Volume 236, number 1, 163–166 FEB 06193 August 1988

A *N*-acetyllactosamine-specific cell-binding activity in a plant pathogen, *Erwinia rhapontici*

Timo K. Korhonen, Kielo Haahtela, Anna Pirkola° and Jaakko Parkkinen+

Department of General Microbiology and +Department of Medical Chemistry, University of Helsinki and *°Finnish Red Cross Blood Transfusion Service, SF-00280 Helsinki, Finland*

Received 17 June 1988

A strain of the phytopathogenic bacterial species, *Erwinia rhapontici*, was found to cause hemagglutination of human erythrocytes that was specifically inhibited by β-galactosides. Of the monosaccharides tested, *N*-acetyl galactosamine and galactose efficiently inhibited the hemagglutination. The most potent inhibitor identified was Galβ1–4GlcNAc that was 30–100-fold more potent than Galβ1–3GlcNac or Galβ1–3GalNAc. Fetuin had no effect on the hemagglutination whereas asialofetuin was inhibitory. No blood group specificity was found for the hemagglutinin. These findings indicate that the *E. rhapontici* strain possesses a novel bacterial cell-binding activity with specificity for terminal *N*-acetyllactosamine residues.

Bacterial adhesion; Hemagglutination; Lactose; *N*-Acetyllactosamine; Phytopathogen; *(Erwinia)*

1. INTRODUCTION

In mammals, bacterial adhesion to host tissues at the infection site is important for initiation of infectious diseases and for establishment of normal bacterial flora in the oral cavity and the intestine [1,2]. The adhesion is thought to increase the infective potential of bacteria by helping them to resist mechanical clearance functions at mucosal surfaces. Bacterial adhesion mechanisms have been under intensive study, and a number of bacterial adhesins have been characterized for biological function, molecular binding specificity, and genetic and serological properties [2–5]. The general scheme emerging is that the invading bacteria have lectin-like proteins on their surfaces, in Gram-negative bacteria often in the form of fimbrial filaments, which bind to specific carbohydrates of epithelial glycoconjugates.

Adhesion seems important also in bacteria-plant interactions. It is essential for the induction of nodulation in legumes by rhizobia [6] and of tumor formation in host plants by agrobacteria [7], as well as for the colonization of grass roots by klebsiellas [8]. Bacterial adhesion to plant tissue has been observed in compatible virulent interactions [7,9] as well as in incompatible ones leading to resistance of the host plant [10,11]. With the exception of the rhizobe-legume interaction [6], the molecular mechanisms of these processes have not been investigated to a considerable extent. We now report identification of a galactoside-specific cell-binding activity in a phytopathogenic bacterium, *Erwinia rhapontici*.

Correspondence address: T.K. Korhonen, University of Helsinki, Department of General Microbiology, Mannerheimintie 172, SF-00280 Helsinki, Finland

2. MATERIALS AND METHODS

2.1. *Bacteria*

The *E. rhapontici* strain 139 belongs to the set of strains described by Christofi et al. [12]. Bacteria were grown at 28°C in static Luria broth as described [13], collected and suspended to one-tenth of the original volume in phosphate buffered saline, pH 7.1 (PBS).

2.2. *Hemagglutination assays*

Hemagglutination and inhibition tests were performed by standard methods [14] in 96-well, round-bottomed microtiter plates (Nunck, Roskilde, Denmark) over crushed ice. For some experiments, erythrocytes were treated with *Vibrio cholerae*

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

sialidase (Koch-Light Laboratories Ltd, Colnbrook, Berks, England) [15]. NeuAcα2–6Galβ1–4GlcNAc was isolated from bovine colostrum [16]. Galβ1–4GlcNAc (*N*-acetyllactosamine) and Galβ-1–3GalNAc were prepared from the corresponding sialylated oligosaccharides [16] by treatment with 0.1 M HCl and purification by ion-exchange chromatography. Other carbohydrates used in inhibition tests (table 1) were from Sigma (St. Louis, USA). Galacturonic acid, fetuin and asialofetuin were also from Sigma. All inhibitors were in PBS adjusted to pH 7.1.

