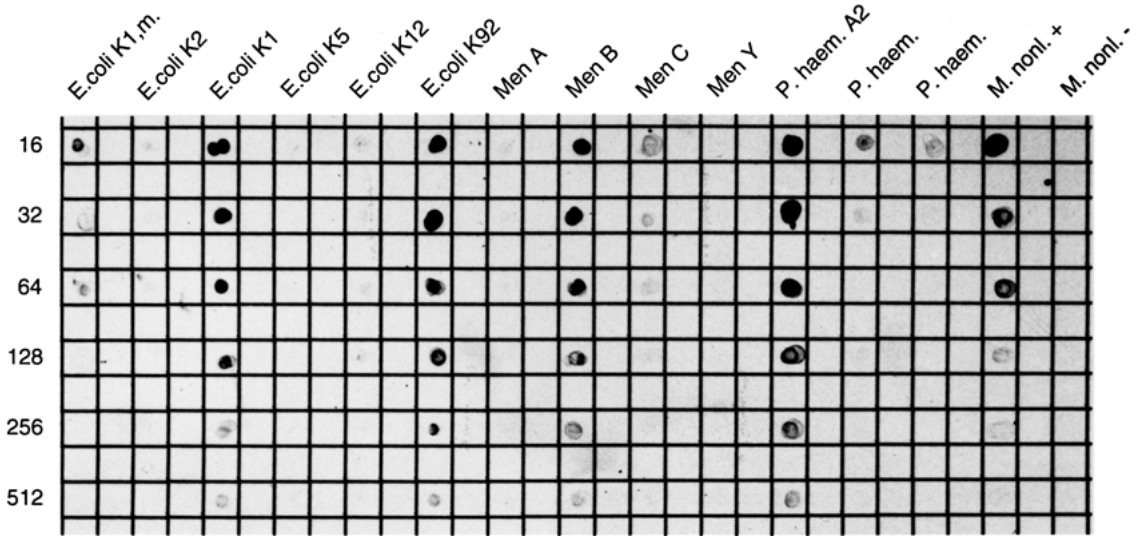

Figure 4. Specificity of phage binding to polysialic acid containing bacteria. Bacterial suspensions of the strains indicated (see Table 1) were adjusted to similar concentrations and spotted (1 μl) in dilutions 1:16 to 1:512 onto a nitrocellulose sheet. Detection was carried out by incubation in the presence of mutant PK1A bacteriophage, followed by anti-bacteriophage antibody and peroxidase-conjugated secondary antibody (*E. coli* K1, m., mutant with low amount of polysialic acid; M. nonl. + and −, *M. nonliquefaciens* strains with or without polysialic acid, respectively).

and kidney had little polysialic acid reactivity. Similar to results obtained using polysialic acid antibodies [26], the much lower amounts polysialic acid present in developing kidney were not revealed in a normal blot analysis. No binding was observed for the less-sialylated adult forms of the NCAM molecule which migrate below the embryonic polysialylated NCAM band [26].

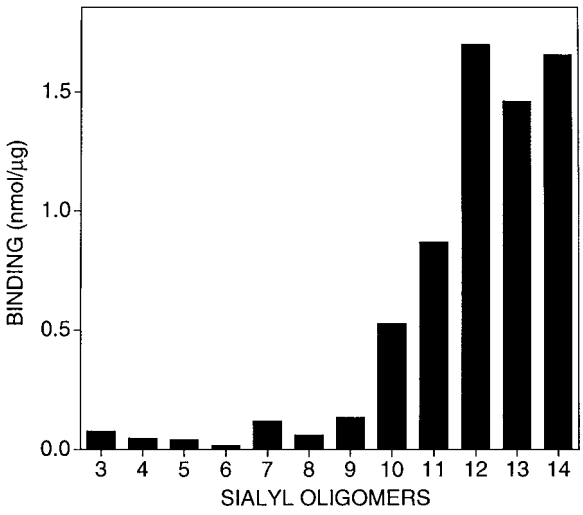

Figure 5. Length-dependence of polysialic acid binding to bacteriophage. Radioactively labelled (^{3}H-reduced) polysialic acid oligomers of the length indicated were incubated overnight 4°C in microtiter wells coated with purified mutant PK1A2 phages. After washing, the bound oligosaccharides were detected by liquid scintillation counting in a microplate liquid scintillation counter.

