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Characterization of a Heat-Modifiable Outer Membrane Protein of *Haemophilus somnus*

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In immunoblot analysis, a murine monoclonal antibody (MAb), 27-1, which was produced to an outer membrane protein (OMP) of *Haemophilus somnus*, showed that a major OMP is heat modifiable, having a molecular mass of 28 kDa when the *N*-lauroylsarcosine-insoluble OMP preparation was solubilized at 60°C and a mass of 37 kDa when the OMP preparation was solubilized at 100°C. The heat-modifiable OMP reacted intensely with convalescent sera obtained from calves with experimental *H. somnus* pneumonia in immunoblot analysis. Immunoelectron microscopic and antibody absorption studies revealed that the MAb 27-1 epitope was not surface exposed on the intact bacterium. However, a decrease in antibody reactivity to the heat-modifiable OMP in immunoblot analysis after absorption of convalescent serum with intact bacterial cells of *H. somnus* suggests that a surface-exposed portion of the heat-modifiable OMP is expressed on the intact bacterium. MAb 27-1 reacted with 45 of 45 strains of *H. somnus* tested in immunoblot analysis. The apparent molecular mass of the antigen varied among strains, and five reactivity patterns demonstrated by MAb 27-1 were observed. MAb 27-1 also reacted with six species in the family *Pasteurellaceae*, *Escherichia coli*, and *Salmonella dublin*, but not with the other eight species of gram-negative bacteria. The heat-modifiable OMP of *H. somnus* showed immunological cross-reactivity with the OmpA protein of *E. coli* K-12 and significant N-terminal amino acid sequence homology with the OmpA proteins of gram-negative bacteria. We conclude that a major, 37-kDa heat-modifiable OMP of *H. somnus*, which elicits an antibody response in *H. somnus*-infected animals, is a common antigen among *H. somnus* strains tested and is structurally related to the OmpA protein of *E. coli*.

Haemophilus somnus is a gram-negative bacterium that causes several diseases in cattle, including pneumonia, abortion, septicemia, arthritis, and thromboembolic meningoencephalitis (13). It also survives as a commensal in the bovine reproductive tract (13). This diverse pathogenic potential of *H. somnus* and the economic importance of the above diseases indicate that this organism is a significant bovine bacterial pathogen.

The emergence of *H. somnus* as an important bovine pathogen has prompted studies of the outer membrane proteins (OMPs) of the organism. Studies of OMPs are important to identify virulence factors, to elucidate structure-function relationships in the outer membrane, to characterize surface antigens, and to understand the immune response to infection. Recent studies have begun to clarify the basic characteristics of the OMPs of *H. somnus* (5, 8, 9, 14, 20, 23, 24, 29, 30).

In a previous study, analysis of OMP fractions of *H. somnus* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has shown that there is a major OMP with an apparent molecular mass of 37 kDa below the most abundant OMP (23); however, little has been known about the characteristics of this OMP. In the OMPs of *H. somnus*, a 40-kDa OMP has been well characterized. The 40-kDa OMP is thought to be an important OMP antigen because this antigen is surface exposed, conserved in all *H. somnus* strains tested (5, 8), and shown to be protective in a passive protection experiment (9). More recently, molecular cloning and analysis of the 40-kDa OMP revealed that this OMP is a lipoprotein (24). Since the 40-kDa lipoprotein and the 37-kDa OMP are demonstrated to be in the same molecular mass range, it is to be clarified whether both OMPs are distinct entities. Therefore, we have developed and used a murine monoclonal antibody (MAb) directed to the 37-kDa OMP of *H. somnus* to characterize this OMP.

In this study, we describe the identification and characterization of a heat-modifiable 37-kDa OMP of *H. somnus*. We also show that this OMP is related to OmpA protein of *Escherichia coli*.

MATERIALS AND METHODS

Bacterial strains. *H. somnus* 8025 (6) was kindly provided by L. Corboz, Institut für Veterinärbakteriologie der Universität Zürich. *H. somnus* NT2301 was from our laboratory collections and was isolated from a case of bovine pneumonia (23). Bacteria used to test MAb reactivity are listed in Tables 1 and 2. The identity of *H. somnus* strains was confirmed as described previously (13). All *H. somnus* strains were gram-negative, nonmotile, and pleomorphic bacilli. All were positive in yellow pigment, oxidase, glucose fermentation, nitrate reduction, δ-aminolevulinic acid utilization, and growth enhancement by supplement of blood or serum and were negative in urease, gelatinase, acetylmethylcarbinol production, β-galactosidase, and growth on MacConkey agar. *E. coli* K-12 strains used were C600 (F^- *thi thr leu lac supE*) and two P400 (F^- *thi argE proA thr leu mtl xyl galK lacY rpsL supE non*) derivatives (18): P460 (same as P400 but *ompA1*) and P2899 (same as P400 but *pyrD34 zcb*::Tn*10-43 zia*::Tn*5 ompA725*). *E. coli* C600 was obtained from T. Sekizaki, National Institute of Animal Health, Tsukuba, Japan. The two P400 derivatives were obtained from R. Morona, Max-Planck-Institut für Biologie via T. Sekizaki. *H. somnus* was cultured on blood agar plates or in broth as described previously (23). *Haemophilus influenzae*,

