

Purification and Partial Characterization of the Major Outer Membrane Protein of *Haemophilus somnus*

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We purified the major outer membrane protein (MOMP), which is the most abundant OMP (with an apparent molecular mass of 40 kDa), from *Haemophilus somnus* strain 8025. The method involves solubilization of the MOMP with Zwittergent 3-14 and further purification accomplished by ion-exchange and molecular-sieve chromatographies. The amino-terminal sequence of the MOMP showed considerable similarity to those of porin proteins from other gram-negative bacteria. The MOMP of *H. somnus* is immunogenic to rabbits and calves. Hyperimmune sera from rabbits and calves reacted with both the MOMP and lipopolysaccharides in enzyme-linked immunosorbent assay (ELISA) and immunoblot analysis. The rabbit antiserum to the MOMP was cross-reactive with whole-cell preparations from strains 8025, D1238, NT2301, and 540 at a band with a molecular mass of 40 kDa in immunoblot analysis, although the reactivity of the rabbit antiserum with strain 540 was lower than those with the other strains tested. Two murine monoclonal antibodies (MAbs) to the MOMP were developed. ELISA with the OMP fractions as the antigens showed that one MAb was cross-reactive with the four strains but that the other MAb was reactive with the three strains other than strain 540. These results indicate that the MOMP of *H. somnus* possesses at least two antigenic determinants and that the MOMP of strain 540 is antigenically different from those of the other strains. The antigenic heterogeneity of the *H. somnus* MOMP has implications regarding the development of a serotyping system with MAbs that is based on the MOMP epitopes.

Haemophilus somnus is widely distributed throughout the world and causes a variety of diseases, including thromboembolic meningoencephalitis, pneumonia, and reproductive disorders in cattle (10). With the recognition of *H. somnus* as an important bovine pathogen, studies are currently under way to understand the pathogenesis and epidemiology of infection due to this bacterium.

H. somnus appears to lack a capsule, pili, and any surface structures other than outer membranes (21), so outer membrane proteins (OMPs) are thought to be important in the interactions between this organism and its host. Over the past years, several *H. somnus* OMPs have been investigated as potential vaccine candidates, virulence factors, and diagnostic antigens (2).

Studies of other gram-negative bacteria demonstrated that the major OMP (MOMP), which represents the most abundant OMP in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), is protective and shows some degree of antigenic heterogeneity among different strains (15, 22). The outer membrane of *H. somnus* contains the MOMP, which has an apparent molecular mass of 41 kDa (3); however, little is known about the characteristics of the MOMP of *H. somnus*. Therefore, we sought to purify the MOMP of *H. somnus* in order to characterize the MOMP.

We report here a method for purification of the *H. somnus* MOMP, determination of the amino-terminal sequence of the purified MOMP, and antigenic characterization of the *H. somnus* MOMP.

MATERIALS AND METHODS

Bacterial strains. *H. somnus* 8025 and 540 were obtained from L. Corboz, Universität Zürich, Zürich, Switzerland. *H.*

somnus 8025 was originally isolated from a case of bovine thromboembolic meningoencephalitis in the United States, and strain 540 was isolated from a case of bovine abortion in Switzerland (4). *H. somnus* D1238 and NT2301 were from our laboratory collections and were isolated in Japan from cases of bovine thromboembolic meningoencephalitis and bovine pneumonia, respectively. Bacteria were stored at -70°C in brain heart infusion broth (Difco Laboratories) containing 20% (vol/vol) glycerol. Bacteria were plated from frozen stocks on brain heart infusion agar (Difco Laboratories) plates containing 5% (vol/vol) bovine blood and 0.5% (wt/vol) yeast extract (Difco Laboratories) and then incubated at 37°C in 7% CO_2 . Broth cultures were prepared in brain heart infusion broth supplemented with 0.1% (wt/vol) Tris and 0.001% (wt/vol) thiamine monophosphate as described previously (5). Cells were harvested by centrifugation and stored at -70°C .

