Brazilian Journal of Microbiology (2008) 39:214-218
ISSN 1517-8382

LECTIN-BINDING PROPERTIES OF *AEROMONAS CAVIAE* STRAINS

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Submitted: April 26, 2007; Returned to authors for corrections: September 22, 2007; Approved: April 29, 2008.

ABSTRACT

The cell surface carbohydrates of four strains of *Aeromonas caviae* were analyzed by agglutination and lectin-binding assays employing twenty highly purified lectins encompassing all sugar specificities. With the exception of L-fucose and sialic acid, the sugar residues were detected in *A. caviae* strains. A marked difference, however, in the pattern of cell surface carbohydrates in different *A. caviae* isolates was observed. Specific receptors for *Tritricum vulgaris* (WGA), *Lycopersicon esculentum* (LEL) and *Solanum tuberosum* (STA) (D-GlcNAc-binding lectins) were found only in ATCC 15468 strain, whereas *Euonymus europaeus* (EEL, D-Gal-binding lectin) sites were present exclusively in AeQ32 strain, those for *Helix pomatia* (HPA, D-GalNAc-binding lectin) in AeC398 and AeV11 strains, and for *Canavalia ensiformes* (Con A, D-Man-binding lectin) in ATCC 15468, AeC398, AeQ32 and AeV11 strains, after bacterial growing at 37ºC. On the other hand, specific receptors for WGA and EEL were completely abrogated growing the bacteria at 22ºC. Binding studies with ^{125}I- labeled lectins from WGA, EEL and Con A were performed. These assays essentially confirmed the selectivity, demonstrated in the agglutination assays of these lectins for the *A. caviae* strains.

Key-words: *Aeromonas caviae*; Lectin; Carbohydrate; Cell surface

INTRODUCTION

Aeromonas species are important opportunistic pathogens, causing diarrhoea, dysentery and extra-intestinal infections (1,2,3). Recent studies revealed that some filamentous structures, outer-membrane proteins (OMPs), capsule and lipopolysaccharides (LPS) of *Aeromonas* spp. could be intestinal adhesins (4,5,6). Some studies demonstrated that *A. caviae* isolates show a high percentage of adherence to and invasion in different host cell lines (6,7,8). *A. caviae* isolates showed higher adherence levels when grown at 22ºC than at 37ºC. Furthermore, these high adherence and invasive proprieties correlated to higher filamentous structures and OMPs expression at 22ºC (6). Similar results were observed with *A. veronii* biovar *sobria* strains (9). In addition, the high percentage of *A. caviae* isolation from children with gastroenteritis associated with the adhesive and invasive characteristics of the isolates may partially reflect the enteropathogenic potential of the *A. Caviae* (1). However, the problems with speciation of the *Aeromonas* spp. may account for much of the confusion surrounding the role of these microorganisms in disease (10). On the basis of the colonization capacity and the problems with speculation of the *Aeromonas* spp., we looked for a study up on the bacterial cell surface carbohydrates in *A. caviae*. Carbohydrates in the forms of glycoproteins and glycolipids have been reported to play an important role in the binding of bacteria to receptors on host epithelial cells (11,12). In recent years, carbohydrate structures have been implicated in cell-to-cell or cell-to-substrate interaction, such as cell adhesion or aggregation (13-15). The specificity, affinity and concentrations of the interacting molecules will determine the degree of success of the bacterial colonization. Much of the works concerning structure and function of cell surface carbohydrates are derived from studies with lectins (16,17). The purpose of this investigation was to evaluate the influence of bacterial growth temperature on lectin binding properties of four *A. caviae* strains.

