The Polysialic Acid Units of the Neural Cell Adhesion Molecule N-CAM Form Filament Bundle Networks*

(Received for publication, August 3, 1998, and in revised form, September 4, 1998)

Jyrki Toikka, Juha Aalto, Jukka Häyrinen, Lauri J. Pelliniemi, and Jukka Finne

From the Department of Medical Biochemistry, University of Turku, FIN-20520 Turku, Finland, the Department of Bio-Organic Chemistry, Bijvoet Research Center, University of Utrecht, NL-3584 CH Utrecht, The Netherlands, and the Laboratory of Electron Microscopy, University of Turku, FIN-20520 Turku, Finland

Polysialic acid is a developmentally regulated component in the neural cell adhesion molecule N-CAM which also occurs as the capsular polysaccharide of bacteria causing meningitis. Polysialic acid has been considered as a repulsive element that regulates intermolecular and intercellular adhesion. Using atomic force microscopy we unexpectedly find that oligomers of polysialic acid assemble with each other into filament bundle networks. Filaments were formed from oligomers containing 12 or more N-acetylneuraminic acid residues, and they were sensitive to sialidase digestion. The networks were also formed by the polysialic acid-containing carbohydrate units of N-CAM. The formation of filament bundles is a novel and unexpected property of polysialic acid and of short carbohydrate oligomers in general and represents a previously unrecognized molecular interaction mechanism which impacts both eukaryotic and prokaryotic cell–cell adhesions.

Polysialic acid is a unique sialic acid polymer that occurs in the neural cell adhesion molecule N-CAM (1–3). During development the polysialylated embryonic N-CAM is replaced by its adult, normally sialylated form (4). Polysialic acid also occurs as the capsular polysaccharide of bacteria causing meningitis (5, 6). The molecular mechanisms by which polysialic acid modulates cell adhesion or participates in the pathogenesis of meningitis are not known. The main function has been thought to be to serve as a repulsive element contributing to the net negative charge of the cell surface.

Atomic force microscopy has become a new method to visualize filaments of biological origin under conditions close to their native state (7–10). Using this technique to image oligomers of polysialic acid we unexpectedly find formation of filament bundles that assemble into networks. This finding suggests an explanation for the unusual properties of polysialic acid and suggests a novel molecular mechanism in the interactions of cell adhesion molecules.

EXPERIMENTAL PROCEDURES

Materials—Oligomers consisting of 6, 9, 12, 15, or 18 residues of α2–8-linked N-acetylneuraminic acid were purified by high performance liquid chromatography from colominic acid (11). The purity of the oligomers was assessed by gel electrophoresis (12) and staining with Alcian blue–silver. Embryonic polysialic acid-containing glycopeptides and normal sialylated embryonic glycopeptides without polysialyl units were isolated as described before (13).

Atomic Force Microscopy—Aliquots of 2 μl containing 2 pmol of polysialic acid oligomer were applied together with 40 pmol of CaCl₂ onto freshly cleaved mica surfaces and allowed to dry at 20 °C. The samples were imaged with no further treatment using Digital Instruments Nanoscope 2 atomic force microscope with a Nanoprobe cantilever with a quoted force constant of 0.38 newton m⁻¹.

Neuraminidase Digestion—Digestion of preformed filament networks was carried out with 5 μl of 0.1 milliunit/ml of Vibrio cholerae neuraminidase (Behringwerke AG) in 50 mM sodium acetate buffer, pH 5.5, containing 9 mM CaCl₂ and 154 mM NaCl at 20 °C for 10 min. Control incubations were carried out under identical conditions but without enzyme.

RESULTS

Oligomers of α2–8–linked N-acetylneuraminic acid residues of defined polymer length, purified from colominic acid by high performance liquid chromatography, were placed on mica surfaces and allowed to dry. Atomic force microscopy revealed the presence of filamentous structures in samples of oligomers of 12 or more sialyl residues, whereas oligomers of 9 residues or shorter did not display these structures (Fig. 1). Individual filaments had a minimum thickness of ~1 nm (Fig. 2) but tended to occur as filament bundles. With increasing chain length, extensive branching of the filament bundles into networks was observed. The filament networks were degraded by sialidase, showing that they consisted of sialic acid (Fig. 3).

