Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 15
Sofia • 2010

Application of Lectin Cytochemistry for Differential Labelling of Surface Polysaccharides of Pathogenic Strains of *Escherichia coli*

*Ts. Paunova, S. Stoitsova, R. Ivanova**

The Stephan Angeloff Institute of Microbiology,
Bulgarian Academy of Sciences, Sofia
**Bul-Bio, NCIPD, Sofia*

The study aimed to identify lectins that can differentiate between surface polysaccharide antigens – exopolysaccharide, capsular antigens and lipopolysaccharides of pathogenic strains of *E. coli*. Initially, the isolated antigens – exopolysaccharide, K and O antigens from four *E. coli* serotypes were subjected to dot-blot analysis with a panel of four lectins with different sugar specificities. After the selection of differential markers, lectin-gold labelling was performed. Changes in the surface exposure of the antigens as a result of growth temperature and differences between individual cells are described.

Key words: Lectin-gold, *E. coli*, polysaccharide antigens, differential labelling.

Introduction

Escherichia coli pathogenic strains are adapted to survive in both external and host environments. They are capable to overcome host resistance and have the capacity to occupy different host niches, both intestinal and extraintestinal. Surface polysaccharides exposed at the surface play a variety of roles in the communication of bacteria with their environment, and changes in their accessibility may affect the interactions. Thus, the exopolysaccharide, colanic acid, when expressed protects the cells from desiccation [2]. Capsules and lipopolysaccharides (LPS) may have different impact in infection, especially with regards to serum resistance and invasion, and are considered virulence factors [6]. This determined our interest in examining the cytochemical characteristics related with the polysaccharide antigens of pathogenic *E. coli* strains that belong to different pathotypes and exploit different host habitats. This study describes an experimental approach based on differential cytochemical labelling of polysaccharide antigens with gold conjugates of glycan-binding proteins, or lectins. Lectins are characterised by specific affinities for definite mono- or oligosaccharide residues at the non-reducing termini of polysaccharides and thus, provided the structure of the antigen is known, its lectin specificity may be predicted [14]. In order to examine surface architecture, our aim was to identify lectins that can differentiate between the antigens.

Materials and Methods

Strains and cultivation. The strains were chosen according to two criteria: known structure of the polysaccharide antigens and occupation of different host niches. *E. coli* O157:H-, strain A2CK, SS, represents a serotype that resides in the intestine and causes severe diarrhoea [13]. E. coli O6:K2:H1 is among the causative agents of urinary tract infections [6], and we used strain Bi7458/41 SS. Strains with K1 capsule are causative agents of neonatal meningitis [8], and were represented by E. coli O1:K1:H7 strain U5/41SS, and E. coli O7:K1, strain Bi759/41 SS. The strains were cultivated for 24 h. on nutrient agar at 20°C or 37°C.

Choice of lectins for differential labelling. The choice of lectins was based on the known chemical structure of the antigens. Polysaccharides were isolated after W e s t p h a l et al. [17] and Ø r s k o v et al. [11]. Lectin affinities of each antigen were tested using a panel of four lectins – concanavalin A (ConA) for mannosyl and glycosyl residues, soybean agglutinin (SBA) with affinity for galactose, wheatgerm agglutinin (WGA) for N-acetylglycosamine and sialic acids, and *Ulex europeaus* agglutinin (UEA-I) for fucose [14]. Each antigen was applied at amounts of 10, 1 and 0,1 μg on nitrocellulose disks and dot-blot was performed with 100 μg/ml of peroxidase conjugates of each of the lectins [15]. Controls included lack of antigen or incubation with the lectins in the presence of 0.2 M of the respective inhibitory sugars.