3. RESULTS

With human O erythrocytes, hemagglutination titers of 16–64 were observed for cell suspensions ($\sim 10^{10}$ cells/ml) of the *E. rhapontici* strain 139, the variation probably reflecting day-to-day differences in the bacterial cultures. Of the monosaccharides tested (table 1), only galactose and *N*-acetylgalactosamine effectively inhibited the hemagglutination, the latter being 2.5-fold more effective than the former. Arabinose and *N*-acetylglucosamine were slightly inhibitory, being one-hundred times less effective than galactose. It is noteworthy that galacturonic acid did not inhibit the binding.

To characterize the binding specificity in more detail, several oligosaccharides were tested for inhibition of the binding (table 1). Hemagglutination by the *Erwinia* cells was effectively inhibited by various disaccharides containing β-galactose at the nonreducing terminus. Oligosaccharides with a terminal α-galactose residue were far less active inhibitors, and those containing terminal β- or α-glucose, sialic acid or β-*N*-acetylglucosamine residues had no inhibitory activity.

The most potent inhibitor tested was Galβ1–4GlcNAc. It was 10-fold more effective than lactose and nearly 40-fold more effective than galactose. Galβ1–6GlcNAc was about 3-fold more active than lactose whereas Galβ1–3GlcNAc was some 10-fold less active than lactose. Galβ1–3GalNAc was also less active than lactose but about 4-fold more effective than Galβ1–3GlcNAc. Neutral β-galactose-containing disaccharides with a manno- or galactopyranose at the reducing end were equally effective inhibitors with lactose whereas those with a fructo- or arabinofuranose were about 2-fold more effective.

Of the glycoproteins tested, fetuin had no inhibitory effect whereas asialofetuin was a potent inhibitor of hemagglutination (table 1) supporting a masking effect for sialic acid on the binding. In accordance with this, neuraminidase treatment of erythrocytes increased the hemagglutination titer from 64 to 512.

To assess the possible ABO, Lewis, P or Rh blood group specificity of the *Erwinia* hemagglutinin, hemagglutination titers were determined using erythrocytes of the following phenotypes: A, B, O, A^h and O^h for the ABO system; Le(a+b−), Le(a−b+) and Le(a−b−) for the Lewis system; P_1^+, P_1^- and pp for the P system, and D+ and D− for the Rh system. No significant differences in the titers were detected (not shown).

4. DISCUSSION

The novel cell-binding activity detected in the phytopathogenic bacterium *E. rhapontici* is specific for β-galactoside residues as indicated by the following findings. Firstly, sialidase treatment of erythrocytes, which exposes further galactose residues on the erythrocyte surface [15], increased their agglutinability by the bacteria. Secondly, hemagglutination by the *Erwinia* cells was inhibited by galactose, *N*-acetylgalactosamine, and more effectively by a number of oligosaccharides containing terminal β-galactose residues. Oligosaccharides with a terminal α-galactose residue were far less active whereas oligosaccharides with other monosaccharides at the nonreducing terminus had no inhibitory activity. In accordance, asialofetuin that contains terminal β-galactose residues on its sugar chains [17] was a potent inhibitor.

Inhibition studies with different disaccharides containing β-galactose residues at the nonreducing terminus indicated that the *E. rhapontici* hemagglutinin has remarkably higher affinity for the Galβ1–4GlcNAc sequence than for the Galβ1–3GlcNAc or Galβ1–3GalNAc sequences. The difference between the inhibitory effect of Galβ1–4GlcNAc and Galβ1–6GlcNAc was less, about 4-fold. This suggests that the *E. rhapontici* hemagglutinin may be a useful lectin in recognizing *N*-acetyllactosamine termini in mammalian glycoconjugates.

The substitution of β-galactose residues by sialic acid abolished the binding activity. Also, the substitution of galactose at C-3 by a β-*N*-acetylglucosamine residue abolished the binding (table 1). However, it remains to be resolved

whether some other substituents at galactose are compatible with the binding of *E. rhapontici* as some bacteria, e.g. P-fimbriate *E. coli* [18], recognize internal sugar structures.

