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The effects of deoxycholate and sodium dodecyl sulphate on the serological reactivity of antigens isolated from six Bacteroides reference strains

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Abstract

The detergents sodium dodecyl sulphate (SDS) and sodium deoxycholate (NaD) are frequently used as solvents for macromolecular polysaccharide complexes in immunochemical and serological techniques. The influence of the disaggregating surfactants on the serological reactivity of endotoxins isolated from six serotype specific reference strains of the Bacteroides fragilis group was investigated by comparing haemagglutinating and precipitating reactivities of antigen solutions in phosphate buffered saline (PBS), NaD and SDS. All antigens were phenol/water extracted endotoxins. Solutions of antigens isolated from serotypes A, B, C and D in PBS exhibited mainly serotype specificity and a few well known low-titer cross reactions; solutions in NaD showed additional cross reactivity, which was enhanced by solubilization of the antigens in SDS. In immunoelectrophoresis endotoxins isolated from serotypes A and C and dissolved in NaD or SDS showed additional precipitation lines compared to solutions of the same antigens in PBS.

These changes in the serological reactivity are of relevance for investigations where the serological specificty of antigens is in question.

Introduction

During our biological and immunochemical investigations of cell wall antigens of the Bacteroides fragilis group we were frequently confronted with the poor solubility of the phenol/water extracted antigenic fractions. In the B. fragilis group several polysaccharide surface antigens have been identified: capsular polysaccharide (Kasper 1976a), lipopolysaccharide (Kasper 1976b; Hofstad 1976; Poxton & Brown 1986) and other carbohydrate antigens (Hofstad 1981; Cousland & Poxton 1984; Reid et al. 1987). These are all present in the water-soluble phase after phenol/water extraction. We tried to enhance the solubility of this phase by applying disaggregating surfactants such as sodium deoxycholate (NaD) (Kasper 1976b) and sodium dodecyl sulphate (SDS), which are known to dissociate polysaccharide complexes into their subunits (Ribi et al. 1966; Oroszlan & Mora 1963; Galanos et al. 1977). The observation of changes in the serological behaviour of the thus solubilized antigens prompted to a more detailed investigation of the influence of detergents on the haemagglutinating and precipitating reactivities of the antigens.

Materials and methods

Bacterial strains

We used six culture collection strains of the Bacteroides fragilis group, representing six Bacteroides serotypes (Beerens et al. 1971): B. thetaiotaomicron NCTC 10582 (serotype A), B. ovatus ATCC 8483 (serotype B), B. vulgatus ATCC 8482 (serotype C), B. distasonis ATCC 8503 (serotype D), B. fragilis NCTC 9343 (serotype E_1) and B. fragilis IPL E 323 (serotype E_2).

Extraction and isolation of crude endotoxin (WL-fraction)

Bacteria were cultivated in yeast-broth medium (Werner 1968) at 37° C for 48 h. The cultures were centrifuged at 1500 × g and the deposit collected. The bacteria were extracted with aqueous phenol according to the method of Westphal et al. (1952). The water phase was dialysed against tap water and distilled water, filtered through a Schott G 5 filter, concentrated and lyophylized.

Dissolution of the WL-fraction

For dissolution in PBS 2 mg of WL-fraction were suspended in 1 ml PBS (pH 7.4). The suspension was kept in a boiling waterbath for 2 min and, if not completely dissolved, stirred at room temperature for 4 h. Undissolved material was removed by centrifugation (10 min 2000 × g) and the supernatant used in serological tests as antigen PBS. For serotypes A, B and D the undissolved residues were dissolved in 25 µl Triethylamine + 500 µl PBS. After stirring for 30 min at room temperature the solutions were used for sensitization of formalinized sheep erythrocytes. For dissolution in NaD 2 mg of WL-fraction were suspended in 250 µl sodium deoxycholate buffer pH 8.1 and dissolved by slow stirring at 20° C. An opalescent precipitate formed in several solutions was dissolved by adjusting the pH to 9,0 with 0.1 M NaOH. The solutions were then centrifuged at 1500 × g for 10 min and the clear supernatants precipitated with 5 volumes of cold absolute ethanol. The precipitates were kept overnight at 4° C, centrifuged at 2000 × g (10 min), washed twice with cold absolute ethanol, dissolved in 1 ml PBS (pH 7.4) and used in serological tests as antigen Deox. For dissolution in SDS 5 mg of WL-fraction were suspended in 2 ml of a 1% aqueous solution of SDS and the mixtures kept in a boiling waterbath for 30 min. The opalescent solutions were centrifuged at 1500 × g for 10 min and the supernatants lyophilized. The resulting material was treated with 1 ml absolute ethanol in a waterbath at 50° C, centrifuged at 2000 × g for 10 min and the residue washed twice with 1 ml absolute ethanol at room temperature. The sediment was then dissolved in 2 ml PBS (pH 7.4) and the opalescent solution used in serological tests as antigen SDS.