Lectin-gold labelling. Bacteria were collected from the agar plates and suspended in PBS. The cell suspensions were applied onto Formvar- and carbon-coated nickel electron microscopy grids. The samples were exposed for 30 min at UV light and air dried. The labeling protocol included blocking of the grid-mounted bacteria face-down on drops of 2% BSA in PBS, 2 hours incubation on drops of 50 μg mL^{-1} lectin-gold in PBS (or PBS containing 0.2 mM $CaCl_2$ for ConA), and washes. The lectins were conjugated with 10 or 20 nm gold grains. The control for carbohydrate specificity included pre-absorption of the lectin-gold conjugates with 0.2 M of the respective monosaccharide prior to labelling. Negative staining was performed for 1 min with 0.5% uranyl acetate in methanol. The whole procedure was done at room temperature. Observations were made on Opton 10C electron microscope.

Results

Selectivity of lectin-antigen interactions. Dot-blot experiments (data not illustrated) showed that the exopolysaccharide colanic acid interacted with SBA and UEA-I which confirmed our previous results [15]. The O157 LPS reacted with ConA and WGA. Out of the four lectins tested, the O6 LPS antigen was labeled with ConA, and the K2 capsule – with SBA only. Thus, SBA and ConA discriminated between the O6 LPS and K2 capsule. Unexpectedly, polysialic K1 capsules from both K1 strains included in the study did not interact with WGA.

E. coli O157: temperature-related changes in lectin affinities. When the strain was cultivated at 20°C, it expressed extracellular filamentous material that was labeled with SBA (Fig. 1a) and UEA-I, similarly to the isolated preparation of colanic acid. Cells cultivated at 37°C revealed a surface-associated intensive ConA binding (Fig. 1b). Unlike this, ConA labeling of cells grown at 20°C was by exception. No WGA labeling of native cells was observed, however short (10 min.) treatment of bacterial suspensions on boiling water bath was sufficient to expose WGA-binding sites (Fig. 1c). This was observed with all cells at both growth temperatures compared.

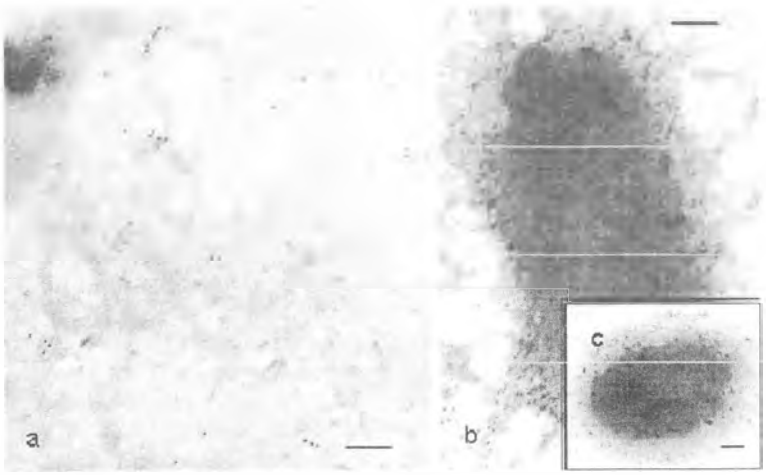

Fig. 1. *E. coli* O157
a – SBA-gold labelling of the exopolysaccharide; b – ConA-gold labelling of cell grown at 37°C; c – WGA-gold of a cell grown at 20°C and boiled for 10 min. prior to application onto the grid. Bars = 0.2 μm

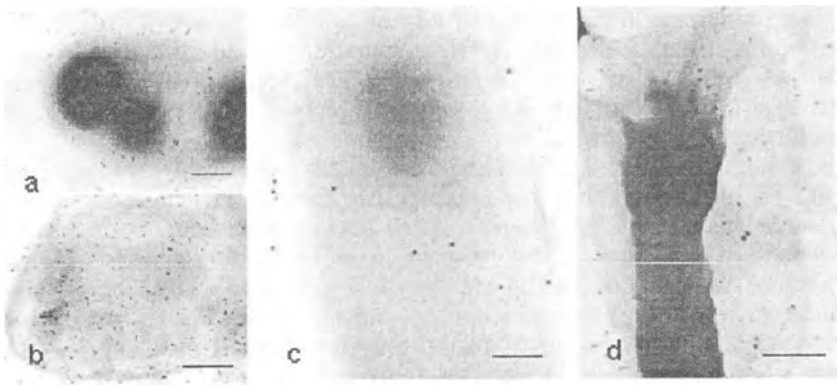

Fig. 2. *E. coli* O6:K2:H1.
(a, b) SBA-10 nm gold. (c) ConA-20 nm gold. (d) Double labelling with SBA-10 nm gold and ConA-20 nm gold. Bars = 0.2 μm