Binding properties related to the present one have been identified in bacteria. Bacterial species belonging to the normal bacteria flora of human intestine have been found to adhere to lactosylceramide [19]; at least in some cases also the ceramide part of the lactosylceramide molecule was thought to participate in the binding. Also a strain of *Staphylococcus saprophyticus* was reported to bind to lactosylceramide [20]. This binding was inhibited by oligosaccharides containing the Galβ1–4GlcNAc sequence. Strains of *Actinomyces naeslundii* and *Actinomyces viscosus* which colonize human oral cavities carry the so-called type-2 fimbriae with lactose-sensitive binding properties [2,5,21]. Binding of the type-2 fimbriae is effectively inhibited by Galβ1–3GalNAc but not by Galβ1–4GlcNAc, hence their binding properties differ significantly from those of the *Erwinia* hemagglutinin.

Table 1

Inhibition of hemagglutination of human O erythrocytes by *E. rhapontici* 139

Inhibitor	MIC[a] (mM)	Relative inhibitory potential[b]
Monosaccharides		
GalNAc	0.58	2.5
Gal	1.4	1.0
GlcNAc	100.0	0.01
Ara	100.0	0.01
Glc, Man, Xyl, Fru, Rha, GalA	>100.0[c]	<0.01
Oligosaccharides		
Galβ1–4GlcNAc	0.038	38.2
Galβ1–6GlcNAc	0.14	10.7
Galβ1–3GlcNAc	4.3	0.3
Galβ1–3GalNAc	1.2	1.2
Galβ1–4Frc	0.19	7.6
Galβ1–3Ara	0.19	7.6
Galβ1–6Gal	0.30	4.8
Galβ1–4Man	0.38	3.8
Galβ1–4Glc	0.38	3.8
Galα1–6Glc	11.6	0.13
Galα1–6Glcα1–2Fru	21.3	0.07
Galα1–6Galα1–6Glcα1–2Fru	50.0	0.03
GlcNAcβ1–6Gal	>4.3[c]	<0.34
NeuNAcα1–6Galβ1–4GlcNAc	>7.0[c]	<0.21
Glcα1–4Glc, Glcα1–1Glc, Glcα1–4Glc, Glcα1–2Fru	>100[c]	<0.01
Glycoproteins		
Fetuin	>3.3 mg/ml[c]	
Asialofetuin	0.057 mg/ml	

[a] Minimal inhibitory concentration required to prevent hemagglutination

[b] Inhibitory potential of galactose is taken as 1.0

[c] No inhibition at the highest concentration indicated

Of the plant lectins binding to β-galactosides [22], the *E. rhapontici* hemagglutinin most resembles the *Erythrina cristagalli* lectin [23]. Both agglutinins have a higher affinity for *N*-acetyllactosamine than for lactose, which however is 4–6 times better in inhibition than galactose. As found for the *Erwinia* hemagglutinin in the present study, the *E. cristagalli* lectin lacks blood group specificity and interacts with asialofetuin but not with fetuin [22,23]. The *E. cristagalli* lectin however interacts with Galβ1–3GlcNAc more efficiently than does the *Erwinia* hemagglutinin.

To our knowledge, this is the first report on a specific recognition of carbohydrates by a phytopathogenic bacterium. Adhesion of zoospores of the fungus *Pythia aphanidermatum* to root surfaces of cress is dependent on recognition of fucose-containing carbohydrates in root surface mucilage [24]. Terminal β-galactose residues are present in plant cell wall polysaccharides, e.g. in side chains of xyloglucans and probably also of rhamnogalacturonans [25], indicating that the binding specificity described here might have a physiological function in mediating bacterial adhesion to plant surfaces. In the genus *Erwinia*, hemagglutination of human erythrocytes has been found only in *E. rhapontici* [12] which is thought to have a host range restricted to rhubarb [26]. At present it is not known whether bacterial adhesion contributes to this host specificity. The virulence of Erwinias largely results from the action of excreted pectinolytic enzymes [26] which damage plant cell walls. As galacturonic acid did not inhibit the hemagglutination (table 1), it appears likely that pectin is not recognized by the *E. rhapontici* hemagglutinin. Fimbriae causing mannose-resistant hemagglutination have been characterized on *E. rhapontici* [27] but our ongoing studies have suggested that the *N*-acetyllactosamine-specific hemagglutinin is non-fimbrial, its molecular nature remains to be characterized.

Acknowledgements: This study was supported by the Academy of Finland, the Finnish Ministry for Agriculture and Forestry and the Sigrid Juselius Foundation.

