RELATION BETWEEN ENCAPSULATION OF STREPTOCOCCI OF SEROLOGICAL GROUP B AND ADHERENCE PROPERTIES OF THE BACTERIA TO DEAE-SEPHACEL

HUBUNGAN ANTARA KEBERADAAN KAPSUL DENGAN SIFAT PERLEKATAN BAKTERI STREPTOKOKUS GRUP B PADA DEAE-SEPHACEL

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ABSTRACT

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Determination of surface charge of group B streptococci by ion exchange chromatography on DEAE-Sephacel revealed that bovine and human group B streptococcal isolates with protein surface antigens alone, or bacteria with protein antigen in combination with polysacharide antigens, adhered strongly to the gel matrix. In contrary, cultures with polysaccharide antigens alone showed no comparable adherence properties. Removal of neuraminic acid from bacterial surface enhanced, but pronase treatment reduced the adherence values. The importance of type specific capsular sialylation for group B streptococcal surface charge could be confirmed with group B streptococci of serotype III and their transposon mutagenized asialocapsular mutants. In contrary to the encapsulated parent strains the asialo capsular mutants adhered strongly to the gel matrix. Comparable differences were observed with unencapsulated group B streptococcal variant strains and its isogenic encapsulated parent strains. The capsule material seemed to mask 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 importance of capsular sialylation for individual isolates of this bacterial species.

Key Words: group B streptococci, ion exchange chromatography, neuraminic acid, capsular sialylation

ABSTRAK

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Penentuan karakter permukaan bakteri streptokokus grup B menggunakan *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, biakan bakteri yang memiliki hanya antigen polisakarida tidak menunjukkan sifat perlekatan yang berarti. Penghilangan kapsul asam neuraminat dari permukaan sel bakteri meningkatkan nilai perlekatan tetapi sebaliknya perlakuan dengan pronase menyebabkan penurunan nilai perlekatan. 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. Berlawanan dengan sifat bakteri induknya, mutan bakteri ini menunjukkan nilai perlekatan yang kuat pada matriks jel. Hasil yang sama diperoleh pula dengan menggunakan bakteri berkapsul dan bakteri varianya yang tidak berkapsul. Kapsul membungkus komponen protein yang bertanggungjawab terhadap sifat perlekatan pada matriks jel. Penentuan sifat permukaan sel bakteri streptokokus grup B dengan menggunakan *ion-exchange chromatography* dapat membantu pemahaman tentang pentingnya peran kapsul pada bakteri.

Kata-kata Kunci: streptokokus group B, *ion exchange chromatography*, asam neuraminat, penentu sifat permukaan

INTRODUCTION

Streptococci of serological group B play an etiological role in bovine mastitis and human neonatal infections, particularly in septicaemia and meningitis (Jelinkova, 1977; Baker, 1980; Hahn, 1980). Though 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 distinct type specific cell surface antigens. Up to now, group B streptococci can be classified into seven major polysaccharide antigens Ia, Ib, II, III, IV, V and VI and three protein antigens c, R and X (Jelinkova, 1977; Henrichsen *et al.*, 1984; Jelinkova and Motlova, 1985). This polysaccharide and protein antigens

might occur singly or in combination. Part of the cultures were nontypable (Wibawan and Lämmler, 1990a; Wibawan *et al.*, 1992b; Lämmler *et al.*, 1993). A direct transmission of group B streptococci between bovines and humans remains uncommon (Brglez, 1981; Nielsen, 1987). Biochemical characteristics and the occurrence of this specific type antigens supported the conclusion that bovine and human group B streptococcal isolates form two distinct biotypes (Finch and Martin, 1984, Lämmler and Blobel, 1987, Wibawan *et al.*, 1991). However, among all polysaccharide serotypes sialic acid residues are present in the type specific capsule and play a role as major immunodeterminant 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 virulence factor of group B streptococci (Edwards *et al.*, 1982; Wessels *et al.*, 1992). The present study was designed to further characterize the relation between encapsulation and surface charge of the bacteria. For this purpose, the group B streptococci were chromatographed on the anion-exchanger DEAE-Sephacel.

MATERIALS AND METHODS

Bacteria Cultures

A total of 89 streptococci of serogical group B were used in this study. This included the 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 latter 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 had been separated from the original high density strain as low density variant by percoll gradient centrifugation. The centrifugation technique and further characteristics of the unencapsulated 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. All cultures had been serogrouped with autoclave 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 by 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). For this, DEAE-Sephacel was washed with 0.05 M phosphate buffer pH 6.8 and placed (1.15 ml) into a pasteur pipette (diameter 7 mm, length 30 mm). A 500 µl of the bacterial suspension adjusted photometrically (Bausch and Lomb, Rochester, New York USA) to 10 % at 620 nm, corresponding to 10^9 bacteri per ml was applied to gel matrix and eluted with 2.5 ml phosphate buffer containing 0.05, 0.1 and 1 M NaCl, respectively. The optical density of the eluate was measured at 540 nm and compared to the optical density of 500 µl 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 µg/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 adhered to the gel. Using an elution buffer containing 0.05 M NaCl almost all bacteria remained on the gel matrix. In contrast, with an elution buffer containing 1 M NaCl almost all bacteria were found in the eluate. 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 adherence properties two bovine (G5 and G28) and two human (FHBS 4 and GHBS 690) group B streptococci with high and low adhe-

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 (M)		
	0.05	0.1	1.0
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. 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)

*The results are presented as mean of tested cultures, with the range of values in paranthesis; elution was performed with phosphate buffer containing 0.1 M NaCl; n = number of cultures with respective serotype

rence values, respectively, were selected. Treatment of the bacteria 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 mainly unaffected. 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 group B streptococcal 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% for strain COH 1-11 and COH 31-21, respectively. The encapsulated parent strains adhered with adherence values of 5% and 40% for strains COH 1 and COH 31r/s, respectively. Comparable differences 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).

Table 3. Adherence of group B Streptococci on DEAE-Sephacel before and after Enzyme Treatment

Treatment	Bovine Cultures		Human Cultures	
	G5	G28	FHBS 4	GHBS 690
None	90*	31	94	25
Neuramindase	94	90	90	81
Neuramindase + 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 Streptococcal Strain	Status	Serotype	% Adherence
COH 1	Encapsulated	III	5*
COH 1-11	Asialo capsular mutant	NT**	97
COH 31 r/s	Encapsulated	III	40
COH 31-21	Asialo capsular mutant	NT	96
5531:LD	Encapsulated variant	III	69
5531:OS	Original strain	NT	96

*Duplicate determinations; **No reaction with monoclonal type III specific antibodies (Wessels *et al.*, 1992); NT = nontypable

DISCUSSION

Studies on bacterial surface characteristics, such as charge and hydrophobic 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 by 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 a 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 mutants and by an 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). Capsular 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 properties of the bacteria to host surfaces. Similar to the hydrocarbon adherence test or the adherence to the 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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