E. coli O6:K2:H1: differential labelling of capsule and LPS (Fig. 2). In this strain, under the experimental conditions applied SBA-binding sites were visualised all over the bacterial surface, with differences in the degree of labeling between individual cells (Fig. 2a, b). ConA labelling was not intensive (Fig. 2c), and this was also demonstrated by double labeling (Fig. 2d).

Discussion

The present results illustrate that lectins may be appropriate tools in examination of the surface architecture of some *E. coli* strains. The interactions of the K2 capsular antigen, and the O6 and the O157 LPS antigens were as predicted by the published structures of the polysaccharides [4, 5, 10, 12, 16]. Both O6 and O157 LPS were recognized by

ConA. In addition, the WGA reactivity of the O157 LPS demonstrated by the dot blot could be due to the non-reducing N-acetylglucosamine residue outer core [1]. The outer core of the O6 LPS contains a non-reducing galactose residue [1], however preliminary dot-blot experiments showed no SBA reactivity of the O6 LPS preparation. These results did not imply any contribution of the residue to the lectin-binding characteristics of the O6 LPS. On the other hand, K2 capsule interacted with SBA in a well-expressed dose-dependent manner. Thus, it can be accepted that in *E. coli* O6:K2 SBA is a differential label of the K2 capsule. Preparations from the capsules of the two chosen K1 strains were not recognized by WGA – the lectin of choice determined by the known structure of this polysialic antigen. This could be due to antigen variation, like the O-acetylation of the K1 antigen recently commented in literature [8]. This indicated that the lectin-based approach well-applicable to the other two serotypes is inappropriate for examinations of the surface architecture of *E. coli* K1.

The four lectins were further applied to test the temperature-related changes of surface polysaccharide expression in *E. coli* O157. This serotype does not synthesize a capsule. The temperature-related difference in expression of the exopolysaccharide was predicted from literature [7, 9] and was confirmed cytochemically in the present experiments. WGA labelling, expectedly due to the outer core oligosaccharide, was insignificant in native cells independently of growth temperature, and was intense after boiling of the samples. This implies that the oligosaccharide outer core of the strain is quite inaccessible to external glycan-binding proteins in the environment such as mammalian lectins that participate in the lectin pathway of complement activation [3]. One novel observation in this study was the growth temperature-related difference in ConA reactivity of the – lack of reactivity after growth at 20°C versus a very intense labeling of cells grown at 37°C. This indicates a possible antigen variability of the O157 LPS which requires further detailed research.

The results illustrated that SBA and ConA can be used as differential labels of capsule and LPS in the uropathogenic strain *E. coli* O6:K2:H1. Under the experimental conditions in this study, SBA labeling varied between individual cells this indicating different state of exposure at the surface of the lectin-binding epitopes. However, the LPS appeared partially covered by the capsules, with only a few ConA-binding sites accessible. Given the different roles of capsule and LPS in the virulence of uropathogenic *E.coli* [6], the present results provide a cytochemical approach to examinations of changes in surface architecture of the strain. Such variations may occur under the action of biogenic factors, after in vivo passages, etc. However, since SBA cannot differentiate between the K2 capsule and colanic acid, SBA labelling of this strain is inappropriate for conditions that promote exopolysaccharide expression. Thus experiments on biofilm architecture related with exopolysaccharide accumulation on bacterial attached microcolonies should preferably use the fucose label, UEA-I, which also interacts with the polymer.