Fluorescence microscopy of cultured cells

In fluorescence microscopy of BHK-21 cells a bright surface fluorescence was observed using the mutant phage as probe,

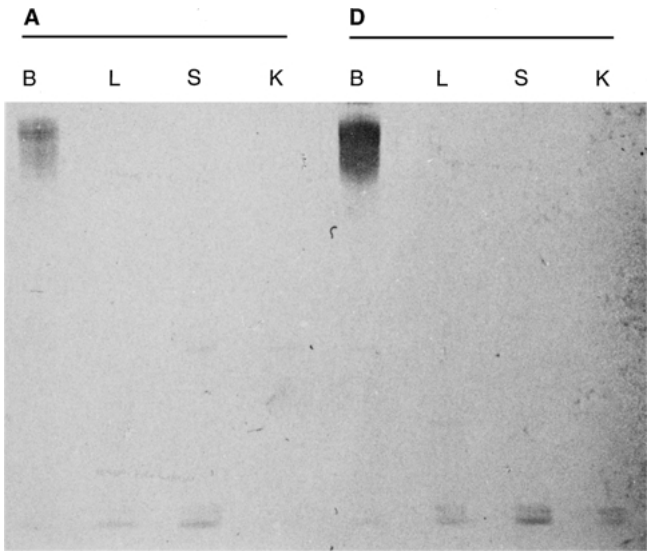

Figure 6. Detection of polysialic acid from Western blots using phage detection. Equal amounts of tissue homogenates of adult (A) or developing (D) rat brain (B), liver (L), spleen (S) and kidney (K) were subjected to SDS polyacrylamide gel electrophoresis. Polysialic acid was detected by incubation of the blots with mutant PK1A2 phage, followed by anti-bacteriophage antibody and peroxidase-conjugated secondary antibody. In control experiments (not shown), the staining was abolished by including polysialic acid (1 mg/ml colominic acid) in the incubation mixture with the PK1A2 phage.

