ISSN : 1411 - 8327

Relation Between Encapsulation and Adherence Properties of Bacteria Streptococci of Serological Group B to DEAE-Sephacel

(HUBUNGAN ANTARA KEBERADAAN KAPSUL DENGAN SIFAT ADHESIFITAS BAKTERI STREPTOKOKUS GRUP B PADA DEAE-SEPHACEL)

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ABSTRACT

The presence of charged antigen on the surfaace of streptococci sero-group B was determined by ion exchange chromatography on DEAE-Sephacel. The study revealed that all bovine and human isolates of group B streptococci with surface protein antigens, either alone or in association with polysacharide antigens, , adhered strongly to the gel matrix. In contrary, all bacterial bulture with polysaccharide antigens alone showed no evident adherence properties. Removal of neuraminic acid capsul from bacdterial surface enhanced the adherent properties, whereas pronase treatment reduced its adherent values. The importance of polysaccaride capsules in determining the surface charge of group B streptococcal charge could be further confirmed using two group B streptococci of serotype III and their tansposon mutant bearing capsule with meuraminic acid. In contrast with the encapsulated parent strains, the mutant strain adhered strongly to the gel matrix. A similar result was observed with unencapsulated group B streptococcal variant strains and its isogenic unencapsulated parent strains. Such capsule seemed to play important role in musking the surface proteins responsible for the adherence to the gel matrix. The determination of surface charge of group B streptococci by ion-exchange chromatography might help to understand the importants of capsular sialylation for individual isolates of this bacterial species.

Key words : encapsulation, adherence properties, streptococci group B, DEAE-sephacel

J Vet 2002 4 (1) : 1 - 8

ABSTRAK

Penentuan karakter permukaan sel bakteri streptokokus grup B menggunakaan *ion-exchange chromatography* DEAE-Sephacel menunjukkan bahwa streptokokus grup B isolat asal sapi maupun manusia yang memiliki antigen protein permukaan, baik sendiri maupun dalam bentuk gabungan dengan antigen polisakarida, menunjukkan adhesivitas yang sangat kuat pada matriks gel DEAE-Sephacel. Sebaliknya, kultur bakteri yang memiliki hanya antigen polisakarida tidak menunjukkan sifat adhesivitas yang berarti. Penghilangan kapsul asam neuraminat dari permukaan sel bakteri, meningkatkan nilai adhesi tetapi sebaliknya perlakuan dengan pronase menyebabkan penurunan nilai adhesi. Pentingnya kapsul polisakarida sebagai penentu sifat permukaan bakteri ditegaskan dengan menggunakan streptokokus grup B serotipe III dan mutannya yang tidak memiliki asam neuraminat , mutan ini dibuat dengan transposon mutagenesis. Terbalik dengan sifat bakteri induknya, mutan bakteri ini menunjukkan nilai adhesi yang kuat pada matriks gel. Hasil yang sama diperoleh pula dengan menggunakan bakteri berkapsul dan bakteri variannya yang tidak berkapsul. Kapsul membungkus komponen protein yang bertanggungjawab terhadap sifat adhesif pada matriks gel. Penentuan sifat permukaan dengan menggunakan *ion-exchange chromatography* dapat membantu pemahaman tentang pentingnya peran kapsul pada bakteri.

