Immunoreactivity of *Brucella melitensis* 16M Soluble Antigen (BmSA) after Ion-exchange Chromatography

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**ABSTRACT**

In the present study, soluble antigen of *Brucella melitensis* 16 M (BmSA) was prepared and purified by ion-exchange chromatography (DEAE-Sepharose) with linear gradient of NaCl (5 mM to 1 M) in Tris-HCl buffer (10 mM, pH 7.5). The fractions obtained constituted three distinct peaks, containing varying amount of protein and carbohydrate. The immune reactivity of these fractions in relation to lipo-polysaccharide (LPS) – protein association by enzyme-linked immunosorbent assay (ELISA) is described.

**Keywords:** *Brucella melitensis*, ELISA, ion-exchange chromatography, lipo-polysaccharide (LPS), soluble antigen (BmSA)

*Brucella*, a Gram negative, facultative intracellular pathogenic bacterium possess a mosaic of antigens with lipo-polysaccharide (LPS) being the immunodominant (Dubray, 1985; Wright and Nielson, 1990; Lapaque *et al.*, 2005). The heterogeneity of LPS and other antigen of brucellae have been investigated in order to understand the immune reactivity of the organism and host response. Such studies analyzing the structural association of LPS with other components such as protein could be vital in understanding the role played by the LPS-protein association in modulating the host immune response against *Brucella*. In the present study, an attempt was made to purify a soluble antigen prepared from *Brucella melitensis* 16M (BmSA) by ion-exchange chromatography and to assess the immune reactivity of the obtained fractions in relation to LPS-protein association by enzyme-linked immunosorbent assay (ELISA).

*B. melitensis* 16M maintained at the *Brucella* laboratory, Division of Veterinary Public Health, IVRI, Izatnagar was used in this study. The culture was tested for purity and biochemical characters before use. Thereafter, it was grown on potato-infusion agar in Roux flask and harvested in sterile phosphate buffer saline (PBS; pH 7.2) and washed thrice with the same buffer. The washed cells were used to prepare soluble antigen BmSA as per the method of Berman *et al.* (1980). Briefly, the cells were suspended in double distilled water in 1:4 ratio (w/v), shaken for 2 h, autoclaved for 20 min at 121°C and centrifuged at 17,000xg for 20 min at 4°C. The supernatant fluid was removed, stored at -25°C without preservatives and designated as *B. melitensis* soluble antigen (BmSA).

The antiserum was raised in three adult healthy rabbits by inoculating s/c 1 ml of sonicated *B. melitensis* 16M cells (1x10⁷/ml) in Freund’s incomplete adjuvant. A booster dose of the same was...
Fig. 1. Ion-exchange chromatographic profile of BmSA

Fig. 2. Protein and carbohydrate content in various fractions of peaks obtained on ion-exchange chromatography of BmSa

Fig. 3. Immunoreactivity of various fractions obtained on ion-exchange chromatography in plate ELISA
given after 15 days. The rabbits were bled by heart puncture seven days after booster, serum separated, pooled and stored at −20°C in aliquots.

The BmSA was purified by ion-exchange chromatography following the method of Denoel et al. (1997) with suitable modification. Briefly, BmSA (10 mg) was applied on a DEAE-sepharose (Sigma) column (bed volume 21 ml) equilibrated with 5 mM NaCl in tris- HCl buffer (10 mM; pH 7.5) and the column was washed with 105 ml of the same buffer. It was then eluted with 120 ml linear gradient of NaCl (5 mM to 1M) in tris-HCl buffer at a flow rate of 20 ml/h. Elutes were collected in 2 ml volume and their absorbance was recorded at 280 nm.

The chemical analysis of fractions for protein and carbohydrate was performed. The protein content was determined from their absorbance at 260 and 280 nm, while the carbohydrate content was estimated by phenol-sulphuric acid method (Dubois et al., 1956).

The ELISA was performed as described by Reizu-Boj et al. (1986) with reference serum as primary antibody, anti-rabbit IgG HRPO (Sigma) as secondary antibody and OPD (Sigma) as substrate. The optical density (OD) was taken at 492 nm in ELISA reader (ECIL, India).

Many attempts have been made to study the association of protein and LPS of Brucella. Several workers have reported that association of protein with LPS, results in heterogeneity to Brucella antigens (Moreno et al., 1987; Sewa et al., 1991). In the present study, BmSA on ion-exchange chromatography got separated into three distinct peaks (Fig. 1). Various workers have reported different elution profiles with gel-permeation or ion-exchange chromatography, probably due to variation in antigen of Brucella used in the chromatography (Bhongbhibhat et al., 1970; Hoffman and Houle, 1986; Gupta, 1993).

The BmSA fractions obtained after ion-exchange chromatography were found to contain varying amounts of protein and carbohydrate (Fig. 2). Gupta et al. (1995), too, have reported similar association of protein and LPS in case of B. abortus S99 and believed this to affect the humoral immune response against Brucella. Even the use of high salt concentration reportedly failed to release protein tightly complexed with LPS in native state of the cell (Osborn and Wu, 1980; Moriyon and Berman, 1983).

Such strong association between protein and LPS of various Gram negative bacteria has been reported which is particularly strong in case of Brucella spp. (Hoffman et al., 1986; Moreno et al., 1987; Moriyon et al., 1987). LPS is the major component of the outer cell membrane of Gram negative bacteria (Cardoso et al., 2006) including Brucella and reportedly has profound effect on immune system besides being of great significance in pathophysiology of disease process (Ritting et al., 2003).

As evident from Fig. 3, various fractions of chromatography were positive to ELISA. Whereas, the OD at 492 nm of negative control was 0.080. The ELISA positivity did not correlate with peak obtained (Fig. 1). A close perusal of figures 2 and 3 reveal the association of carbohydrate content of the fraction and its immune reactivity in ELISA. As the protein content of all the fractions was kept constant for coating the plate, the increase in the values of OD appear to be related with increase in carbohydrate or LPS content of fractions. This observation is in agreement with the fact that the LPS is the most immunodominant antigen in all smooth Brucella strains that are of livestock importance (Abdoel et al., 2008). Based on this assumption most of the serological tests which diagnose brucellosis rely on the unique antigen properties of LPS (Munoz et al., 2005; Gall et al., 2006; Glynn, 2008).

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References