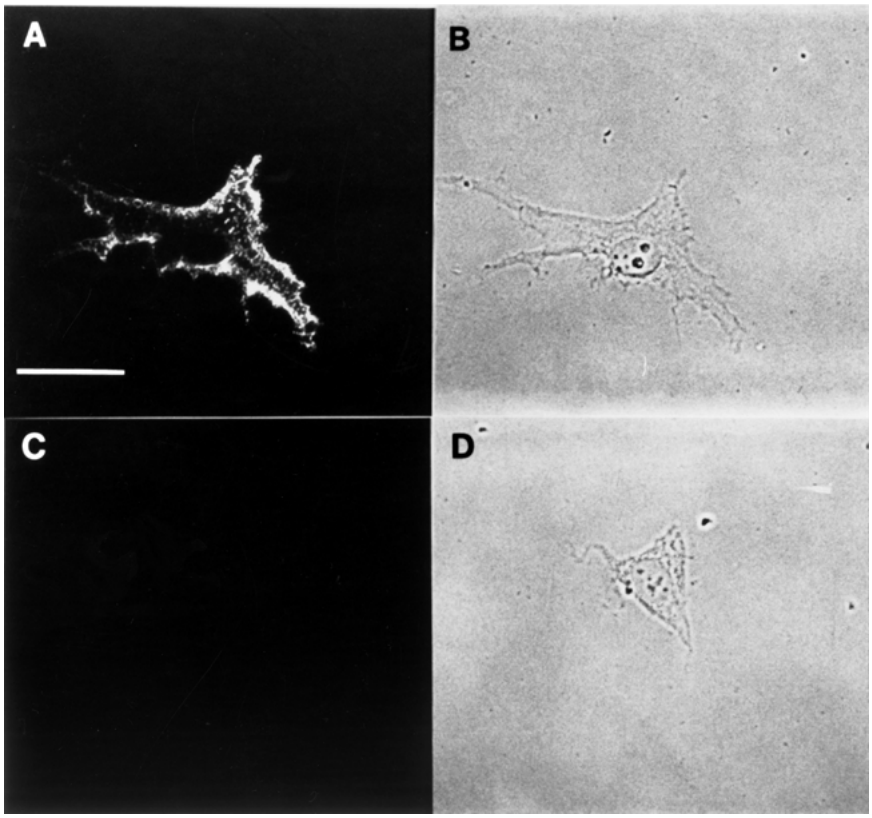

Figure 7. Detection of polysialic acid in fluorescence microscopy of cultured cells using phage detection. BHK-21 cells grown on slides were fixed with paraformaldehyde in PBS and stained by incubation with mutant PK1A2 phage, followed by anti-bacteriophage antibody and rhodamine conjugated anti-rabbit immunoglobulins (A and B), or including polysialic acid (5 mg/ml colominic acid) as inhibitor during phage incubation (C and D). Detection by fluorescence microscopy (A and C) or phase contrast microscopy (B and D) (bar, 10 μm).

and anti-bacteriophage antibodies and rhodamine-conjugated anti-rabbit immunoglobulins for detection (Figure 7). Complete inhibition with polysialic acid in the phage incubation medium (Figure 7C) confirmed the polysialic acid nature of the fluorescent signal.

Light microscopy of tissue sections

In tissue sections of developing rat brain, strong polysialic acid reactivity was revealed using the mutant bacteriophage as probe (Figure 8). Controls including use of polysialic acid as inhibitor of phage binding (Figure 8A), preincubation of the section with active endosialidase (Figure 8D), or performing staining without the mutant phage (not shown) all abolished the reaction, which confirmed that the reactivity was specific for polysialic acid.

Discussion

The results of the present study suggest that bacteriophages with catalytically-disabled endosialidase activity efficiently recognise and remain bound to polysialic acid of both bacteria and eukaryotic cells. Using bacterial strains of different species and capsular types phage binding appeared specific for polysialic acid consisting of N-acetylneuraminic acid in α2–8, or alternating α2–8 and α2–9 linkages. This parallels the substrate specificity of the active endosialidase [29]. In mammals, polysialic acid with alternating α2–8 and α2–9 linkages have not been described, and the phages can therefore be regarded specific for the α2–8 linked form of polysialic acid. The Western blot results confirm the specificity, showing developmental regulation and tissue distribution of polysialic acid similar to that reported previously using antibodies [26]. The sensitivity of Western blot detection using the phage method with peroxidase-conjugated antibodies was similar to that of the antibody method with radiolabelled protein A using conventional exposure times. With prolonged exposure times, the latter method was able to reveal also the low amounts of polysialic acid present in developing kidney [26].

Interestingly, a long oligosialyl segment of about ten residues is required for the binding of the inactive phage to polysialic acid. Under "physiological" conditions, the active enzyme requires a minimum of eight sialyl residue for efficient cleaving of polysialic acid [31]. The requirement of a long segment resembles the properties of the horse H46 [21,32] and mouse 735 monoclonal antibodies [33,34].