Kata Kunci : kapsul, sifat adhesifitas, streptokokus group B, DEAE-sephacel

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INTRODUCTION

Streptococci of serological group B is one of the causative agent in bovine mastitis and in human neonatal infecions, particularly in septicaemia and meningitis (Jelinkova, 1977; Hahn, 1980, Baker, 1980). Although less common other infectious processes caused by group B streptococci have been described (Wilkinson, 1978; Gallagher and Watanakunakorn, 1986). The antigenic classification of group B streptococci is based on the occurrence of distionct type specific cell surface antigens. Up to now, group B streptococci can be classified into seven antigenic types based on the known major polysaccharide antigens i.e. Ia, Ib, II, III, IV, V and VI and the three proteion antigens c, R and X (Jelinkova, 1977; Henrichsen *et al.*, 1984; Jelinkova and Motlova, 1985; Bopp, 1994). This polysaccharide and protein antigens might occur singly or in combination , and cultures were nontypable (Wibawan and Lämmler, 1990a; Wibawan *et al.*, 1992b; Laemmler *et al.*, 1993). A direct transmission of group B streptococci between bovine and human was uncommon (Brglez, 1981; Nielsen, 1987). This was suppoted by their biochemical characteristic and the presence of this specific type antigens (Finch and martin, 1984, Lemmler and Blobel, 1987, Wibawan *et al.*, 1991). However, among all seven polysaccharide serotypes, sialic acid residues are present in the type specific capsule and play a role as major immuno determinant for type Ia, II and III organisms (Shigeoka *et al.*, 1983,; Molinari *et al.*, 1987). In addition, capsular sialylation is thought to be a critical virulent factor of group B streptococci (Edwards *et al.*, 1982; Wessels *et al.*, 1992). This study was designed to further characterize the relation between encapsulation and surface charge of the bacteria. For this, the group B streptococci were chromatographed on the anion-exchanger DEAE-Sephacel.

MATERIALS AND METHODS

Bacterial Cultures

A total of 89 streptococci isolates of serogical group B were used in this study. This included group B streptococcal reference cultures 090 (Ia), H36B (Ib), 18RS21 (II), 6313 (III), 3139 (IV), SS1169 (V), A909 (Ic), 25/60 ® and 24/60 (X), 30 cultures isolated from bovine mastitis and 42 cultures from human routine specimen, the type III group B streptococcal strain COH 1, COH 31r/s and their asialo capsular mutants COH 1-11, COH 31-21, respectively. The later were kindly provided by M.R. Wessels (Channing Laboratory, Boston, MA, USA). The mutant strain COH 1-11 and COH 31-21 were constructed by transposon mutagenesis with Tn916, a 16,4 kb transposon encoding tetracyclin resistance (Wessels *et al.*, 1989, 1992). In addition, the encapsulated group B streptococcus 5531:LD with type antigen III and the isogenic nonencapsulated original strain 5531:OS were included. Both strains were kindly provided by M. Sellin (Department of Clinical Bacteriology, University of umea, Sweden). Strain 5531:OS was originally isolated from human endocarditis and appeared to be nontypeable (NT) by routine procedure. The encapsulated strain was separated from the original high density strain as low density variant by percoll gradient centrifugation. The centrifugation technique and further characteristics of the unencapsulated strain original strain and the encapsulated variant had been described recently (Sellin *et al.*, 1992). The cultures were maintained on sheep blood agar and subcultured every four weeks. For cultivation in fluid media, the bacteria were incubated in Todd-Hewitt broth

(THB, Gibco, Karlsruhe, Germany) for 18 h at 37°C under shaking conditions. All cultures had been serogrouped with autoclaved extracts and group B specific antiserum (Wellcome, Burgwedel, FRG), serotyped with monospecific antisera Ia, Ib, II, III, IV, c, R and X and further characterized as described (Wibawan and Lämmler, 1990a, 1990b).

Ion Exchange Chromatography

Retention of group B streptococci on the anion exchanger DEAE-Sephacel (Pharmacia, LKB, Freiburg, Germany) was basically performed as described by Kabir and Ali (1983). Briefly, DEAE-Sephacel was washed with 0.05 M phospphate buffer pH 6.8 and placed (1.15 ml) into a pasteur pipette (diameter 7mm, length 30 mm). A 500ml of the bacterial suspension diluted with 2.5 ml buffer. The data were expressed as percentage of the bacteria that adhered to DEAE-Sephacel.

In parallel experiments the photometrically adjusted bacteria (1 ml) were treated with 0.1 U/ml neuraminidase (neuraminidase type V from *Clostridium perfringens;* Sigma, Deisenhofen, Germany) for 1 h at 37°C (Wibawan and Lämmler, 1991). The neuraminidase pretreated bacteria were subsequently incubated with 50 mg/ml of proteolytic enzyme (Pronase E, Merck, Darmstadt, Germany), washed, adjusted photometrically and used in the DEAE-Sephacel adherence test.