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MATERIALS AND METHODS

Bacterial strains and growth conditions

Three nonfimbriated *A. caviae* strains, confirmed by transmission electron microscopy under fimbrial expression conditions (6) and the reference strain (ATCC 15468) obtained from American Type Culture Collection, were used in this study. We previously demonstrated that these three nonfimbriated strains grown at 22ºC show a high percentage of adherence to and invasion in different host cell lines (6,7). The strain AeC398 was isolated from stools of children with gastroenteritis in the Laboratory of Bacteriology, Hospital Pedro Ernesto, Universidade do Estado do Rio de Janeiro (UERJ) (18). The lettuce (AeV11) and the Brazilian white cheese (AeQ32) *A. caviae* isolates were gently supplied by Dra M.L. Pena-Queiróz, Faculdade de Ciências Médicas (UERJ). The strains were cultivated onto blood agar and incubated at 37ºC overnight. A sweep of colonies was inoculated into Tryptic Soy Broth (TSB) without glucose (Difco Lab., Detroit, MI, USA) and incubated at 37ºC for 24h or at 22ºC for 48h (6).

Lectins

Twenty lectins (Sigma Chemical Co., St. Louis, MO, USA) of various specificities were used for this study. All lectins were tested for the presence of glycosidase using a- and b-p-nitrophenyl glycosides of D-Gal, D-GalNAc and D-GlcNAc (19).

Agglutination assays

Agglutination assays were performed with a Terasaki microtitrator, using 25-ml loops and suspensions containing 2 x 10^8 bacteria ml^{-1}. Equal volumes of the cell suspension and the lectin dilution were mixed, incubated at room temperature (24ºC) for 1 h. Bacterial agglutination was always scored visually with a hand lens after gently resuspending the settled cells and by observations using a phase contrast microscope. Agglutination inhibition assays were done at room temperature with 4 agglutinating units of lectin and 0.1 M concentration of each specific carbohydrate. In certain experiments washed bacteria suspended in phosphate-buffered saline (PBS) 10 mM, pH 6.0, have been treated with 0,1 U of *Clostridium perfringens* sialidase for 60 min at 37ºC, the cells washed four times with PBS, pH 7.2, and ressuspended at suitable concentrations for agglutination or binding assays. (20).

Lectin radioiodination and binding studies

Triticum vulgaris (WGA), *Euonymus europaeus* (EEL) and *Canavalia ensiformes* (Con A) lectins were labeled with ^{125}I by the method of Hunter (21), in the presence of 0.1 M of the specific inhibitory sugar. Specific activities ranged from 2 to $4x10^4$ cpm μg^{-1} of lectin. The lectin binding studies were performed as follows: 10^6 bacterial cells were incubated with increasing concentrations of iodinated lectin in 150 µl of PBS-0.5% BSA. The amount of iodinated lectin bound to the cells was determined using a gamma counter (Beckman Instruments, Inc., Palo Alto, CA, USA). Specificity of binding was ascertained by performing parallel binding determinations in the presence of 0.1 M of each of the specific sugar inhibitor for each concentration of lectin used (20).

