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Isolation, Purification, and Partial Characterization of *Brucella abortus* Matrix Protein

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Peptidoglycan sacculi with peptidoglycan-associated proteins were prepared from cell envelopes of *Brucella abortus* by extraction with sodium dodecyl sulfate (SDS) at 50°C. On extraction of these preparations with SDS at 100°C, a protein was obtained whose removal from peptidoglycan was confirmed by electron microscopy. Incubation of the 50°C SDS-extracted cell envelopes with 50 mM $MgCl_2$ in SDS-2-β-mercaptoethanol at 37°C also extracted the protein, along with lipopolysaccharide. At temperatures below 60°C, the protein did not bind SDS strongly and had an apparent molecular weight greater than 92,000 in SDS-polyacrylamide gel electrophoresis. At higher temperatures, SDS bound strongly, and the apparent molecular weight was 38,000. Urea at 5 M did not alter the electrophoretic mobility of this 38,000-molecular-weight form. Immunoelectrophoresis in detergents with antisera to cell envelopes, carbohydrate staining of SDS-polyacrylamide gels, and production of anti-lipopolysaccharide antibodies by mice immunized with the purified protein indicated that lipopolysaccharide was present in free and protein-bound forms. Sequential gel filtration in SDS-EDTA and SDS-NaCl removed most lipopolysaccharide. After further purification by preparative SDS-polyacrylamide gel electrophoresis, a gas-liquid chromatographic analysis showed residual lipid tightly associated with the protein. The results suggested that the interactions between matrix proteins and other outer membrane components are stronger in *B. abortus* than in *Escherichia coli*, which was used as a control throughout.

Extensive work on the major outer membrane proteins of gram-negative bacteria, primarily *Enterobacteriaceae* (6, 33), has shown them to include a set of proteins with molecular weights (MWg) of about 40,000 (40K) that are tightly associated with the peptidoglycan (36). Because of their regular arrangement on the peptidoglycan, these proteins are often referred to as matrix proteins (36, 39). They constitute the diffusion pores through which hydrophilic substances of low MW penetrate the outer membrane (26, 27) and are essential for assembly of the outer membrane (29, 44). Analogous proteins have been described for other gram-negative bacteria (14, 21, 23).

There has been relatively little work on the outer membrane proteins of *Brucella* spp. Dubray and Bezard (8) have isolated three proteins of MW 37K, 25K, and 15K by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of lysozyme-digested peptidoglycan sacculi of *Brucella abortus* that had been prepared by extracting the cell envelopes with SDS at 100°C and found them to be protective immunogens in a mouse model for brucellosis. Verstreate et al. (42) have identified three clusters of proteins in the outer membrane of *B. abortus*, two of which (MWs of 43K to 41K and 30K) could be isolated by ion-exchange chromatography after extraction of lysozyme-digested cell envelopes with the dipolar ionic detergent Zwittergent 314 (Calbiochem, La Jolla, Calif.). In a preliminary report (I. Moriyon and D. T. Berman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, K171, p. 166), we indicated that a 38K protein of cell envelopes of *B. abortus* behaved as a peptidoglycan-associated protein as defined by Lugtenberg et al. (21), and a similar finding has been reported by Verstreate et al. (42) for their group 2 proteins. In the present report, we extend these findings and describe a method for the isolation of the matrix protein(s) of *B. abortus* in a nondenatured state. A partial characterization of the 38K protein is also presented.

MATERIALS AND METHODS

Bacterial cultures and cell envelope preparation. The characteristics of *B. abortus* rough (R) strain 45/20 and

smooth (S) strain 1119-3 and methods of preparing cell envelopes have been described previously (13, 25). Briefly, logarithmic-phase cells were harvested by centrifugation from cultures in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) dialysate in flasks on a rotatory shaker. After being washed with saline, the cells were suspended in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.5). DNase and RNase (100 μg/ml each) were added, and the cells were broken in a colloid mill (Mini-Mill; Greerco Corp., Hudson, N.H.). Cell envelopes were recovered by ultracentrifugation and stored at −20°C in HEPES. *E. coli* K-12 1485 F^+ cell envelopes were prepared by disruption in a French pressure cell as described by Diedrich et al. (5).