Antisera

The preparation of rabbit antisera against the six Bacteroides serotype reference strains was as previously described by Meisel-Mikołajczyk and Grzelak-Puczyńska (1981).

Haemagglutination tests

Sensitization of formalinized sheep erythrocytes and haemagglutination tests were performed as described by Beckmann et al. (1985). Antigen/antibody mixtures were incubated in microtiter plates at 37° C for 3 h and the results read after overnight storage at 4° C.

Immunoelectrophoresis

Electrophoresis on microscopic slides was performed in agarose gel (1% agarose in Laurell buffer at pH 8.3 (Laurell 1965) as described by Beckmann et al. (1985). The immune precipitates were stained according to the method of Weeke (1973) with Coomassie Brilliant Blue G 225.

Results

The six WL-fractions exhibited distinct solubilities in PBS. WL-fractions isolated from serotypes C, E_1 and E_2 formed opalescent solutions whereas WL-fractions obtained from serotypes A, B and D were not completely dissolved at a concentration of 2 mg/ml PBS. Solubilization of the residues in PBS was achieved by addition of triethylamine. In NaD and SDS all WL-fractions were soluble. This solubility pattern was repeatedly observed with different batches isolated at different times from the same culture collection strains.

After fixation of formalized sheep erythrocytes all antigenic solutions were investigated in haemagglutination tests. The results of homologous and heterologous reactions are summarized in Table 1.

The PBS-insoluble residues of endotoxins A, B and D, solubilized in PBS by addition of triethylamine, reacted with the same titers as the PBS-soluble fraction. No additional cross-reactions were observed.

We investigated the influence of the solvents on the precipitation line pattern of the antigenic fractions by means of the immunoelectrophoresis test for the antigens isolated from serotype A, C and E_2. The results are shown in Figs. 1 and 2.

In Fig. 1, immunoelectrophoresis tests with antigens isolated from serotype A, the solution in PBS showed one main precipitation line, and one weak line, also present after solubilization in NaD and SDS. After treatment with either surfactants a third precipitation line could be observed. The WL-fraction of serotype C (Fig. 2), soluble in all three solvents, developed an additional precipitation line after solubilization in NaD and, even stronger, in SDS. Weak precipitation lines in PBS, only faintly visible in the unstained agarose gel, were fully developed after SDS-treatment.

Table 1. Haemagglutinating reactivity of phenol/water-extracted Bacteroides antigens dissolved in PBS, deoxycholate and SDS.

Antigen serotype	Solvent	Reciprocal haemagglutination titres with antisera against Bacteroides serotype reference strains[a]					
		A	B	C	D	E_1	E_2
A	PBS[b]	320	–[o]	–	–	40	–
	NaD[c]	1280	–	10	–	160	–
	SDS[d]	10240	–	40	20	160	–
B	PBS	–	320	–	–	–	–
	NaD	–	640	20	10	10	–
	SDS	–	1280	20	10	40	–
C	PBS	–	–	10240	–	–	–
	NaD	–	–	10240	10	–	–
	SDS	–	10	10240	40	10	20
D	PBS	–	–	–	5120	–	20
	NaD	–	–	–	10240	20	20
	SDS	–	10	20	40960	40	20
E_1	PBS	–	–	10	10	1280	40
	NaD	–	–	20	20	1280	160
	SDS	–	–	20	20	5120	160
E_2	PBS	–	–	40	40	2560	10240
	NaD	–	–	40	40	2560	10240
	SDS	–	80	40	40	5120	10240

[a] : serotype
A : B. thetaiotaomicron NCTC 10582
B : B. ovatus ATCC 8483
C : B. vulgatus ATCC 8482
D : B. distasonis ATCC 8503
E_1 : B. fragilis NCTC 9343
E_2 : B. fragilis IPL E 323

[b] PBS : phosphate buffered saline
[c] NaD : sodium deoxycholate buffer
[d] SDS : 1% aqueous sodium dodecyl sulphate
[o] : haemagglutination titre < 1:10

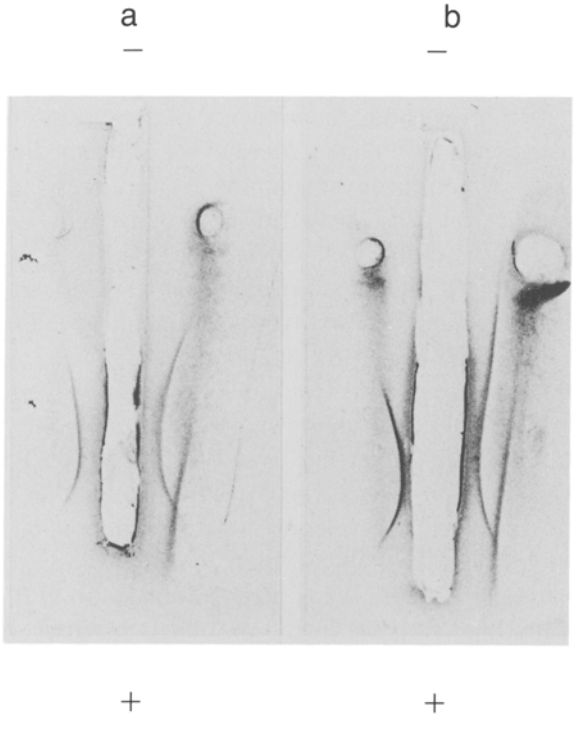

Fig. 1. Immunoelectrophoresis of antigen WL from B. thetaiotaomicron (serotype A) dissolved in PBS (*a* and *b* left), NaD (*a* right) and SDS (*b* right). Middle: antiserum B. thetaiotaomicron.