The purified polysialylated glycopeptides of the neural cell adhesion molecule N-CAM of embryonic brain also formed extensive branched filament bundle networks (Fig. 4A) similar to those obtained from polysialic acid. Control specimens of glycopeptides without polysialic acid from the same source did not form these structures (Fig. 4B).

DISCUSSION

Polysialic acid is an exceptional polysaccharide in that a long oligosialyl fragment, ~8–10 sialyl residues, is needed for the interaction of polysialic acid with most of its antibodies (13–15). Similarly, a minimum of 8 residues is required for the most efficient cleavage of polysialic acid by endosialidases (14, 16). The requirement of the unusually long segment of polysialic acid for the interaction with these proteins is suggested to be related to conformational factors (15, 17). A helical coil structure may be stabilized for oligomers of sufficient chain length, as supported by NMR studies (18). It is possible that the attaining of the helical coil epitope is also a prerequisite for the formation of the filament structure observed in the present study. Thus, only fragments of sufficient length would fulfill the physical requirements for the association with each other into filaments. With regard to the interaction mechanisms of polysialic acid with proteins, the possibility remains that the interaction in fact is with the filamentous form of polysialic acid.
Formation of Filament Bundle Networks by Polysialic Acid

Polysialic acid occurs abundantly at the cell surface of developing neural and other cells (2). One of its biological roles has been suggested to be associated with a barrier function between cells (20). Due to its negative charge, polysialic acid has been viewed mainly as a repulsive element between cells and molecules (21, 22). The potential of association into bundle networks suggests that polysialic acid could also participate in associative interactions. In developing brain, the length of the polysialic acid chains undergoes temporal and topical modulation. The observed length-dependent formation of filament bundle networks may offer a molecular mechanism for the modulation of cell interactions involved in these processes.

The formation of filament networks by polysialic acid could also be a mechanism to enhance its postulated barrier function. In addition to its presence on the eukaryotic cell surface, polysialic acid forms the polysaccharide capsule of some important bacterial pathogens causing sepsis and meningitis, such as Neisseria meningitidis group B, Escherichia coli K1, and Pasteurella hemolytica A2 (6, 23). It is conceivable that the stability of the surface layer of the cells would be enhanced significantly by a filamentous network.

Filaments of protein and nucleic acid have been examined by atomic force microscopy (7–10). Some polysaccharides have also been reported to form filamentous networks (24, 25). In addition hyaluronic acid, an abundant extracellular component, may form filaments (26). However, polysialic acid and other cell surface carbohydrates have mainly been considered as inert or repulsive molecules in cellular and molecular interactions. Although carbohydrate–carbohydrate interactions have in some cases been proposed to mediate cell adhesion (27, 28), no data are available on the supramolecular organization of the complexes involved. The results of the present study unexpectedly suggest that carbohydrate oligomers as short as 12 residues long may associate into long filamentous structures. The filament network formation has to be considered as a novel potential mechanism by which N-CAM and other cell surface carbohydrates may mediate their interactions with other molecules and cells.

Acknowledgments—We thank Drs. M. Radmacher and J. Mäki for decisive guidance and technical help.

REFERENCES

1. Finne, J. (1982) J. Biol. Chem. 257, 11966–11970
2. Rutishauser, U. (1996) Curr. Opin. Cell Biol. 8, 679–684
3. Kiss, J. Z., and Rougon, G. (1997) Curr. Opin. Neurobiol. 7, 640–646
4. Finne, J., Finne, U., Deagostini-Bazin, H., and Goridis, C. (1983) Biochem. Biophys. Res. Commun. 112, 482–487
5. Finne, J., Leitonen, M., and Makela, P. H. (1983) Lancet 2, 355–358
6. Jennings, H. J., Katzenellenbogen, E., Lugowski, C., Michon, F., Roy, R., and Kasper, D. L. (1984) Pure Appl. Chem. 56, 893–905
7. Gale, M., Pollanen, M. S., Markiewicz, P., and Geh, M. C. (1995) Biophys. J. 68, 2124–2128

FIG. 1. Formation of filament network bundles by oligomers of polysialic acid. Oligomers consisting of 6, 9, 12, 15, or 18 residues of α2–8-linked N-acetylnemaminic acid were imaged with atomic force microscopy. The oligomers were also analyzed by gel electrophoresis and staining with Alcian blue–silver (staining intensity of hexasaccharide low due to diffusion out from the gel); the ladder obtained from colominic acid is shown for reference (scale bar, 200 nm).