Preparation of OMP fractions. *N*-Lauroylsarcosine (Sarkosyl [Sigma])-insoluble OMP was prepared as described previously (5). Frozen cells (about 20 g [wet weight]) were suspended in 50 ml of 50 mM Tris-hydrochloride buffer (pH 7.8) containing 2 mM MgCl_2 and sonicated for 3 min in 15-s bursts in an ice bath. After the sonicated cell suspension was centrifuged at $10,000 \times g$ for 15 min to remove cell debris, the supernatant was centrifuged at $230,000 \times g$ for 70 min at 4°C . The pellet was suspended in 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) and extracted with an equal volume of 2% (wt/vol) Sarkosyl in 10 mM HEPES buffer for 20 min at room temperature. The insoluble residue was collected by centrifugation at $125,000 \times g$ for 110 min at 4°C .

Purification of MOMP. *H. somnus* 8025 was used to purify the MOMP of *H. somnus*. Purification steps were performed at room temperature unless otherwise stated. Preliminary experiments showed that 0.5% (wt/vol) and 1.0% (wt/vol)

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Zwittergent 3-14 (Calbiochem-Behring Corp.) in 50 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA resulted in the most effective extraction of the MOMP from the OMP fraction, whereas other detergents {1% [wt/vol] and 2% [wt/vol] sodium deoxycholate; 1.25% [wt/vol] heptyl-thio-glucoside; 0.5% [wt/vol], 1% [wt/vol], and 2% [wt/vol] Zwittergent 3-12; 1% [wt/vol] 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate [CHAPS]; and 1% [wt/vol] decanoyl-*N*-methylglucamide [MEGA 10] in 50 mM Tris-hydrochloride [pH 8.0]–10 mM EDTA} were less effective in extraction of the MOMP. Thus, the MOMP was solubilized by suspending the Sarkosyl-insoluble OMP fraction in 50 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA–0.5% (wt/vol) Zwittergent 3-14 (buffer A). After incubation at room temperature for 30 min, the insoluble fraction was removed by centrifugation at $105,000 \times g$ for 1 h. The soluble fraction (6.7 mg of protein) was applied to a DEAE-Sephacel (Pharmacia) column (1.5 by 8 cm) equilibrated with buffer A. The column was washed with 2 column volumes of buffer A, and the MOMP was eluted in 1.2-ml fractions with a linear gradient of 0 to 0.5 M NaCl in buffer A with a total volume of 130 ml. The fractions containing the MOMP were identified by SDS-PAGE and pooled. DEAE-Sephacel column chromatography was repeated six times. The proteins in the total pooled fractions (8.4 mg of protein) were precipitated by the addition of cold ethanol to a final concentration of 80% (vol/vol). The precipitate was allowed to collect overnight at -20°C and recovered by centrifugation at $20,000 \times g$ for 30 min. The pellet was suspended in a minimum volume of buffer B (10 mM Tris-hydrochloride (pH 8.0), 200 mM NaCl, 10 mM EDTA, 0.05% [wt/vol] Zwittergent 3-14) and applied to a Sephacryl S200 HR (Pharmacia) column (2.6 by 64 cm) equilibrated with buffer B. The MOMP was eluted in 2.2-ml fractions with buffer B at a flow rate of 12 ml/h. The fractions containing the purified MOMP were identified by SDS-PAGE, pooled, and precipitated with ethanol as described above. The final pellet was solubilized in 50 mM Tris-hydrochloride (pH 7.5)–5 mM EDTA–0.5% (wt/vol) Zwittergent 3-14, aliquoted, and stored at -70°C .

Protein determination. The protein concentration was measured as the A_{280} or by the bicinchoninic acid method (19) by using Micro BCA protein assay reagent (Pierce) with bovine serum albumin as a standard.

SDS-PAGE. Samples for electrophoresis were solubilized in SDS sample buffer (11) at 100°C for 5 min. SDS-PAGE was carried out on a 12.5% separating gel with the discontinuous buffer system described by Laemmli (11). The gels were stained with 0.25% (wt/vol) Coomassie brilliant blue R-250 (Merck) in 50% (vol/vol) methanol–10% (vol/vol) acetic acid and then destained with 10% (vol/vol) methanol–7.5% (vol/vol) acetic acid.