Preparation of Rosenbusch envelopes and extraction of matrix proteins. Peptidoglycan sacculi with their associated and covalently linked proteins (Rosenbusch envelopes) were prepared by the SDS differential heat extraction method of Rosenbusch (36). Briefly, cell envelopes were suspended in 10 mM Tris-hydrochloride (pH 7.5)–2% SDS–0.7% 2-β-mercaptoethanol–10% glycerol (SDS-βM-buffer) at 5 mg of cell envelope protein per ml, incubated at 50°C for 30 min, and then centrifuged at 100,000 × *g* for 1 h. Where indicated, the pellet was reextracted under identical conditions.

Matrix proteins were extracted by three different methods. First, Rosenbusch envelopes were resuspended in SDS-βM buffer and extracted at 100°C for 1 to 2 min (36). After centrifugation at 100,000 × *g* for 1 h, supernatant fluids containing the matrix proteins were collected and stored at 4°C. Second, Rosenbusch envelopes were extracted in SDS-βM buffer containing 0.5 M NaCl at 37°C for 30 min, and matrix proteins were recovered after centrifugation as described above (30). Third, similar extractions were performed in SDS-βM buffer containing 50 mM $MgCl_2$. When necessary, proteins were concentrated by precipitation with acetone and either redissolved in SDS-βM buffer or dialyzed extensively, first against 20% acetone in water and then against distilled water, and stored at −20°C.

Electron microscopy. Rosenbusch envelopes were negatively stained with 1% neutral phosphotungstic acid and examined with a Hitachi HU 11 E electron microscope.

Gel filtration chromatography. Gel filtration was performed in Sephacryl S-300 (Pharmacia Fine Chemicals, Piscataway, N.J.) in columns (2.6 by 85 cm). The buffer systems used were 10 mM Tris-hydrochloride (pH 7.5)–0.25% SDS (Bio-Rad Laboratories, Richmond, Calif.)–5 mM EDTA–0.05% NaN_3 (SDS-EDTA buffer) and 10 mM Tris-hydrochloride (pH 7.5)–0.25% SDS–100 mM NaCl–0.05% NaN_3 (SDS-NaCl buffer). The same buffer systems with SDS replaced by Zwittergent 316 (Calbiochem, La Jolla, Calif.) were also used. Columns were calibrated with bovine serum albumin, egg albumin, and lysozyme (Sigma Chemical Co., St. Louis, Mo.). Stokes radii for these markers in SDS are 7.8, 5.8, and 2.75 nm, respectively (40).

SDS-PAGE. SDS-PAGE was performed by the method of Laemmli (18). Gels were stained for proteins with Coomassie blue (9) and for carbohydrates with Schiff reagent by the method of Zacharius and Zell (46). MW standards were phosphorylase B (94K), bovine serum albumin (67K), aldolase (40K), carbonic anhydrase (29K), hemoglobin (4 × 16K) and cytochrome *c* (12K).

Trichloroacetic acid-extracted, phenol-extracted, and purified phenol-extracted lipopolysaccharides (LPS) were prepared from *B. abortus* 1119-3 as described previously (2, 24) and analyzed by SDS-PAGE. LPS-gels were stained for proteins by the alkaline silver method (32).

Preparative SDS-PAGE. Preparative SDS-PAGE was performed as described by Chai and Foulds (3). Purified proteins were precipitated with trichloroacetic acid at 4°C, dialyzed extensively against 20% acetone and water, and lyophilized.

Limited proteolysis in SDS-PAGE. Matrix proteins of *B. abortus* 45/20 and *Escherichia coli* were first purified by preparative SDS-PAGE. Alternatively, viable cells of *B. abortus* 45/20 and 1119-3 were extrinsically labeled by the [^{125}I]lactoperoxidase catalysis method (22) and extracted with SDS-βM buffer at 100°C. After SDS-PAGE of these extracts, the gels were stained with Coomassie blue, and the selected proteins were cut from the gel. Limited proteolysis was carried out with *Staphylococcus aureus* V-8 protease (Miles Laboratories, Inc., Elkhart, Ind.) during SDS-PAGE (4, 7), and the gels were stained with Coomassie blue or autoradiographed on Kodak XAR-5 X-ray film.