RESULTS

The group B streptococcal type reference strains were chromatographed on DEAE-Sephacel and eluted from the gel matrix with phosphate buffer containing increasing concentrations of NaCl. The adherence pattern of the bacteria were expressed as percentage of the bacteria remaining adherent to the gel. Using an elution buffer containing 0.05 M NaCl, almost all bacteria remained on the gel matrix. However, elution with an elution buffer containing 1 M NaCl eluted almost all bacteria. Using 0.1 M NaCl in the elution buffer the group B streptococcal reference strains Ib, Ic, R and X remained on the gel matrix, the reference strains Ia, II, III, IV and V were mainly found in the eluate (Table 1).

The additional experiments were performed with elution buffer containing 0.1 M naCl. Screening of previously serotyped and characterized group B streptococci from bovines and humans revealed that cultures with type antigen patterns IV/X, NT/X and NT from bovines and cultures of serotypes Ia/c, II/R, III/R and NT/R from humans adhered strongly to DEAE-Sephacel. In contrast, most of the cultures of serotypes II and III from humans adhered weakly to DEAE-Sephacel. The bacteria were mostly found in the eluate (Table 2).

For further characterization of the adherent properties of the two bovine (G5 and G28) and two human (FHBS 4 and GHBS 690) group B streptococci with high and low adherence values, respectively, were selected. Treatment with neuraminidase significantly increased the adherence values of group B streptococcal cultures G28 and GHBS 690. In contrast, the adherence values of group B streptococcal cultures G5 and FHBS 4 were generally not affected by such treatment.. Pronase treatment of the neuraminidase-pretreated bacteria reduced the adherence values of all four group B streptococci (Table 3).

The role of encapsulation of group B streptococci in inhibiting the adherence of bacteria to the gel matrix could be confirmed with the encapsulated type III

Table 1. Adherence of group B streptococcal reference strains on DEAE- Sephacel after elution with phosphate buffer containing various concentrations of NaCl

Reference strains	Concentration of NaCl (mol 1^{-1})		
	0.05	0.1	1
090 (Ia)	79*	31	11
H36B (Ib)	93	93	9
A909 (Ic)	97	97	7
18 RS 21 (II)	92	32	9
6313 (III)	96	51	5
3139 (IV)	54	14	5
SS 1169 (V)	94	57	7
25/60 (R)	94	91	5
24/60 (X)	94	91	16

* % Adherence (duplicate determinations)

Table 2. The DEAE-Sephacel adherence values of group B streptococci isolated from bovines and humans

Serotype	n	% Adherence*
Bovine cultures		
II	5	73** (37-94)
IV	7	59 (9-75)
IV/c	4	66 (53-84)
IV/X	4	83 (56-99)
NT/X	5	84 (75-87)
NT	5	92 (87-94)
Human cultures		
Ia/c	9	91 (77-97)
II	6	62 (38-83)
II/R	5	94 (86-97)
III	7	64 (41-85)
III/R	9	91 (83-97)
NT/R	6	95 (86-99)

* Eluted with hosphate buffer containing 0.1 M of NACl

** The results are presented as mean of tested cultures, with the range of values in paranthesis

n = number of cultures with respective serotype

Table 3. Adherence of group B streptococci on DEAE-Sephacel before and after enzyme treatment

Treatment	Bovine culteres		Human cultures	
	G5	G28	FHBS 4	GHBS 690
None	90*	31	94	25
Neuraminidase	94	90	90	81
Neuraminidase+ pronase	35	6	42	31

* % Adherence (duplicate determinations)

Table 4. Adherence values of encapsulated and unencapsulated group B streptococcal strains on DEAE-Sephacel

Group B strepto coccal strain	Status	Serotype	% Adherence
COH 1	encapsulated	III	5
COH 1-11	asialo capsular mutant	(III)*	97
COH 31 r/s	encapsulated	III	40
COH 31-21	asialo capsular mutant	NT	96
48:LD	low density variant	IV	16
48:OS	high density original strain	NT/X	92
5531:LD	low density variant	III	69
5531:OS	high density original strain	NT	96