FIG. 2. Surface contour display of polysialic acid filaments in high resolution. Purified oligomers of polysialic acid (degree of polymerization 18) were imaged by atomic force microscopy under conditions of Fig. 1. The surface scan contour image displays the filaments as two ridges, cross-sectioned by the display program in the front and their anastomosis farther back in the image.

FIG. 3. Degradation of polysialic acid filaments by sialidase. Filament bundle networks were formed from polysialytic acid oligomers (degree of polymerization 18). A, digestion with V. cholerae neuraminidase. B, control incubation under identical conditions but without enzyme. After incubation the samples were rinsed lightly with distilled water, allowed to dry, and examined by atomic force microscopy. The large particles in both panels are crystals of precipitated buffer (scale bar, 200 nm).

FIG. 4. Formation of filament network bundles by polysialyl glycopeptides of embryonic brain N-CAM. A, embryonic polysialic acid-containing glycopeptides without polysialyl units. The elongated structures in panel B apparently result from artifactual movement of the tip, which is expected in a specimen of small loosely bound particles (scale bar, 200 nm).

Acid. Alternatively, the filamentous form may represent an additional form of polysialic acid. The filamentous form could also correspond to the postulated “intermolecular epitope” of polysialic acid, implicated as a potential vaccine epitope against group B meningococci (19).

Polysialic acid occurs abundantly at the cell surface of developing neural and other cells (2). One of its biological roles has been suggested to be associated with a barrier function between cells (20). Due to its negative charge, polysialic acid has been viewed mainly as a repulsive element between cells and molecules (21, 22). The potential of association into bundle networks suggests that polysialic acid could also participate in associative interactions. In developing brain, the length of the polysialic acid chains undergoes temporal and topical modulation. The observed length-dependent formation of filament bundle networks may offer a molecular mechanism for the modulation of cell interactions involved in these processes.

The formation of filament networks by polysialic acid could also be a mechanism to enhance its postulated barrier function. In addition to its presence on the eukaryotic cell surface, polysialic acid forms the polysaccharide capsule of some important bacterial pathogens causing sepsis and meningitis, such as Neisseria meningitidis group B, Escherichia coli K1, and Pasteurella hemolytica A2 (6, 23). It is conceivable that the stability of the surface layer of the cells would be enhanced significantly by a filamentous network.

Filaments of protein and nucleic acid have been examined by atomic force microscopy (7–10). Some polysaccharides have also been reported to form filamentous networks (24, 25). In addition hyaluronic acid, an abundant extracellular component, may form filaments (26). However, polysialic acid and other cell surface carbohydrates have mainly been considered as inert or repulsive molecules in cellular and molecular interactions. Although carbohydrate–carbohydrate interactions have in some cases been proposed to mediate cell adhesion (27, 28), no data are available on the supramolecular organization of the complexes involved. The results of the present study unexpectedly suggest that carbohydrate oligomers as short as 12 residues long may associate into long filamentous structures. The filament network formation has to be considered as a novel potential mechanism by which N-CAM and other cell surface carbohydrates may mediate their interactions with other molecules and cells.

Acknowledgments—We thank Drs. M. Radmacher and J. Mäki for decisive guidance and technical help.