Analytical methods. 2-Keto-3-deoxyoctulosonic acid (KDO) was measured by the thiobarbituric acid method corrected for interference by 2-deoxy sugars (25, 43). Protein was determined by the method of Lowry et al. (20). When KDO and proteins were measured with material that otherwise would be dissolved or extracted in SDS-βM buffer, 2-β-mercapthoethanol and glycerol were left out of the buffer. For fatty acid determination, 1 to 2 mg of purified protein was hydrolyzed with 2 N HCl as described by Kates (16), and the methyl esters were analyzed in a Varian 3700 gas-liquid chromatography apparatus equipped with a CDS 111 integrator with known amounts of archidate as internal standards. Fatty acid standards were obtained from Supelco, Inc., Bellefontaine, Pa., and Sigma.

Antisera. Antisera to cell envelopes of *B. abortus* 1119-3, *B. abortus* 45/20, and *E. coli* were raised in New Zealand white rabbits as described by Smyth et al. (38). Antibodies to matrix proteins were produced in outbred ICR mice. Protein bands cut from SDS-polyacrylamide gels after a brief staining were homogenized in 0.15 M NaCl and administered intraperitoneally in Freund complete adjuvant, at 5 μg/ml in 0.2 ml per animal, weekly for 4 weeks. Four days after the last injection, ascites was induced with sarcoma 180 cells, and ascitic fluid was collected (37).

Purification of rabbit IgG. The immunoglobulin G (IgG) fractions of rabbit antisera were purified by chromatography on protein A-Sepharose 4B (35), dialyzed against 20 mM phosphate-buffered saline (pH 7.2)–0.05% NaN_3, and concentrated by ultrafiltration on an Amicon OM 20 membrane.

Immunoelectrophoresis. Immunoelectrophoresis was carried out in 0.04 M sodium barbital-hydrochloride buffer (pH 8.6) containing 1% Triton X-100 (Eastman Kodak, Rochester, N.Y.; scintillation grade) in both reservoirs and gels (1% agarose, Sigma type I) for 60 min at 5 to 10 V/cm with running tap water as the coolant.