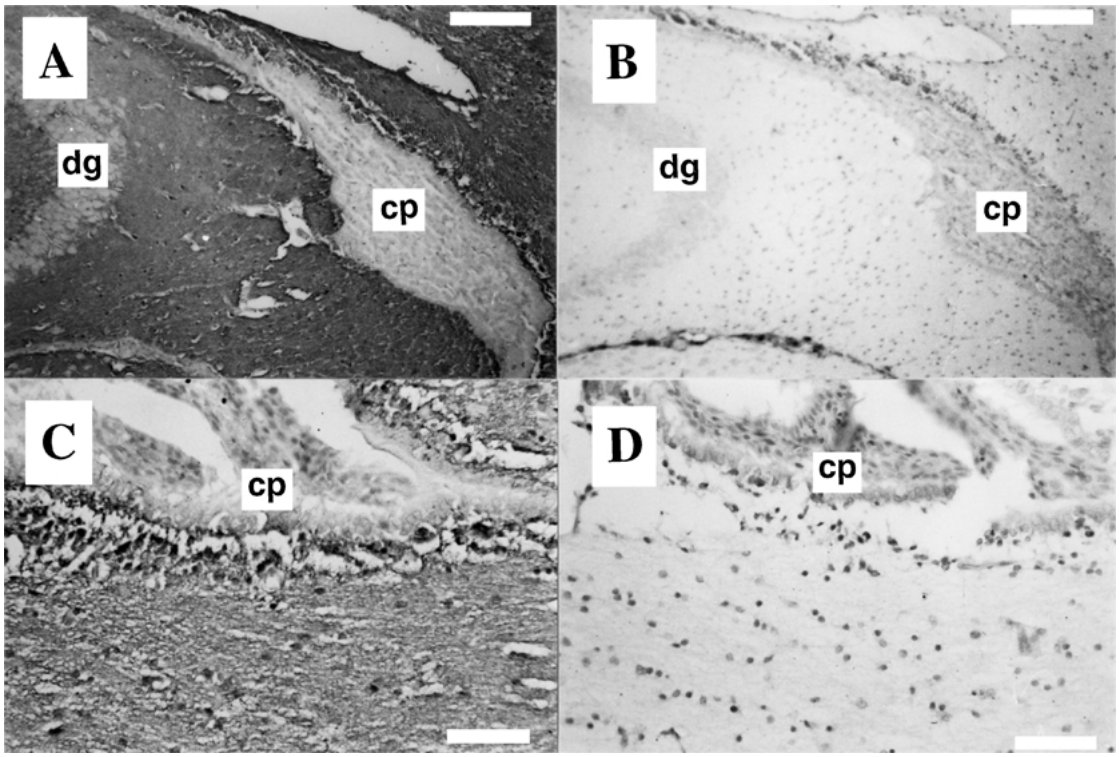

Figure 8. Detection of polysialic acid in light microscopy of tissue sections using phage detection. Paraffin sections of 8 day old rat brain were stained by incubation with mutant PK1A2 phage, followed by anti-bacteriophage antibody and peroxidase-conjugated secondary antibody, and counter-stained with hematoxylin. In B, binding of the PK1A2 phage was inhibited by including polysialic acid (10 mg/ml colominic acid) in the incubation mixture, and in D, by preincubation with wild-type phage (22.5 μg/ml) containing active endosialidase. A, The neuropil in the hippocampal region stains strongly positive for polysialic acid, while the nuclei and cell bodies are negative (dg, dentate gyrus; cp, choroid plexus)(bar, 142 μm). C, The white matter and periventricular glia are positive for polysialic acid, while the choroid plexus (cp) remains negative (bar, 57 μm).

The binding of the mutant bacteriophage to both bacterial and eukaryotic polysialic acid suggests not only structural but also close conformational similarity of these polysaccharides, as also supported by their immunological crossreactivity [10]. The reasons for this molecular mimicry, especially in the pathogenesis of meningitis, remain at present unknown. Whether the fact that the polysialic acid chains mimicked by the pathogens occur in a neural cell adhesion molecule is an important factor remains an intriguing possibility. The possibility of using the mutant bacteriophages and molecules derived from it as specific probes for the polysialic acid units of the host opens further possibilities for their study. More generally, reagents derived from bacteriophages recognising specific target molecules could be used in selected cases as molecular probes for the study of eukaryotic molecules. Phage probing was in the present study found to be applicable to dot immunobinding assays, probing of Western blots, fluorescence microscopy of cultured cells, and histochemistry of tissue sections. An advantage of the use of phages is that there is an unlimited access to the polysialic acid binding reagent in bacterial culture, and that the use of animals for antibody production is avoided. Other uses of phages as substitutes for antibodies may be found in cases where the molecule studied is not readily available due to low abundance, poor solubility, difficulties in purification, or has low immunogenic activity.