Amino-terminal sequencing. The purified MOMP was electrophoresed by SDS-PAGE. After electrophoresis, the method of Matsudaira (12) was followed, with modifications. The MOMP was transferred to a polyvinylidene difluoride membrane sheet (Fluorotrans; Pall) in transfer buffer composed of 25 mM Tris, 192 mM glycine, 0.02% (wt/vol) SDS, and 20% (vol/vol) methanol, as described previously (7), at 100 V for 3 h by using a blotting apparatus (Mini Protean system; Bio-Rad Laboratories). After the MOMP was transferred, the membrane was washed extensively in deionized water and stained with 0.1% (wt/vol) Coomassie brilliant blue R-250 for 5 min. The membrane was destained with 60% (vol/vol) methanol for 2 min, washed with deionized water for 5 min, and then hung to dry. The section of the membrane containing the MOMP was excised and applied to a

gas-phase sequencer (model 477A; Applied Biosystems). N-terminal sequencing was performed by H. Hirano, National Institute of Agrobiological Resources, Tsukuba, Japan.

Antisera. The purified MOMP was freed from detergent by ethanol precipitation. For experiments with rabbits, the precipitated protein was suspended in phosphate-buffered saline (PBS) to a concentration of 200 $\mu\text{g/ml}$ and emulsified with 2 volumes of Freund's complete adjuvant (Difco) and then 3-ml volumes were injected subcutaneously at multiple sites on the rabbits' backs. Booster immunizations with the same antigen in Freund's incomplete adjuvant (Difco) were given on days 20, 40, and 60, and sera were collected on day 70. For experiments with calves, the precipitated protein (500 $\mu\text{g/ml}$) in PBS was emulsified with 2 volumes of Freund's incomplete adjuvant and then 3-ml volumes were injected subcutaneously at four sites. Booster immunizations with the same antigen in Freund's incomplete adjuvant were given on days 28 and 56, and sera were collected on day 77.

ELISA. Anti-OMP, Anti-lipopolysaccharides (anti-LPS), and anti-MOMP antibodies were quantitated by enzyme-linked immunosorbent assay (ELISA). For measurement of anti-OMP antibody, the Sarkosyl-insoluble OMP fractions from the four strains were diluted to 5 μg of protein content per ml in carbonate coating buffer (pH 9.6). For measurement of anti-LPS antibody, LPS was prepared from strain 8025 by the hot phenol method (23) and diluted to 10 $\mu\text{g/ml}$ in 20 mM PBS (pH 7.2) containing 20 mM MgCl_2 . For measurement of anti-MOMP antibody, purified MOMP was precipitated with ethanol to remove detergent, suspended in PBS, and diluted to 5 $\mu\text{g/ml}$ in carbonate coating buffer (pH 9.6). ELISA plates (type H; Sumitomo Bakelite Co.) were coated with 100 μl of OMP, LPS, or MOMP solution overnight at 37°C . After being coated, the plates were washed with PBS containing 0.05% (vol/vol) Tween 20 and then blocked with a blocking agent made from milk (Block Ace; Snow Brand Milk Products Co.). The plates were washed with PBS–0.05% Tween 20 and incubated at room temperature for 2 h with 100 μl of antiserum or murine hybridoma culture supernatants diluted with Block Ace solution. Then the plates were washed with PBS–0.05% Tween 20 and incubated at room temperature for 1 h with peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) (Cappel Laboratories); peroxidase-conjugated goat anti-bovine IgG (Jackson Immunoresearch Laboratories); or peroxidase-conjugated goat anti-mouse IgG, IgM, and IgA (Cappel Laboratories) diluted with Block Ace solution. After being washed, the plates were incubated at room temperature for 30 min with 200 μl of 0.012% (vol/vol) hydrogen peroxide and 0.04% (wt/vol) *o*-phenylenediamine in 100 mM phosphate-citrate buffer (pH 5.0) and the reaction was stopped by the addition of 50 μl of 2 M H_2SO_4 . The plates were read with a microplate reader (model MTP-100; Corona Electric Co.) in the dual-wavelength mode (492 and 660 nm).

Immunoblot analysis. For immunoblot analysis, whole bacterial cells were used as the antigens. The 24-h cultures of the four strains grown on blood agar plates were washed twice in physiological saline and suspended in SDS sample buffer at 10 mg (wet weight) per ml. The protein concentration of whole bacterial lysates was 1.2 mg/ml. After SDS-PAGE, the whole-cell antigens were electrotransferred to a nitrocellulose membrane sheet (Bio-Rad Laboratories) as described above. The sheet was blocked with PBS containing 3% (wt/vol) Teleostean gelatin (Sigma) at 37°C for 1 h. After blocking, the sheet was incubated with antisera or