RESULTS AND DISCUSSION

In our effort to find potential differences in surface carbohydrates of the four *A. caviae* strains grown at different temperatures, we have analyzed lectin binding properties by agglutination and lectin-binding assays. The lectin agglutination reaction of *A. caviae* (ATCC 15468, AeC398, AeQ32 and AeV11 strains) was considered most specific with bacterial cells that were agglutinated at the lowest lectin concentration (Table 1). Visual observation of agglutination showed roughly the same titters as those determined by phase contrast microscopy. The two L-fucose-binding lectins, *Lotus tetragonolobus* (LTL) and *Ulex europaeus* I (UEL-I), and the two sialic acid-binding lectins, *Aaptos papillata* II (APL-II) and *Limulus polyphemus* (LPL), did not agglutinate the *A. caviae* strains. The results shown in Table 1 suggest the occurrence of qualitative and quantitative differences in bacterial surface carbohydrate composition, as detected by lectins, between *A. caviae* strains grown at 37ºC and 22ºC. Four lectins with affinity for D-GlcNAc were tested. *Lycopersicon esculentum* (LEL) and *Solanum tuberosum* (STA) lectins agglutinated strongly ATCC 15468 strain of *A. caviae* grown at 37ºC or at 22ºC but were unreactive with the three *A. caviae* isolates. *Triticum vulgaris* (WGA), however, interacted selectively and strongly with ATCC 15468 strain of *A. caviae* grown at 37ºC. Agglutination was inhibited by 0.1 M D-GlcNAc. Among the five lectins with affinity for D-GalNAc tested, only *Helix pomatia* (HPA) lectin showed activity. HPA recognised *A. caviae* AeC398 strain grown at 37ºC and *A. caviae* AeV11 strain grown at both temperatures. Agglutination was inhibited by 0.1 M D-GalNAc. Six D-Gal-binding lectins were tested. *Euonymus europaeus* (EEL) selectively agglutinated *A. caviae* AeQ32 strain grown at 37ºC. Agglutination was inhibited by 0.1 M D-Gal. Among the two lectins tested (Con A and *Lens culinaris*), which have binding sites for D-Man-like residues, only Con A reacted with all *A. caviae* strains grown at 37ºC and *A. caviae* ATCC 15468 and AeQ32 strains grown at 22ºC. Agglutination was inhibited by 0.1 M α-methyl-D-mannoside. Because of the dual specificity of the WGA binding site, with affinities for both D-GlcNAc polymers and sialic acid residues, neuraminidase treatment of *A. caviae* ATCC 15468 strain was performed. Such treatment did not abolish the strong agglutinating activity of WGA. To verify if the differences detected in the cell agglutination pattern with lectins (Table 1) were compatible with the expression of their respective cell

Table 1. Activity of lectins of various specificities for *A. caviae* strains grown at 37ºC and 22ºC.[a]

	Minimum lectin concentration (μg ml^{-1}) required to agglutinate							
	ATCC 15468 strain		AeC398 strain		AeQ32 strain		AeV11 strain	
	37ºC	22ºC	37ºC	22ºC	37ºC	22ºC	37ºC	22ºC
D-GlcNAc-binding lectins								
Triticum vulgaris (WGA)	15.6	–[b]	–	–	–	–	–	–
Lycopersicon esculentum (LEL)	15.6	15.6	–	–	–	–	–	–
Solanum tuberosum (STA)	15.6	15.6	–	–	–	–	–	–
D-GalNAc-binding lectin								
Helix pomatia (HPA)	–	–	125	–	–	–	15.6	15.6
D-Gal-binding lectin								
Euonymus europaeus (EEL)	–	–	–	–	31.2	–	–	–
D-Man-binding lectin								
Canavalia ensiformis (Con A)	125	125	62.5	–	125	62.5	125	–

[a] Average of three experiments.
[b] Agglutination was not observed even at the highest concentrations tested (500. μg ml^{-1}).

surface receptors, the binding of ^{125}I- labeled WGA, EEL and Con A to *A. caviae* strains was determined. All three iodine-labeled lectins discriminated strains of *A. caviae* grown at 37ºC (Fig. 1). WGA interacted more strongly with *A. caviae* ATCC 15468 strain, whereas the binding of EEL was higher with *A. caviae* AeQ32 strain. Con A preferentially bound to two strains of *A. caviae* grown at 37ºC. Although the AeV11 strain bound 2 times less radioactivity than AeC398 strain, the amount of [^{125}I]-Con A bound was nevertheless specific. The binding was specific because it could be reversed with 0.1 M of the corresponding specific saccharide. The binding data in Fig. 1 demonstrate that the differential agglutination of the *A. caviae* strains by WGA, EEL and Con A (Table 1) correlated with the number of exposed [^{125}I]-lectin receptor sites on them. When the binding data were plotted according to the method of Steck and Wallach 1965 (22), as shown in Fig. 2, the average number of lectin-binding sites (n) and the lectin association constant (Ko) were determined. WGA-binding data for *A. caviae* ATCC 15468 strain was n ~ 6.2 x 10^5/bacteria, Ko ~ 2.6 x 10^7 M^{-1}. EEL-binding data for *A. caviae* AeQ32 strain was n ~ 2.0 x 10^5/bacteria, Ko ~ 3.6 x 10^7 M^{-1}. Con A-binding data for *A. caviae* AeC398 and AeV11 strains was n ~ 3.4 x 10^5/bacteria, Ko ~ 2.2 x 10^7 M^{-1} and n ~ 5.5 x 10^5/bacteria, Ko ~ 1.3 x 10^7 M^{-1} respectively. Based on these findings, a new approach for the isolation of cell surface receptor sites can be envisaged. Furthermore, the analysis of the lectin receptors should allow deeper insight in how the *A. caviae* strains exert their biologically relevant functions. The differential expression of carbohydrate structures on the bacterial surface could be in part associated with host specificity that, as is well known, depends on microorganism recognition based on surface saccharide components (17,23). Finally, lectin reactivity in *A. caviae* isolates, as opposed to other organisms, might be used as a diagnostic method that may be eventually useful in epidemiological studies (16,24). It also opens the possibility of