REFERENCES

[1] Beachey, E.H. (1980) Bacterial Adherence. Receptors and Recognition, series B, vol.6, Chapman and Hall, London.
[2] Mergenhagen, S.E. and Rosan, B. (1985) Molecular Basis of Oral Microbial Adhesion, American Society for Microbiology, Washington, DC.
[3] Lark, D.L. (1986) Protein-Carbohydrate Interactions in Biological Systems. The Molecular Biology of Microbial Pathogenicity, Academic Press, London.
[4] Mirelman, D. (1986) Microbial Lectins and Agglutinins: Properties and Biological Activity, John Wiley and Sons, New York.
[5] Sharon, N. (1986) in: The Lectins: Properties, Functions, and Applications in Biology and Medicine (Liener, I.E. et al. eds) pp.494–526, Academic Press, London.
[6] Dazzo, F.B. and Truchet, G.L. (1983) J. Membr. Biol. 73, 1–16.
[7] Lippincott, B.B., Whatley, M.H. and Lippincott, J.A. (1977) Plant Physiol. 59, 388–390.
[8] Haahtela, K., Laakso, T. and Korhonen, T.K. (1986) Appl. Environ. Microbiol. 52, 1074–1079.
[9] Romantschuk, M. and Bamford, D. (1986) Microb. Pathogen. 1, 139–148.
[10] Sequeira, L. (1985) J. Cell. Sci. suppl. 2, 302–316.
[11] Ersek, T., Gaborjanyi, R., Höltzl, P. and Kiraly, Z. (1985) PHytopathol. Z. 113, 260–270.
[12] Christofi, N., Wilson, M.I. and Old, D.C. (1979) J. Appl. Bacteriol. 46, 179–183.
[13] Korhonen, T.K. (1979) FEMS Microbiol. Lett. 6, 421–425.
[14] Korhonen, T.K. and Finne, J. (1985) in: Enterobacterial Surface Antigens: Methods for Molecular Characterisation (Korhonen, T.K. et al. eds) pp.301–313, Elsevier, Amsterdam.
[15] Gahmberg, C.G. and Andersson, L. (1977) J. Biol. Chem. 252, 5888–5894.
[16] Parkkinen, J. and Finne, J. (1987) Methods Enzymol. 138, 289–300.
[17] Nilsson, B., Norden, N.E. and Svensson, S. (1979) J. Biol. Chem. 254, 4545–4553.
[18] Bock, K., Breimer, M.E., Brignole, A., Hansson, G.C., Karlsson, K.-A., Larson, G., Leffler, H., Samuelson, B.E., Strömberg, N., Svanborg Edén, C. and Thurin, J. (1985) J. Biol. Chem. 260, 8545–8551.
[19] Hansson, G.C., Karlsson, K.-A., Larson, G., Lindberg, A., Strömberg, N. and Thurin, J. (1984) in: Glycoconjugates (Chester, M.A. et al. eds) pp.631–632, Rahms, Lund.
[20] Gunnarsson, A.A., Mårdh, B.-A., Lundblad, A. and Svensson, S. (1984) Infect. Immun. 45, 41–46.
[21] McIntire, F.C., Crosby, L.K., Bralow, J.J. and Matta, K.L. (1983) Infect. Immun. 41, 848–850.
[22] Goldstein, I.J. and Poretz, R.D. (1986) in: The Lectins: Properties, Functions, and Applications in Biology and Medicine (Liener, I.E. et al. eds) pp.33–247, Academic Press, London.
[23] Kaladas, P.M., Kabat, E.A., Iglesias, J.L., Lis, H. and Sharon, N. (1982) Arch. Biochem. Biophys. 217, 624–637.
[24] Longman, D. and Callow, J.A. (1987) Physiol. Mol. Plant Pathol. 30, 139–150.
[25] Darvill, A.G., Albersheim, P., McNeil, M., Lau, J.M., York, W.S., Stevenson, T.T., Thomas, J., Doares, S., Gollin, D.J., Chelf, P. and Davis, K. (1985) J. Cell Sci. suppl. 2, 203–217.
[26] Pérombelon, M.C.M. (1980) Annu. Rev. Phytopathol. 18, 361–387.
[27] Korhonen, T.K., Kalkkinen, N., Haahtela, K. and Old, D.C. (1987) J. Bacteriol. 169, 2281–2283.