hybridoma culture supernatants diluted with PBS containing 1% (vol/vol) Teleostean gelatin at room temperature for 2 h. The sheet was rinsed with PBS-0.05% Tween 20 and then incubated with peroxidase-conjugated goat anti-rabbit IgG; peroxidase-conjugated goat anti-bovine IgG; or peroxidase-conjugated goat anti-mouse IgG, IgM, and IgA diluted with PBS-1% Teleostean gelatin for 1 h at room temperature. The sheet was rinsed with PBS-0.05% Tween 20 and then with Tris-buffered saline (pH 7.5). The sheet was immersed in Tris-buffered saline containing 0.05% (wt/vol) 4-chloro-1-naphthol (Bio-Rad Laboratories), 16.6% (vol/vol) methanol, and 0.015% (vol/vol) hydrogen peroxide. Color developed within a few minutes, and the reaction was stopped by rinsing the sheet extensively with deionized water.

MAbs. On day 0, a mixture of the OMP preparations from *H. somnus* 8025, D1238, NT2301, and 540 (100 µg of protein per head) or the purified MOMP from strain 8025 (50 µg of protein per head) was used to immunize BALB/c mice intraperitoneally along with Freund's complete adjuvant. On day 14, the same antigens with Freund's incomplete adjuvant were injected into mice intraperitoneally. On day 28, the same antigens were inoculated into mice intravenously. Fusions were done on day 32 by using spleen cells from immunized mice and P3U1 mouse myeloma cells at a cell number ratio of 2:1 in 45% (wt/vol) polyethylene glycol 4000 (Merck) in PBS. The fused cells (2×10^6 cells per ml) were dispensed in 24-well tissue culture plates at 1 ml per well. The cells were grown in RPMI 1640 medium (GIBCO Laboratories) containing 50 µg of penicillin per ml, 50 µg of streptomycin per ml, 1 mM sodium pyruvate, 2 mM L-glutamine, 30 µg of endothelial cell growth supplement (Sigma) per ml, 100 µM hypoxanthine, 16 µM thymidine, 0.4 µM aminopterin, and 15% (vol/vol) fetal bovine serum. On day 7 after fusion, the medium was replaced with medium without aminopterin. On days 12 to 13 after fusion, supernatants from wells containing growing hybridoma cells were tested by ELISA with the OMP fractions, MOMP, and LPS as antigens. The monoclonal antibody (MAb)-producing cells were cloned by limiting dilution, and the cell culture supernatants were used as antibody sources. The isotypes of the MAbs were determined by ELISA with the Mouse Typer subtyping kit (Bio-Rad Laboratories).

RESULTS

Purification of the MOMP. The Zwittergent 3-14-soluble fraction was applied to a DEAE-Sephacel chromatography column with a 0 to 0.5 M NaCl gradient (Fig. 1). The MOMP eluted in one peak between 0.21 and 0.28 M and thus can be separated from a major contaminant which eluted earlier in the gradient (Fig. 1). The next step of the purification procedure was the application of the ion-exchange-purified material to a molecular-sieve chromatography column. The MOMP eluted from a Sephacryl S200 HR column in the first peak (Fig. 2). The protein profile by SDS-PAGE revealed that this peak was essentially pure MOMP (Fig. 3). The yield from 20 g (wet weight) of bacteria was approximately 6.4 mg.

N-terminal amino acid sequence. N-terminal amino acid analysis of the *H. somnus* MOMP showed that the MOMP exhibited considerable homology with the N-terminal sequences of the porin proteins from *Haemophilus influenzae* type b and *Escherichia coli* (Fig. 4). The first 16 residues of the MOMP of *H. somnus* showed a 56% overlap with the *H. influenzae* type b porin sequence and a 43% overlap with the *E. coli* OmpC porin sequence.