ELISA. *B. abortus* S LPS was attached to polysty-

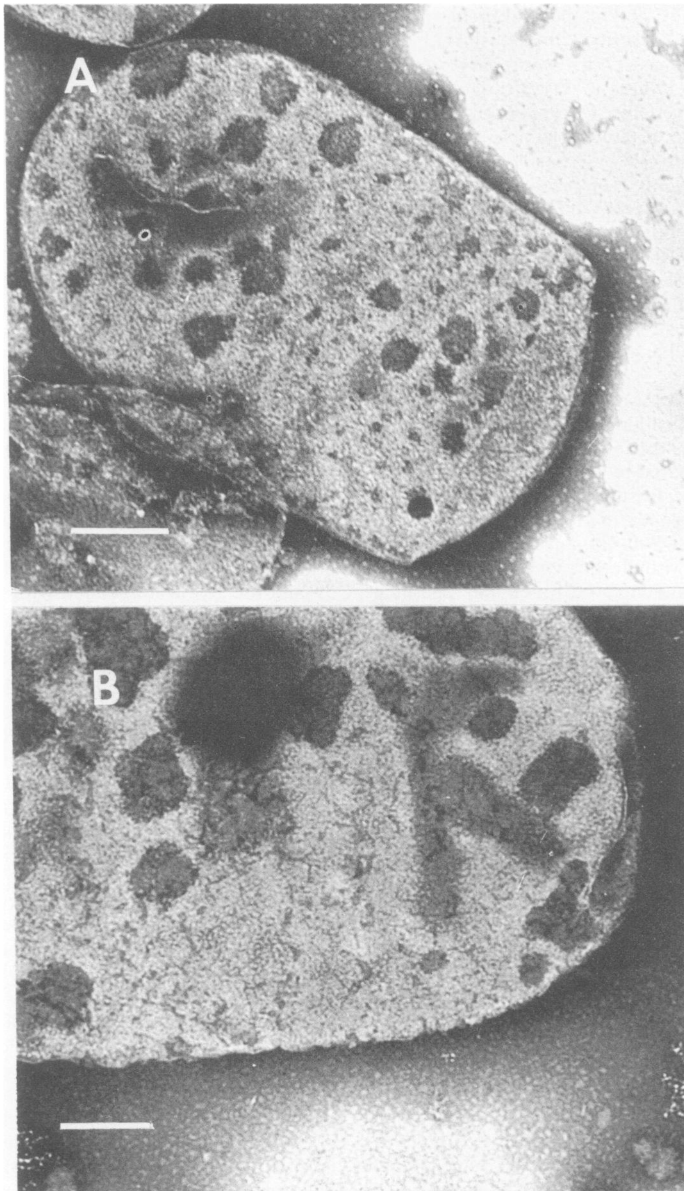

FIG. 1. Electron micrographs of *B. abortus* cell envelopes (A) and *E. coli* K-12 cell envelopes (B) extracted with SDS at 55°C. Bar, 150 nm.

rene microtiter plates by overnight incubation of a 1 μg/ml solution in 0.06 M carbonate buffer (pH 9.6) at 37°C. Rabbit anti-mouse IgG (Miles) was conjugated to horseradish peroxidase (Sigma type VI) (31). Antigen-antibody reactions were allowed to proceed at room temperature overnight, and the enzyme-linked immunosorbent assay (ELISA) was performed as described previously (19).

RESULTS

Differential heat extraction of matrix proteins. To determine the presence in *B. abortus* of a matrix protein(s), we first used sequential SDS-heat extraction (36). Electron micrographs of cell envelopes extracted with SDS-βM buffer at 50°C showed the arrangement of *E. coli* matrix proteins on the peptidoglycan and a similar image for *B. abortus* (Fig. 1). Upon reextraction at 100°C in SDS-βM buffer, both the *E. coli* and the *B. abortus* matrix proteins were removed from the peptidoglycan, and a conspicuous protein with a MW of about 38K was detected by SDS-PAGE in the extracts, along with smaller

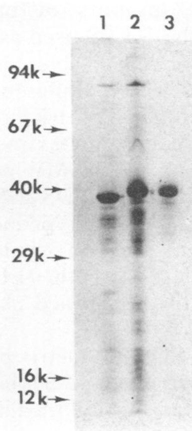

FIG. 2. SDS-PAGE analysis of matrix proteins prepared by the differential SDS-heat extraction method of Rosenbusch (36). Lanes: 1, *B. abortus* 45/20; 2, *B. abortus* 1119-3; 3, *E. coli* K-12.

amounts of several other proteins of similar MW (Fig. 2). As with *E. coli*, *B. abortus* matrix proteins were released from the peptidoglycan by SDS at temperatures above 60°C. However, in contrast to *E. coli*, when Rosenbusch envelopes of *B. abortus* were prepared, it was necessary to repeat the extraction at 50°C to obtain low levels of background in SDS-PAGE. An analysis of the supernatants of successive extractions with SDS-βM buffer at 50°C confirmed that background levels of both protein and KDO

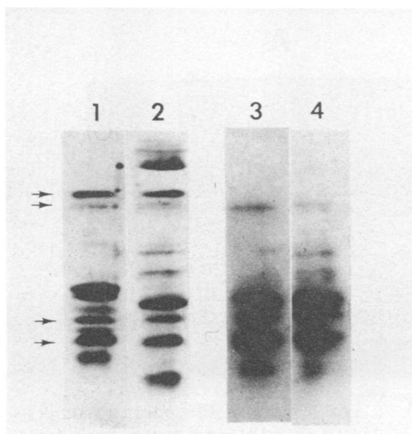

FIG. 3. SDS-PAGE analysis of peptides obtained by limited proteolysis of matrix proteins. Proteins were prepared as described in the text, and proteolysis was carried out during stacking with 2 μg of *S. aureus* V-8 protease. Gels were stained with Coomassie blue (lanes 1 and 2) or autoradiographed (lanes 3 and 4). Lanes: 1, *E. coli* K-12; 2, *B. abortus* 45/20; 3, *B. abortus* 45/20; 4, *B. abortus* 1119-3. Arrows, *E. coli* and *B. abortus* proteolytic fragments of similar MW.