Acknowledgments

We thank the Sigrid Jusélius Foundation and the Academy of Finland for financial support and Terttu Jompero and Liisa Peltonen for technical assistance.

References

1 Jennings HJ, Katzenellenbogen E, Lugowski C, Michon F, Roy R, Kasper DL, Structure, conformation, and immunology of sialic acid-containing polysaccharides of human pathogenic bacteria, *Pure Appl Chem* **56**, 893–905 (1984).

2 Adlam C, Knights JM, Mugridge A, Williams JM, Lindon JC, Production of colominic acid by *Pasteurella haemolytica* serotype A2 organism, *FEMS Microbiol Lett* **42**, 23–5 (1987).

3 Devi SJN, Schneerson R, Egan W, Vann WF, Robbins JB, Shiloach J, Identity between polysaccharide antigens of *Moraxella nonliquefaciens*, group B *Neisseria meningitidis*, and *Escherichia coli* K1 (non-O acetylated), *Infect Immun* **59**, 732–6 (1991).

4 Finne J, Occurrence of unique polysialosyl carbohydrate units in glycoproteins of developing brain, *J Biol Chem* **257**, 11966–70 (1982).

5 Finne J, Finne U, Deagostini-Bazin H, Goridis C, Occurrence of α2-8 linked polysialosyl units in a neural cell adhesion molecule, *Biochem Biophys Res Commun* **112**, 482–7 (1983).

6 Rutishauser U, Polysialic acid and the regulation of cell interactions, *Curr Opin Cell Biol* **8**, 679–84 (1996).

7 Kiss JZ, Rougon G, Cell biology of polysialic acid, *Curr Opin Neurobiol* **7**, 640–6 (1997).

8 Troy FA,II, Polysialylation: From bacteria to brains, *Glycobiology* **2**, 5–23 (1992).

9 Rutishauser U, Polysialic acid at the cell surface: Biophysics in service of cell interactions and tissue plasticity, *J Cell Biochem* **70**, 304–12 (1998).

10 Finne J, Leinonen M, Mäkelä PH, Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development and pathogenesis, *Lancet* **2**, 355–8 (1983).

11 Robbins JB, Vaccines for the prevention of encapsulated bacterial diseases: Current status, problems and prospects for the future, *Immunochemistry* **15**, 839–54 (1978).

12 Frosch M, Görgen I, Boulnois GJ, Timmis KN, Bitter-Suermann D, NZB mouse system for production of monoclonal antibodies to weak bacterial antigens: Isolation of an IgG antibody to the polysaccharide of *Escherichia coli* K1 and group B meningococci, *Proc Natl Acad Sci USA* **82**, 1194–8 (1985).

13 Dubois C, Okandze A, Figarella-Branger D, Rampini C, Rougon G, A monoclonal antibody against *Meningococcus* group B polysaccharides used to immunocapture and quantify polysialylated NCAM in tissues and biological fluids, *J Immunol Methods* **181**, 125–35 (1995).

14 Sato C, Kitajima K, Inoue S, Seki T, Troy FA,II, Inoue Y, Characterization of the antigenic specificity of four different anti-(α2–>8-linked polysialic acid) antibodies using lipid-conjugated oligo/polysialic acids, *J Biol Chem* **270**, 18923–8 (1995).

15 Gross RJ, Cheasty T, Rowe B, Isolation of bacteriophages specific for the K1 polysaccharide antigen of *Escherichia coli*, *J Clin Microbiol* **6**, 548–50 (1977).

16 Lindberg AA, Bacterial surface carbohydrates and bacteriophage adsorption. In *Surface carbohydrates of the prokaryotic cell*, edited by Sutherland I (Academic Press, New York, 1977), pp. 289–356.