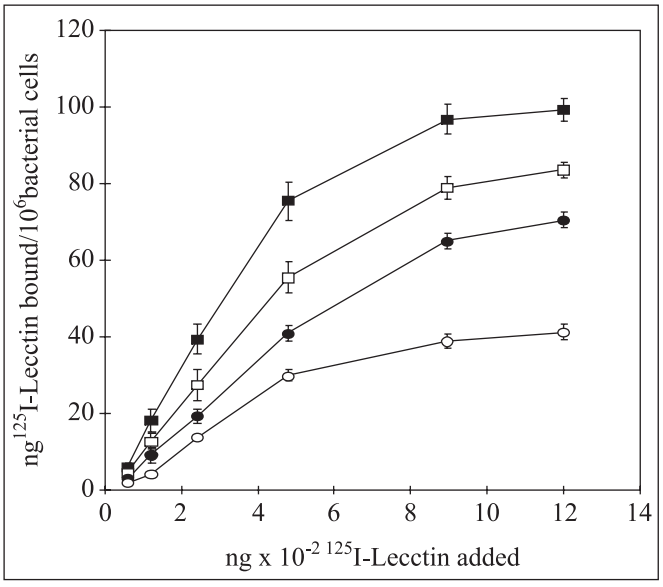

Figure 1. Binding of iodinated lectins *Triticum vulgaris* (WGA), *Euonymus europaeus* (EEL) and *Canavalia ensiformis* (Con A) to *A. caviae* strains grown at 37ºC. WGA-binding to ATCC 15468 strain (■); EEL-binding to AeQ32 strain (□); Con A-binding to AeC398 strain (●) and to AeV11 strain (○). Each point is the mean ± S.E.M. derived from three separate experiments.

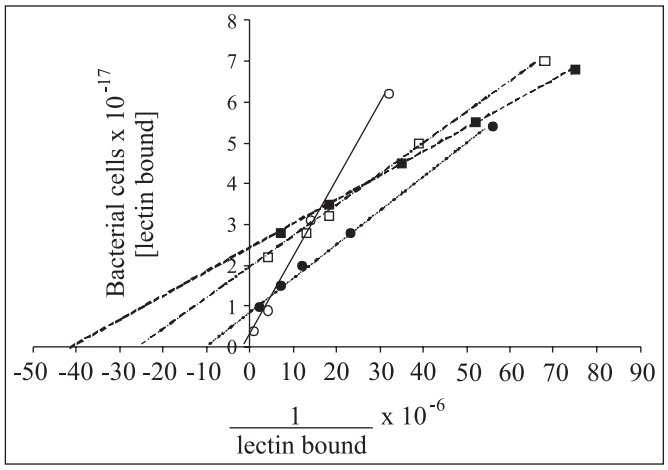

Figure 2. Binding of [^{125}I]-labeled *Triticum vulgaris* (WGA) to ATCC 15468 strain (■), [^{125}I]-labeled *Euonymus europaeus* (EEL) to AeQ32 strain (□) and [^{125}I]-labeled *Canavalia ensiformis* (Con A) to AeC398 strain (●) and to AeV11 strain (○). The data have been plotted by the method of Steck and Wallach (11) according to the equation:

$$C/Lectin\ \text{bound} = 1/K.n \times 1/Lectin\ \text{free} + 1/n$$

Where *C* is the concentration of bacteria, *n* is the number of lectin molecules bound/bacteria, *K* is the lectin association constant and *Lectin* is the concentration of lectin in molar. All points represent the average of triplicate experiments and the standard deviation is lower than 10%.

using lectins to isolate cell surface glycoconjugates by affinity chromatography for analysis of their chemical structure and biological function (25,26). Furthermore, the ability to vary quantitative and qualitatively the surface carbohydrate composition under different environmental conditions could be advantageous to *A. caviae* during different stages of host cell colonization. There are strong evidences that bacterial growth temperature influence *Aeromonas* spp. adherence to host cells and the fimbrial structure, OMPs and smooth-LPS phenotype expression (4,6,9). *A. hydrophila* was found to produce a 43kDa haemagglutinin that binds to H-antigen expressed on the surface of most human erythrocytes and is inhibited by L-fucose (27). Previous studies also demonstrated that differences in *A. caviae* adherence levels correlated with higher expression of the 43kDa OMP at 22ºC (6). *Aeromonas* strains isolated from feces also showed higher mannose-resistant adherence than strains isolated from samples of food and river water (28). Interestingly, in this study, Con A reacted with all *A. caviae* strains grown at 37ºC and was remarkably specific for AeC398 and AeV11 strains of *A. caviae*. The lectin-like reactivity could be associated with the ability of these bacteria to adhere to carbohydrate-rich surfaces such as the human gut, contributing to enteric diseases. Thus, complementary studies are required in order to better understand the role of cell-surface carbohydrates in pathogenesis of *A. caviae*.

ACKNOWLEDGEMENTS

The authors are grateful to CNPq, CAPES, FAPERJ and SR-2/UERJ for providing financial support.

RESUMO

Propriedades lectínicas de amostras de *Aeromonas caviae*

Os carboidratos de superfície celular de quatro amostras de *Aeromonas caviae* foram analisados por aglutinação e ensaios de ligação de lectinas empregando vinte lectinas altamente purificadas com especificidade para açúcares. Com exceção da L-fucose e do ácido siálico, os resíduos de açúcar foram detectados em amostras de *A. caviae.* Entretanto, foi observada uma diferença marcante no padrão de carboidratos de superfície celular em diferentes amostras de *A. caviae*. Receptores específicos para *Tritricum vulgaris* (WGA), *Lycopersicon esculentum* (LEL) e *Solanum tuberosum* (STA), lectinas de ligação a D-GlcNAc, foram encontrados apenas na amostra ATCC 15468, enquanto sítios de *Euonymus europaes* (EEL), lectina de ligação a D-Gal, estavam presentes exclusivamente na amostra AeQ32, sítios de *Helix pomatia* (HPA), lectina de ligação a D-GalNac, nas amostras AeC398 e AeV11 e de *Canavalia ensiformis* (Com A), lectina de ligação a D-Man, nas amostras ATCC 15468, AeC398, AeQ32 e AeV11, após crescimento bacteriano a 37ºC. Por outro lado, receptores específicos para WGA e EEL foram completamente abolidos após o crescimento das bactérias a 22ºC. Estudos de ligação com lectinas WGA, EEL e Con A marcadas com ^{125}I também foram realizados. Esses ensaios confirmaram a seletividade, demonstrada em ensaios de aglutinação dessas lectinas para as amostras de *A. caviae.*

Palavras-chaves: *Aeromonas caviae*; Lectina; Carbohidrato; Superficie celular

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