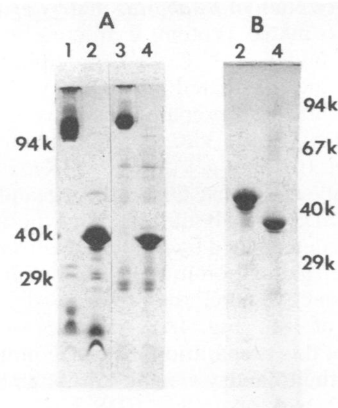

FIG. 4. SDS-PAGE analysis without urea (A) or in the presence of 5 M urea (B) of matrix proteins extracted from Rosenbusch envelopes with 50 mM $MgCl_2$ in SDS-βM buffer at 37°C. Lanes: 1, *E. coli* K-12 incubated at 37°C; 2, *E. coli* K-12 incubated at 100°C; 3, *B. abortus* 45/20 incubated at 37°C; 4, *B. abortus* 45/20 incubated at 100°C.

were reached more easily for *E. coli* than for *B. abortus* (not shown). Even after repeated extraction at 50°C, *B. abortus* matrix protein preparations also contained an 88K protein (Fig. 2). A protein of similar MW was removed from *E. coli* cell envelopes during the first extraction with SDS at 50°C.

The apparent MW of *B. abortus* 45/20 matrix proteins in SDS-PAGE was somewhat less than that of *B. abortus* 1119-3 or *E. coli* matrix proteins (Fig. 2). Peptide profiles after limited proteolysis of matrix proteins from both S and R *B. abortus* were almost identical and were distinct from that of *E. coli* matrix protein (Fig. 3). The accessibility, in this experiment, of matrix proteins of *B. abortus* to extrinsic labeling by the [^{125}I]lactoperoxidase catalysis method indicated their exposure on the surface of both R and S strains.

Extraction of matrix proteins by NaCl and $MgCl_2$. Although *E. coli* matrix proteins were extracted in a nondenaturated state from Rosenbusch envelopes by 0.5 M NaCl in SDS-βM buffer at 37°C (30), *B. abortus* matrix proteins were not. However, $MgCl_2$ in SDS-βM buffer at 37°C extracted the matrix proteins, as well as LPS and smaller amounts of other proteins, from Rosenbusch envelopes of both *B. abortus* and *E. coli*. The best yields in a single extraction (60% of the matrix protein extracted at 100°C) were obtained at $MgCl_2$ concentrations of 50 mM or greater for *B. abortus* and 100 mM for *E. coli*. When SDS was replaced by Zwittergent 316, 10 to 20 mM $MgCl_2$ was enough to extract the matrix proteins from Rosenbusch envelopes of both bacteria.

Characterization of *B. abortus* matrix proteins. *B. abortus* matrix proteins extracted by $MgCl_2$–SDS-βM buffer at 37°C were precipitated with acetone and incubated in SDS-βM buffer at 37°C. When these preparations were examined by SDS-PAGE, the 38K protein remained at the top of the tracking gel (MW, >94K) (Fig. 4A). After dialysis against 20% acetone and water, retained SDS was about 0.001% of the total dry weight as determined by gas-liquid chromatography. After incubation in SDS-βM buffer at temperatures above 60°C, the protein had an apparent MW of 38K (Fig. 4A). After acetone-water dialysis of this preparation, SDS accounted for 2 to 3% of the total dry weight. Although both the MW shift and reversible SDS binding of the matrix proteins incubated at 37°C were similar for *E. coli* (30, 36), only traces of SDS were detected in matrix proteins of *E. coli* incubated in SDS-βM buffer above 60°C after acetone-water dialysis. Furthermore, although the mobility of *E. coli* matrix proteins denatured in SDS at 100°C decreased in SDS–5 M urea gels with respect to that in SDS gels, *B. abortus* matrix proteins had the same mobility in both gels (Fig. 4B).