REFERENCES

1. Finne, J. (1982) J. Biol. Chem. 257, 11966–11970
2. Rutishauser, U. (1996) Curr. Opin. Cell Biol. 8, 679–684
3. Kiss, J. Z., and Rougon, G. (1997) Curr. Opin. Neurobiol. 7, 640–646
4. Finne, J., Finne, U., Deagostini-Bazin, H., and Goridis, C. (1983) Biochem. Biophys. Res. Commun. 112, 482–487
5. Finne, J., Leitonen, M., and Makela, P. H. (1983) Lancet 2, 355–358
6. Jennings, H. J., Katzenellenbogen, E., Lugowski, C., Michon, F., Roy, R., and Kasper, D. L. (1984) Pure Appl. Chem. 56, 893–905
7. Gale, M., Pollanen, M. S., Markiewicz, P., and Geh, M. C. (1995) Biophys. J. 68, 2124–2128

FIG. 1. Formation of filament network bundles by oligomers of polysialic acid. Oligomers consisting of 6, 9, 12, 15, or 18 residues of α2–8-linked N-acetylnemaminic acid were imaged with atomic force microscopy. The oligomers were also analyzed by gel electrophoresis and staining with Alcian blue–silver (staining intensity of hexasaccharide low due to diffusion out from the gel); the ladder obtained from colominic acid is shown for reference (scale bar, 200 nm).
Formation of Filament Bundle Networks by Polysialic Acid

8. Lyubchenko, Y. L., Jacobs, B. L., Lindsay, S. M., and Stasiak, A. (1995) Scanning Microsc. 9, 705–724
9. Rohrer, A. E., Chaney, M. O., Kao, Y. M., Webster, S. D., Stine, W. B., Haverkamp, L. J., Woods, A. S., Csetter, R. J., Tusby, J. M., Krafft, G. A., Bonnell, B. S., and Emmerling, M. R. (1996) J. Biol. Chem. 271, 20631–20635
10. Marsh, T. C., Vesenka, J., and Henderson, E. (1995) Nucleic Acids Res. 23, 696–700
11. Hallenbeck, P. C., Yu, F., and Troy, F. A. (1987) Anal. Biochem. 161, 181–186
12. Pelkonen, S., Hayrinen, J., and Finne, J. (1988) J. Bacteriol. 170, 2646–2653
13. Hayrinen, J., Jennings, H., Raff, H. V., Rougon, G., Hanai, N., Gerardy-Schahn, R., and Finne, J. (1989) J. Infect. Dis. 171, 1481–1490
14. Finne, J., and Makela, P. H. (1985) J. Biol. Chem. 260, 1265–1270
15. Jennings, H. J., Roy, R., and Michon, F. (1985) J. Immunol. 134, 2651–2657
16. Pelkonen, S., Pelkonen, J., and Finne, J. (1989) J. Virol. 63, 4409–4416
17. Hayrinen, J., Bitter-Suermann, D., and Finne, J. (1989) Mol. Immunol. 26, 523–529
18. Brisson, J.-R., Baumann, H., Imberty, A., Perez, S., and Jennings, H. J. (1992) Biochemistry 31, 4996–5004
19. Jennings, H. J., Gamian, A., Michon, F., and Ashton, F. E. (1989) J. Immunol. 142, 3585–3591
20. Acheson, A., and Rutishauser, U. (1988) J. Cell Biol. 106, 479–486
21. Sadoul, R., Hirn, M., Deagostini-Bazin, H., Rougon, G., and Goridis, C. (1983) Nature 304, 347–349
22. Hoffman, S., and Edelman, G. M. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 5762–5766
23. Adlam, C., Knights, J. M., Mugridge, A., Williams, J. M., and Lindon, J. C. (1987) FEMS Microb. Lett. 42, 23–25
24. Kirby, A. R., Gunning, A. P., Morris, V. J., and Ridout, M. J. (1995) Biophys. J. 68, 360–363
25. Round, A. N., MacDougall, A. J., Ring, S. G., and Morris, V. J. (1997) Carbohydr. Res. 303, 251–253
26. Scott, J. E., Cummings, C., Brass, A., and Chen, Y. (1991) Biochem. J. 274, 699–705
27. Popescu, O., and Miseric, G. N. (1997) Nature 386, 231–232
28. Song, Y., Withers, D. A., and Hakomori, S. (1998) J. Biol. Chem. 273, 2517–2525