* = The antigen preparation obtained by hot acid extraction reacted with type III specific polyclonal antiserum but did not reacted with type III monoclonal antibodies (Wessels et al 1992)

NT = non typeable

group B streptocoaccal strains COH 1 and COH 31 r/s and their asialo capsular mutants COH 1-11 and COH 31-21. Both mutants adhered to the gel matrix with adherence values of 97% and 96% respectively for strain COH 1-11 and COH 31-21. The encapsulated parent strains adhered with adherence values of 5% and 40% for strains COH 1 and COH 31r/s, respectively. A clear difference in adherence values could be observed with the low density encapsulated variant 5531:OS and its isogenic, unencapsulated parent strain 5531:LD. The unencapsulated strain adhered with adherence values of 96%, the low density variant adhered with values of 69% (Table 4).

DISCUSSION

Studies on bacterial surface characteristics, such as charge and hydriphobic properties have provided valueable insights on the nature of bacterial surface. The surface hydrophobicity of streptococci of serological group B has been studied by salt aggregation and hexadecane adherence tests and hydrophobic interaction chromatography on phenyl-sepharose (Wibawan and Lämmler, 1990a, 1992; Wibawan *et al.*, 1992a). All these tests revealed a close type specificity of the surface charge with a generally hydrophilic surface of encapsulated group B streptococci and a hydrophobic surface mostly among group B streptococci with protein type antigens. In addition, the differences in the surface charge seemed to be closely related to chain formation of the group B streptococci and growth properties of the bacteria in fluid media and soft agar (Wibawan and Lämmler, 1990b; Wibawan *et al.*, 1992b). In the present study adherence properties of group B streptococci of various serotypes could be studied with the anion exchanger DEAE-Sephacel. The ion exchange chromatography technique with whole bacterial cells was originally described to study surface characteristics of *Vibrio cholerae* (Kabir and Ali, 1983). Ion exchange adherence studies of previously serotyped and characterized group B streptococci revealed high adherence values for cultures with protein antigens either alone or in combination with polysaccharide antigens. Cultures with polysaccharide antigens alone were mainly found in the eluate. This results are in agreement with previous hexadecane- and phenyl-sepharose adherence studies indicating close relationship between surface charge and the degree of sialylation of group B streptococcal microcapsule (Wibawan and Lämmler, 1992; Wibawan *et al.*, 1992a). Removal of microcapsule enhanced the adherence of the bacteria to the gel matrix, pronase treatment reduced the adherence values. As already described for hydrophobic surface proteins (Wibawan and Lämmler, 1992) the proteins responsible for the adherence to the anion exchanger seemed to be masked by capsular neuraminic acid. This could be additionally confirmed by two encapsulated group B streptococci, their asialo capsular mutant and by encapsulated variant of an originally unencapsulated parent strain. It was of interest that the ion exchange technique used in this study also allowed a differentiation between various degrees of encapsulation. Group B streptococcus COH 1 with adherence values of 5% has been described as being highly encapsulated, strain COH 31r/s with adherence values of 40% has been described as being poorly encapsulated (Wessels *et al.*, 1989, 1992). Capsualr sialylation of group B streptococci is well known as major virulence factor of this bacterial species. Group B streptococcal microcapsule has been shown to block the

phagocytosis of the bacteria (Wibawan and Lämmler, 1991; Wessels *et al.*, 1992). However, the adherence of group B streptococci to epithelial cells seemed to be inversly proportional to the degree of encapsulation (Wibawan *et al.*, 1992a). As already described for hydrophobic surface proteins of group B streptococci (Wibawan *et al.*, 1992a), the proteins detectable by ion exchange chromatography might support adherence propertie of the bacteria to host surfaces. Similar to the hydrocarbon adherence test or to use of hydrophobic matrix phenyl-sepharose (Wibawan and Lämmler, 1992; Wibawan *et al.*, 1992a,b) the adherence to the anion-exchanger DEAE-Sephacel used in this study might help to differentiate the capsular types or the unencapsulated, cell-adherent types of group B streptococci.

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