Immunological properties of the MOMP. The antibody

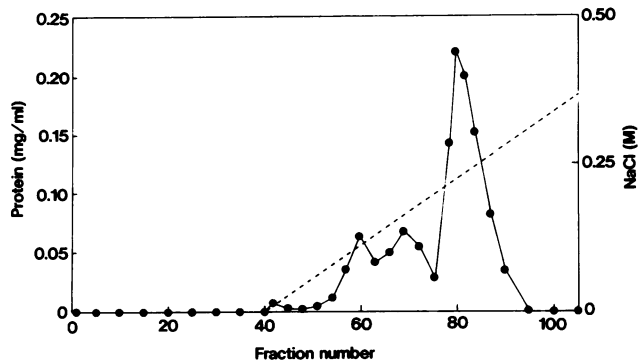


FIG. 1. Elution profile of the *H. somnus* MOMP on a DEAE-Sephacel column. The sample (6.7 mg of protein) was applied to a column (1.5 by 8 cm) packed with DEAE-Sephacel in a buffer (50 mM Tris-hydrochloride [pH 8.0], 10 mM EDTA, 0.5% [wt/vol] Zwittergent 3-14). The column was washed with the same buffer and the bound proteins were eluted in 1.2-ml fractions with a linear gradient of 0 to 0.5 M NaCl. Fractions were assayed for protein concentration (solid line) by the bicinchoninic acid method (21) and for NaCl concentration (dashed line) by measuring conductivity. SDS-PAGE analysis revealed that the MOMP was in the major peak, eluting at between 0.21 and 0.28 M.

response to the purified MOMP of strain 8025 increased greatly in rabbits and calves after the course of injections of the purified MOMP, as measured by ELISA (Table 1). There was also a large increase in the antibody response to the purified LPS of strain 8025 in the hyperimmunized rabbits and calves. It is therefore clear that the purified MOMP preparation contained some LPS. To assess whether the antibody bound in the anti-MOMP ELISA was really anti-MOMP or was predominantly anti-LPS, the reactivity of anti-MOMP serum was tested by immunoblot analysis with whole bacteria of strain 8025 as the antigen. The antiserum reacted with both the MOMP and LPS, although the intensity of the reaction with the MOMP was higher than that with the LPS (Fig. 5). No antibodies directed against other antigens were detected. We therefore conclude that the

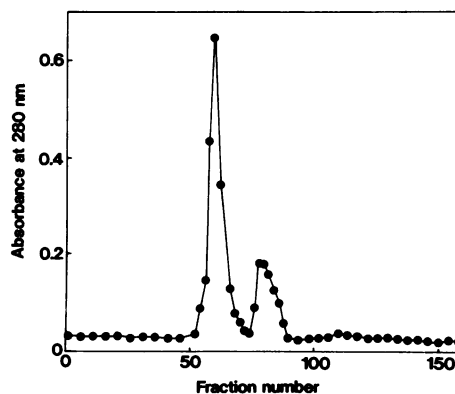


FIG. 2. Elution profile of the *H. somnus* MOMP on a Sephacryl S200 HR column. The sample (8.4 mg of protein) was applied to a column (2.6 by 64 cm) packed with Sephacryl S200 HR which had been equilibrated with a buffer (10 mM Tris-hydrochloride [pH 8.0], 200 mM NaCl, 10 mM EDTA, 0.05% [wt/vol] Zwittergent 3-14). The flow rate was 12 ml/h. Fractions of 2.2 ml were monitored by A_{280} . SDS-PAGE analysis revealed that the MOMP was in the first peak, and no protein was detected in the second peak.

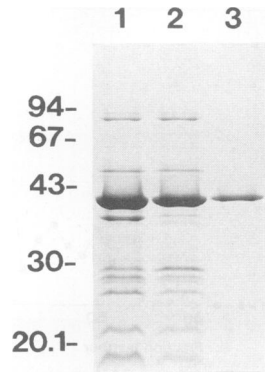


FIG. 3. SDS-PAGE showing the purification of the *H. somnus* MOMP. Lanes: 1, *N*-lauroylsarcosyl-insoluble OMP (10 μ g of protein); 2, Zwittergent 3-14 extract (6.3 μ g of protein); 3, pooled Sephacryl S200 fractions (2 μ g of protein). Molecular mass standards (in kilodaltons) are noted on the left.