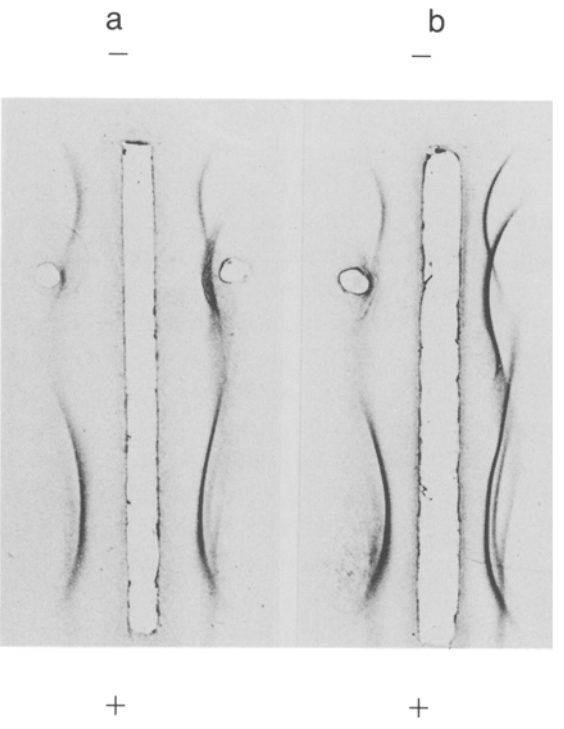

Fig. 2. Immunoelectrophoresis of antigen WL from B. vulgatus (serotype C) dissolved in PBS (*a* and *b* left), NaD (*a* right) and SDS (*b* right). Middle: antiserum B. vulgatus.

For WL E_2 no change in the complex precipitation line pattern was observed after treatment with NaD and SDS.

Discussion

Cross reactivity between lipopolysaccharides isolated from strains of the species Bacteroides fragilis has frequently been observed (Cousland & Poxton 1983; Hofstad 1977; Weintraub et al. 1985; Cousland & Poxton 1984). Incidentally also cross reactions between phenol/water extracted antigens of several serotype specific strains of the Bacteroides fragilis group were reported (Hofstad 1977; Meisel-Mikołajczyk & Gałkowska 1976; Babb & Communs 1981; Linko-Kettunen et al. 1984). Our results obtained with endotoxins dissolved in PBS are in accordance with these findings.

Typical endotoxins of gram negative bacteria are known to form a heterogenous population of aggregates with a particle size of at least 1×10^6 Daltons which are dissociated by the surfactant NaD and SDS into subunits of about 1×10^4 Daltons (Oroszlan 1963; Ribi et al. 1966; Galanos et al. 1977). After removal of NaD these subunits reaggregate to form a relatively uniform endotoxin with a particle size of $0.5–1 \times 10^6$ Daltons (Ribi et al. 1966). Kasper (1976b) demonstrated that the high molecular weight lipopolysaccharide isolated from Bacteroides fragilis dissociated in the presence of NaD into its monomers of approximately 12000 Daltons which aggregated after removal of the surfactant to particles with a higher molecular weight.

Responsible for the aggregation are primarily hydrophobic forces in the lipid A – part of the lipopolysaccharide, especially expressed in R-type lipopolysaccharides which lack the hydrophilic O-specific polysaccharidic side chains and are often

poorly soluble in aqueous solutions (Galanos et al. 1977). The lipopolysaccharides isolated from species of the B. fragilis group are not yet fully immunochemically analyzed, with the exception of B. fragilis. Investigations of the lipopolysaccharides of the species B. fragilis by Poxton & Brown (1986) and Weintraub et al. (1985) indicate that the lipopolysaccharide(s) are mainly of the R-type. R-type lipopolysaccharides in Bacteroides would explain the poor solubility of some of our endotoxins in aqueous solutions.

Our results show that dissociation of the Bacteroides endotoxins, a change in the physico-chemical state of antigens, can expose serologically reactive epitopes which are unaccessible in highly aggregated lipopolysaccharides present in aqueous solutions. This view is supported by the investigations of Brade et al. (1987) about the influence of the environment and the physico-chemical properties of lipid A on the exposure of antigenically reactive structures.

The results of this study indicate that the effect of detergents on the serological reactivity of phenol/water extracted endotoxins should be considered whenever antigenic solutions in detergents are used for determinations of serological specificities in antigens of the B. fragilis group.

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