17 Pelkonen S, Aalto J, Finne J, Differential activities of bacteriophage depolymerase on bacterial polysaccharide: Binding is essential but degradation is inhibitory in phage infection of K1-defective *Escherichia coli*, *J Bacteriol* **174**, 7757–61 (1992).

18 Korhonen TK, Valtonen MV, Parkkinen J, Väisänen-Rhen V, Finne J, Ørskov F, Ørskov I, Svenson SB, Mäkelä PH, Serotypes, hemolysin production, and receptor recognition of *Escherichia coli* strains associated with neonatal sepsis and meningitis, *Infect Immun* **48**, 486–91 (1985).

19 Pelkonen S, Finne J, A rapid turbidimetric assay for the study of serum sensitivity of *Escherichia coli*, *FEMS Microbiol Lett* **42**, 53–7 (1987).

20 Gupta DS, Jann B, Schmidt G, Golecki JR, Ørskov I, Ørskov F, Jann K, Coliphage K5, specific for *E. coli* exhibiting the capsular K5 antigen, *FEMS Microbiol Lett* **14**, 75–8 (1982).

21 Finne J, Mäkelä PH, 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**, 1265–70 (1985).

22 Pelkonen S, Häyrinen J, Finne J, Polyacrylamide gel electrophoresis of the capsular polysaccharides of *Escherichia coli* K1 and other bacteria, *J Bacteriol* **170**, 2646–53 (1988).

23 Pelkonen S, Finne J, Polyacrylamide gel electrophoresis of capsular polysaccharides of bacteria, *Methods Enzymol* **179**, 104–10 (1989).

24 Wedege E, Svenneby G, Effects of the blocking agents bovine serum albumin and Tween 20 in different buffers on immunoblotting of brain proteins and marker proteins, *J Immunol Methods* **88**, 233–7 (1986).

25 Laemmli UK, Cleavage of the structural proteins during the assembly of the head of bacteriophage T4, *Nature* **227**, 680–5 (1970).

26 Finne J, Bitter-Suermann D, Goridis C, Finne U, An IgG monoclonal antibody to group B meningococci crossreacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues, *J Immunol* **138**, 4402–7 (1987).

27 Kyhse-Andersen J, Electroblotting of multiple gels: A simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose, *J Biochem Biophys Methods* **10**, 203–9 (1984).

28 Sternberger LA, The unlabeled antibody method. Hormone receptor, Golgi-like and dual color immunocytochemistry, *J Histochem Cytochem* **27**, 1658–9 (1979).

29 Kwiatkowski B, Boschek B, Thiele H, Stirm S, Endo-*N*-acetylneuraminidase associated with bacteriophage particles, *J Virol* **43**, 697–704 (1982).

30 Cremer H, Lange R, Christoph A, Plomann M, Vopper G, Roes J, Brown R, Baldwin S, Kraemer P, Scheff S, Barthels D, Rajewsky K, Wille W, Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning, *Nature* **367**, 455–9 (1994).

31 Pelkonen S, Pelkonen J, Finne J, Common cleavage pattern of polysialic acid by bacteriophage endosialidases of different properties and origins, *J Virol* **63**, 4409–16 (1989).

32 Jennings HJ, Roy R, Michon F, Determinant specificities of the groups B and C polysaccharides of *Neisseria meningitidis*, *J Immunol* **134**, 2651–7 (1985).

33 Häyrinen J, Bitter-Suermann D, Finne J, Interaction of meningococcal group B monoclonal antibody and its Fab fragment with α2-8-linked sialic acid polymers: Requirement of a long oligosaccharide segment for binding, *Mol Immunol* **26**, 523–9 (1989).

34 Häyrinen J, Jennings H, Raff HV, Rougon G, Hanai N, Gerardy-Schahn R, Finne J, Antibodies to polysialic acid and its *N*-propyl derivative: Binding properties and interaction with human embryonal brain glycopeptides, *J Infect Dis* **171**, 1481–90 (1995).

Received 25 March 2002; revised 3 September 2002;
accepted 9 September 2002