LPS in matrix protein preparations. As stated above, KDO was released during the preparation of Rosenbusch envelopes until it reached a background level. Upon extraction with $MgCl_2$–SDS-βM buffer at 37°C, or at 100°C in SDS, additional KDO was released (data not shown). An immunoelectrophoretic analysis with antisera to whole cell envelopes revealed a major anodally migrating component in matrix protein preparations dissolved in SDS (Fig. 5A). A second precipitate line, formed by a diffuse smear from the first one, was observed only in preparations from S *B. abortus*. An SDS-PAGE analysis of the two precipitin lines showed that the 38K protein was present in the anodal precipitate but not in the second precipitate (Fig. 5B). This second precipitate was identified as S LPS by immunoelectrophoresis (Fig. 5A). The reaction of identity between these two precipitin lines suggests that interactions of LPS with the protein persist during electrophoresis and that the LPS exists in both loosely and more firmly protein-bound states.

SDS-PAGE of matrix proteins of the R strain extracted with $MgCl_2$–SDS-βM buffer showed that carbohydrate-staining material also existed in free and protein-associated forms (data not shown). This association existed with proteins incubated at either 37 or 100°C. Evidence that the carbohydrate-staining material represented, at least in part, LPS associated with the protein was obtained by detection, by ELISA, of antibody to S LPS in ascitic fluids of mice immunized with purified matrix protein. The possibility that small amounts of matrix protein contaminating the S LPS used as the antigen in ELISA could be responsible for these results (10) was tested in two different ways. First, although preparations of trichloroacetic acid-extracted and crude phenol-extracted *Brucella* S LPS both contained a 38K-MW protein, demonstrable in silver-stained SDS gels, this protein was absent from purified phenol-extracted S LPS (not shown). Second, similar titers were obtained regardless of whether ELISA was done with the crude or the purified phenol-extracted LPS.

When $MgCl_2$–SDS-βM matrix protein extracts were chromatographed in Sephacryl S-300 in SDS-EDTA buffer, most of the matrix protein of *B. abortus* was recovered in the void volume, with a small fraction of the same protein eluting in a second peak (Fig. 6A). A third peak contained the 30K protein, and LPS was partitioned among the three peaks. When the first and second peaks were pooled and rechromatographed in SDS-NaCl buffer, most of the protein eluted in the second peak, and most of the LPS eluted after the protein (Fig. 6B). The *B. abortus* protein peak eluted at the same position as the nondenatured *E. coli* matrix protein (5.3 nm). Although Zwittergents with alkyl chain lengths greater than 10 are very effective in dispersing LPS (25), when Zwittergent 316 was substituted for SDS, the protein standards, matrix protein, and LPS were all eluted in the void volume regardless of the ionic strength of the buffer (not shown).

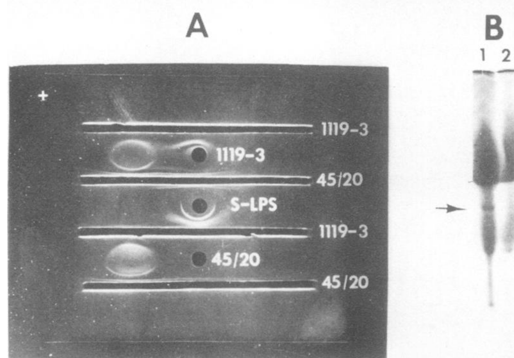

FIG. 5. (A) Immunoelectrophoretic analysis of *B. abortus* 1119-3 S LPS and matrix proteins of the indicated strains of *B. abortus* (wells); troughs contained rabbit IgG against whole crude cell envelopes of the indicated strains. (B) SDS-PAGE of the anodic precipitin line of *B. abortus* 1119-3 (lane 1) and the *B. abortus* 1119-3 precipitin line closer to the well (lane 2). The smears in SDS-PAGE were due to the presence of Triton X-100 and represent the heavy and light chains of the IgG. The matrix protein in lane 1 is indicated by the arrow.