Acknowledgements. This study was supported by the National Research Fund at the Ministry of Education and Science, Republic Bulgaria, Project L-1402/04.

References

1. Amor, K., D. Heinrichs, E. Fridrich, K. Ziebell, R. Johnson, C. Whitfield. Distribution of core oligosaccharide types in lipopolysaccharides from *Escherichia coli*. – Infect. Immun., **68**, 2000, 1116-1124.
2. Clarke, A., H. Strating, N. Blackburn. Pathways for the O-acetylation of bacterial cell wall polysaccharides. – In: Glycomicrobiology. New York, Dordrecht, London, Moscow, 2000, 187-224.

3. Goldsby, R., T. Kindt, B. Osborne. Kuby Immunology. Fourth Edition. New York, W.H. Freeman & Co, 2000. 669 p.
4. Jann, B., A. Shashkov, H. Kochanowski, K. Jann. Structural comparison of the O6 specific polysaccharides from *E. coli* O6:K2:H1, *E. coli* O6:K13:H1, and *E. coli* O6:K54:H10. – Carbohydrate Res., **263**, 1994, 217-225.
5. Jann, K., B. Jann, M. Schmidt, W. Vann. Structure of the *Escherichia coli* K2 capsular antigen, a teichoic acid-like polymer. – J. Bacteriol., **143**, 1980, 1108-1115.
6. Johnson, J. Virulence factors in *Escherichia coli* urinary tract infection. – Clin. Microbiol. Rev., **4**, No 1, 1991, 80-128.
7. Junkins, A., M. Doyle. Demonstration of exopolysaccharide production by enterohaemorrhagic *Escherichia coli*. – Curr. Microbiol., **25**, 1992, 9-17.
8. King, M., S. Steenbergen, E. Vimr. Going for baroque at the *Escherichia coli* K1 cell surface. – Trends Microbiol., **15**, 2007, 196-202.
9. Mao, Y., M. Doyle, J. Chen. Insertion mutagenesis of *wca* reduces acid and heat tolerance of enterohemorrhagic *Escherichia coli* O157:H7. – J. Bacteriol., **183**, 2001, 3811-3815.
10. Nishiuchi, Y., M. Doe, H. Hotta, K. Kobayashi. Structure and serologic properties of O-specific polysaccharide from *Citrobacter freundii* possessing cross-reactivity with *escherihia coli* O157:H7. – FEMS Immunol. Med. Microbiol., **28**, 2001, 63-171.
11. Ørskov, I., F. Ørskov, B. Jann, K. Jann. Serology, chemistry, and genetics of O and K antigens of *Eschericia coli*. – Bacteriol. Rev., **41**, 1977, 667-710.
12. Perry, M., L. MacLean, D. Griffith, Structure of the O-chain polysaccharide of the phenol-phase soluble lipopolysaccharide of *Escherichia coli* O157:H7. – Can. J. Biochem. Cell Biol., **64**, 1986, 21-28.
13. Roberts, T. Maximizing the usefulness of food microbiology research. – Emerg. Infect. Dis., **3**, 1997, 523-528.
14. Slifkin, M., Doyle R. Lectins and their application to clinical microbiology. – Clin. Microbiol. Rev., **3**, 1990, 197-218.
15. Stoitsova, S., R. Ivanova, I. Dimova. Lectin-binding epitopes at the surface of Escherichia coli K-12: examination by electron microscopy, wit special reference to the presence of a colonic acid-like polymer. – J. Basic Microbiol., **44**, 2004, 296-304.
16. Vinogradov, E., J. Conlan, M. Perry. Serological cross-reaction between the lipopolysaccharide O-polysaccharide antigens of *Escherichia coli* O157:H7 and strainsof *Citrobacter freundii* and *Citrobacter sedlakii*. – FEMS Microbiol. Lett., **190**, 1998, 157-161.
17. Westphal, O., K. Jann. Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedures. – Methods in Carbohydrate Chemistry, **5**, 1965, 83-91.