hyperimmune sera really contained antibodies directed to both the MOMP and LPS. To compare the antigenicities of MOMP from different strains, the cross-reactivity of rabbit antiserum was tested with whole-cell antigens of strains 8025, D1238, NT2301, and 540 in immunoblot analysis. The antiserum reacted with the four strains at a band with a molecular mass of 40 kDa, but strain 540 showed a lower intensity at the band (Fig. 6A). To analyze antigenic determinants on the MOMP, MABs directed to the MOMP were produced. The isotypes of MABs 57-16-2 and 68-4-3 were identified as IgG2a and IgG1, respectively. In immunoblot analysis with whole bacteria of strain 8025 as the antigen, MAB 68-4-3 recognized a single band with a molecular mass of 40 kDa (Fig. 6B, lane 1); however, MAB 57-16-2 did not react with any band (data not shown). To determine whether the two MABs recognize the MOMP, they were tested in ELISA with the purified MOMP and LPS from strain 8025 as the antigens. Both reacted with the purified MOMP alone (Table 2). The cross-reactivity of the MABs with the four strains was tested by ELISA and immunoblot analysis. MAB 57-16-2 reacted with the four strains in ELISA (Table 2) but not with the four strains in immunoblot analysis (data not shown). MAB 68-4-3 reacted with the three strains other than strain 540 in both tests (Table 2 and Fig. 6B). These results indicate that the MOMP of *H. somnus* possesses at least two antigenic determinants and that the MOMP of strain 540 is antigenically different from those of the other strains.

DISCUSSION

In this study, we have developed a method for purification of the *H. somnus* MOMP. We applied in principle the

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P2   A V V Y N N E G T N V E L G G R
MOMP T T V Y N Q N G T K V D V G G R
OmpC A E V Y N K D G N K L D L Y G K
  
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FIG. 4. Comparison of the N-terminal amino acid sequence of the *H. somnus* MOMP with those of other bacterial porin proteins. Identical residues are indicated by dashes. The N-terminal amino acid sequences of the other bacterial porin proteins were determined from the DNA sequences of the cloned porin genes for *H. influenzae* type b P2 (9, 14) and *Escherichia coli* OmpC (13).

TABLE 1. Serum antibody response to the *H. somnus* MOMP and LPS measured by ELISA in rabbits and calves immunized with the MOMP^a

Serum	Animal	Anti-MOMP response	Anti-LPS response
Preimmune	Rabbit	0.311 \pm 0.168	0.296 \pm 0.063
	Calf	0.157 \pm 0.072	0.256 \pm 0.047
Immune	Rabbit	2.233 \pm 0.390	2.419 \pm 0.302
	Calf	2.123 \pm 0.316	2.641 \pm 0.102

^a Data are expressed as the A_{492S} (means \pm standard deviations) for animals ($n = 2$) in each group of three experiments. Sera were diluted to 1:500. Peroxidase-conjugated goat anti-rabbit IgG and peroxidase-conjugated goat anti-bovine IgG were diluted to 1:12,000 and 1:10,000, respectively.

method for P2 (15), the porin of *H. influenzae* type b, to purification of the *H. somnus* MOMP, since both proteins are the most abundant in SDS-PAGE. Thus, purification of the *H. somnus* MOMP to apparent homogeneity was achieved by ion-exchange and molecular-sieve chromatographies. The purified MOMP, however, contains an amount of LPS which promotes the immune response in rabbits and calves (Table 1). This indicates that the MOMP closely associates with LPS as described for porins (6, 18); therefore, the present purification procedures could not separate the MOMP and LPS completely.

The present data from protein sequence analysis indicate that the MOMP of *H. somnus* exhibits considerable N-terminal sequence homology with the porin proteins of other gram-negative bacteria. This indicates that the MOMP of *H. somnus* is probably a porin protein. The porin proteins of gram-negative bacteria have been demonstrated to form water-filled channels which allow the diffusion of low-molecular-mass solutes and to serve as receptor sites for the binding of phages and bacteriocins (17). Whether the MOMP of *H. somnus* performs similar functions has not been determined.

If the MOMP of *H. somnus* is considered a vaccine candidate, the immunogenicity of the protein should be evaluated. The present data indicated that the MOMP of *H. somnus* is immunogenic to animals, and the antisera to the

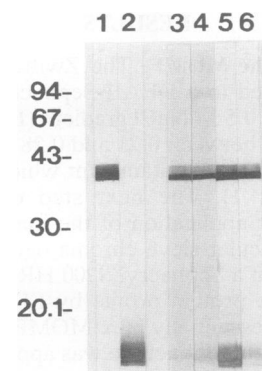


FIG. 5. Immunoblot analysis of whole cells (7.2 μ g of protein per lane) from *H. somnus* 8025 reacted with calf and rabbit hyperimmunized sera to the *H. somnus* MOMP and MAB controls. Lanes: 1, MAB 68-4-3 to the *H. somnus* MOMP; 2, MAB 2-15-5 to *H. somnus* LPS; 3, serum from calf no. 1; 4, serum from calf no. 2; 5, serum from rabbit no. 1; 6, serum from rabbit no. 2. Hybridoma culture supernatants were diluted to 1:5. Sera were diluted to 1:4,000. All peroxidase conjugates were diluted to 1:1,000. Molecular mass standards (in kilodaltons) are noted on the left.