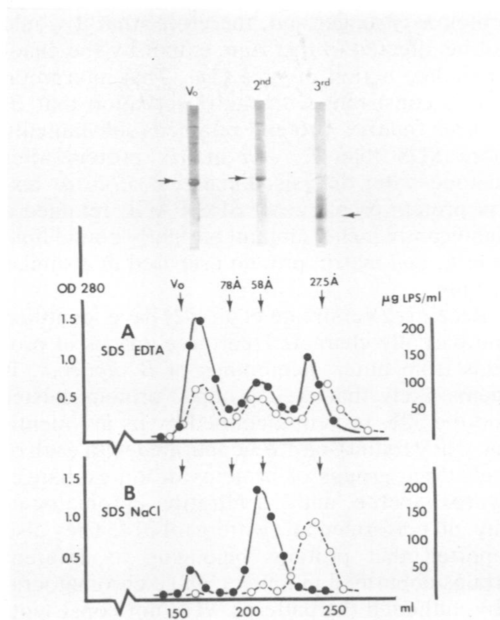

FIG. 6. Sephacryl S-300 gel filtration of matrix proteins. (A) *B. abortus* 45/20 matrix proteins extracted with 50 mM $MgCl_2$ in SDS-βM buffer, equilibrated, and chromatographed in SDS-EDTA buffer. (B) V_0 and second peaks of (A) pooled, equilibrated, and chromatographed in SDS-NaCl buffer. Symbols: ○, KDO (LPS); ●, protein. OD 280, Optical density at 280 nm.

KDO measurements showed that LPS remained associated with the protein after repeated gel filtration in SDS-NaCl buffer (1.6 μg of KDO per mg of protein). In an attempt to remove this LPS, the protein was denatured by boiling in SDS at 100°C and purified further by preparative SDS-PAGE, and its fatty acid content was then determined by gas-liquid chromatography (Table 1). Although these preparations contained significant amounts of fatty acids that are also present in the LPS, the relative proportions of the acids differed from those reported for the LPS or cell envelopes of *B. abortus* (1, 24). *E. coli* matrix protein preparations purified and analyzed by the same methods contained traces of β-hydroxymyristic acid and seven to nine times less total fatty acid per milligram of protein than that in preparations from *B. abortus*.

TABLE 1. Fatty acid composition of *B. abortus* S LPS and *B. abortus* matrix protein purified by sequential gel filtration in SDS-EDTA and SDS-NaCl buffers followed by preparative SDS-PAGE[a]

Fatty acid[b]	% of total fatty acid in:		
	LPS	Prepn I	Prepn II
A	5.4	12.3	5.2
16:0	76.4	29.9	31.0
16:1	0.4	6.3	8.5
B		4.6	3.6
18:0	6.9	8.6	9.4
18:1	5.4	21.5	25.9
C		5.6	9.5
D		3.8	2.5
E	1.3	2.1	1.2
F	7.3	5.6	3.1

[a] Results obtained with two different matrix protein preparations (I and II) are presented. The total fatty acid content was 15 μg per mg of protein for preparation I and 18 μg per mg of protein for preparation II.

[b] Letters represent unknown fatty acids.

DISCUSSION

Our results show that cell envelopes of *B. abortus* contained a protein similar to the matrix protein of other gram-negative bacteria (14, 21, 23, 36). The 38K protein was not extracted by SDS at temperatures below 60°C and remained closely associated with the peptidoglycan. At temperatures above 60°C, the protein underwent denaturation, was extracted from the peptidoglycan, exhibited its 38K MW in SDS-PAGE, and bound SDS strongly. Matrix proteins present in S and R strains had slightly different apparent MWs. Since as many as three proteins with MWs clustered around 38K were observed, it is possible that the two strains studied might express different major proteins. Alternatively, tightly bound LPS, along with differences in the carbohydrate content of S and R LPS (24), could explain different mobilities for an otherwise identical protein. In view of the presence of such a tightly protein-bound LPS and the identical proteolytic patterns given by both proteins, we favor the second alternative.