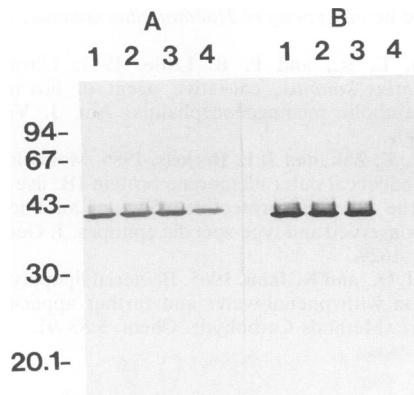


FIG. 6. Immunoblot analysis of whole-cell preparations (3 μ g of protein per lane) from *H. somnus* strains reacted with hyperimmunized rabbit serum to the *H. somnus* MOMP and MAb directed to the MOMP. (A) Blot probed with serum from rabbit no. 1 (1:8,000). Peroxidase-conjugated goat anti-rabbit IgG was diluted to 1:2,000. (B) Blot probed with MAb 68-4-3 (1:5). Peroxidase-conjugated goat anti-mouse IgG, IgM, and IgA was diluted to 1:1,000. Lanes: 1, strain 8025; 2, strain D1238; 3, strain NT2301; 4, strain 540. Molecular mass standards (in kilodaltons) are noted on the left.

purified MOMP reacted extensively with the MOMP in ELISA and immunoblot analysis. The *H. somnus* 41-kDa MOMP described previously (3) appears to be same as the 40-kDa MOMP in the present study because *H. somnus* possesses the single most abundant OMP with a molecular mass of nearly 40 kDa. However, the previous study demonstrated that the 41-kDa MOMP of *H. somnus* was not recognized by bovine convalescent serum and bovine hyperimmune serum with whole formalized *H. somnus* in immunoblot analysis (3). The reason for the lack of response of the bovine sera to the MOMP in the previous study has not been determined. The immunogenicity of the native MOMP in bacterial cells may be different from that of the purified MOMP. If the native MOMP elicit only the antibodies directed to the epitopes which are denatured in immunoblot analysis, the immune response of animals to the MOMP cannot be detected. In fact, the MOMP possesses an antigenic determinant to which MAb 57-16-2 binds in ELISA but not in immunoblot analysis.

The biological activity of the antibodies to the *H. somnus* MOMP remains to be determined. The antibodies to porin proteins of *H. influenzae* (8, 15, 16) and *Neisseria gonor-*

rhoeae (22) showed bactericidal and protective effects. The antibodies to the *H. somnus* MOMP may be protective.

Immunological diversity among *H. somnus* strains has been reported (1, 20); however, the molecule on which the antigenic differences reside has remained to be determined. The data in this study indicate that the MOMP of *H. somnus* shows antigenic heterogeneity among different strains and that there are at least two antigenic determinants on the MOMP molecule. The strain specificities of the MAbs have implications regarding the feasibility of developing a serotyping system with MAbs that is based on MOMP epitopes. The establishment of a serotyping system for *H. somnus* based on MOMP epitopes would provide a better understanding of the immunological and molecular bases of the epidemiology of *H. somnus* infection.

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TABLE 2. Immunological analysis of MAb reactivity to the *H. somnus* MOMP by ELISA^a

Antigen and strain	MAb reactivity	
	57-16-2	68-4-3
MOMP	0.642 \pm 0.224	0.628 \pm 0.101
LPS	0.013 \pm 0.002	0.005 \pm 0.001
OMP		
8025	2.664 \pm 0.026	2.629 \pm 0.055
D1238	2.671 \pm 0.077	2.652 \pm 0.042
NT2301	2.629 \pm 0.026	2.610 \pm 0.064
540	2.110 \pm 0.084	0.005 \pm 0.003

^a Data are expressed as the A_{492S} (means \pm standard deviations) for three experiments. Hybridoma culture supernatants were diluted to 1:2. Peroxidase-conjugated goat anti-mouse IgG, IgM, and IgA was diluted to 1:12,000.

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