In contrast to *E. coli* matrix proteins, *B. abortus* matrix proteins were not extracted by NaCl in SDS-βM buffer at 37°C. Both *E. coli* and

B. abortus matrix proteins were extracted by $MgCl_2$ in SDS-βM buffer at 37°C. The *B. abortus* 45/20 matrix proteins prepared by extraction with $MgCl_2$ in SDS-βM buffer were in a nondenatured state, as shown by (i) their ability to react with antibodies produced against cell envelopes, (ii) the fact that, in contrast to the proteins denatured by SDS at 100°C, SDS could be completely removed, and (iii) their identity with nondenatured (31) *E. coli* matrix proteins with respect to mobility in SDS-PAGE and behavior during gel filtration in SDS. Since nondenatured *E. coli* matrix protein has a Stokes radius of 5.3 nm in SDS (45), this should also be valid for *B. abortus* matrix protein (40). A Stokes radius of 5.3 nm and association with the peptidoglycan imply that the protein should span the outer membrane, which is in agreement with the accessibility of the 38K protein to extrinsic labeling.

LPS contaminating the matrix protein preparations was in both free and protein-bound states in the presence of detergents. Most contaminant proteins and LPS were removed by sequential gel filtration in SDS-EDTA and SDS-NaCl buffers. The differences observed between the protein distributions in SDS-EDTA and SDS-NaCl buffers were presumably due to different states of aggregation of the protein itself (I. Moriyon and D. T. Berman, unpublished data), and similar observations have been made for enterobacterial matrix proteins (28, 41). The remaining LPS was tightly bound and could not be removed by preparative SDS-PAGE. Although *B. abortus* LPS lacks fatty acids that, like β-hydroxymyristic acid of enterobacterial LPS, can be used unequivocally as LPS markers, most of its fatty acids consist of palmitic, stearic, and oleic acids (1, 24). The relative proportions of these fatty acids associated with *Brucella* matrix protein were different from those of either the LPS or the cell envelopes (1, 24), and they probably represent a mixture of lipids from both origins.

We have previously interpreted the resistance of the *B. abortus* cell envelope to nonionic detergents and EDTA (25) as indicating that it is more hydrophobic than that of *E. coli*. Some of the differences between *B. abortus* and *E. coli* matrix proteins may also be understood in that context. First, for *B. abortus*, but not for *E. coli*, SDS extraction at 50°C had to be repeated to reduce the amounts of contaminating LPS and protein. This could be the result of stronger hydrophobic associations among the envelope components. Second, urea reduces the interaction of SDS with *E. coli* OmpC matrix protein (45), and this could account for its apparent MW shift in urea gels (34). It is possible that the interaction between SDS and *B. abortus* matrix protein is stronger and, therefore, that it would not be affected to the same extent by the chaotropic-like action of urea (11). This interpretation is consistent with the observation that *B. abortus* matrix protein retained substantially more SDS than *E. coli* matrix protein after acetone-water dialysis. Finally, *B. abortus* matrix protein prepared by SDS-PAGE retained a significantly higher amount of tightly bound lipid than *E. coli* matrix protein prepared in a similar fashion.

Recently, Verstreate et al. (42) have identified and partially characterized three groups of proteins from outer membranes of *B. abortus*. It seems likely that their group 2 protein cluster and the 38K protein identified by us are identical. They found that LPS copurified with each of their three groups of proteins in ion-exchange, hydroxyapatite, and gel filtration chromatography, all performed in Zwittergent 314. They also reported that proteins belonging to different groups copurified in ion-exchange chromatography, although the patterns were not consistent. Our results suggest that the high micellar weight of the Zwittergents could be responsible, at least in part, for those associations. Similar problems have been encountered by others (12) in the purification of *E. coli* outer membrane proteins in the presence of Triton X-100.

The extraction and purification procedures described in this work provide an alternative method to those described by Dubray and Bezard (8) and Verstreate et al. (42) for obtaining *B. abortus* matrix proteins in a nondenatured state for investigation of their immunological and biological properties. It will be necessary in such investigations to take into account effects that could be attributable to the tightly bound LPS (10, 15, 17).

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