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TABLE 1. Reactivity of MAb 27-1 with 45 *H. somnus* strains from diseased animals and carriers in immunoblot analysis[a]

Reactivity pattern	No. of strains assigned	Molecular mass[b] (kDa)
A	5	37.3, 28.5
B	28	37, 28
C	7	36.7, 28.5
D	3	36.3, 27.8
E	2	35.5, 28

[a] Seven strains including strain 8025 were obtained from L. Corboz. One strain (ATCC 25836 [1]) was obtained from the American Type Culture Collection, Rockville, Md. The other 37 strains including strain NT2301 were from our laboratory collections.

[b] Relative molecular mass estimates of antigen bands reactive with MAb 27-1.

Haemophilus parainfluenzae, and *Haemophilus haemoglobinophilus* were grown on chocolate agar plates in 7% CO_2. The *Campylobacter* species bacteria were cultured on blood agar plates in a microaerophilic environment (5% O_2, 10% CO_2, and 85% N_2). Other bacteria except *E. coli* K-12 strains were cultured on blood agar plates at 37°C in 7% CO_2. *E. coli* K-12 strains were cultured on LB agar plates at 37°C. Tetracycline was added to the medium at 10 μg/ml for cultivating strain P2899. Bacteria were stored at −70°C in brain heart infusion broth containing 20% (vol/vol) glycerol.

MAb. BALB/c mice were immunized intraperitoneally with live *H. somnus* 8025 (1.5×10^7 CFU) on day 0 and boosted with live bacteria of the same strain (3×10^7 CFU) on day 28. On day 32 after the initial immunization, spleen cells were recovered and fused with P3U1 mouse myeloma cells to obtain antibody-producing hybridomas as described previously (23). Supernatants from hybridoma cell cultures were tested by enzyme-linked immunosorbent assays with formalin-treated *H. somnus* whole cells (27) as the antigen, as described previously (23). A hybridoma cell line was cloned by sequential limiting dilutions and labeled MAb 27-1. The isotype of MAb 27-1 was identified as immunoglobulin G2a by using the Mouse Typer Subisotyping kit (Bio-Rad Laboratories). The hybridoma culture supernatant from a serum-free medium, GIT medium (Nihon Pharmaceutical Co.), was used as the antibody source.

TABLE 2. Cross-reactivity of MAb 27-1 with a variety of gram-negative bacteria in immunoblot analysis

Species	No. of strains		Molecular mass[b] (kDa)
	Tested	Positive[a]	
Histophilus ovis	3	3 (+)	35, 26[c]
Haemophilus agni	1	1 (+)	35, 26[c]
Haemophilus influenzae[d]	1	1 (+)	36
Haemophilus parainfluenzae[d]	1	1 (w)	38
Haemophilus haemoglobinophilus[d]	1	1 (+)	38, 29[c]
Actinobacillus seminis	1	1 (+)	36, 31[c]
Actinobacillus lignieresii[d]	1	0	
Pasteurella multocida[d]	3	0	
Pasteurella haemolytica[d]	3	0	
Brucella abortus	1	0	
Moraxella bovis[d]	1	0	
Campylobacter species[e]	3	0	
Escherichia coli	2	2 (+)	35
Salmonella dublin	2	2 (+)	36

[a] Intensity of reaction is shown in parentheses: w, weak reaction; +, intense reaction.

[b] Relative molecular mass estimates of antigen bands reactive with MAb 27-1.

[c] Two bands were detected.

[d] Type strain was tested or test strains included type strain.

[e] One strain each of *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, and *C. sputorum* subsp. *bubulus*.

Sera from calves with experimental *H. somnus* pneumonia. In each of a 6-week-old (calf 9001) and 8-week-old (calves 9007 and 9111) Japanese black male calves, a suspension of 10^9 CFU of *H. somnus* NT2301 prepared from 18-h cultures on blood agar plates was inoculated intrabronchially into a lung lobe by using a 14-gauge needle and polyethylene tube (about 1 mm in diameter) as described previously (12). Clinical signs of mild pneumonia developed. Sera were collected before and weekly after inoculation.

SDS-PAGE and immunoblot analysis. *N*-Lauroylsarcosine (Sarkosyl)-insoluble OMP and whole-cell antigens prepared as described previously (23) were subjected to SDS-PAGE on 12.5% separating gels and then either stained with Coomassie brilliant blue or subjected to immunoblot analysis by a previously described method (23).

Immunoelectron microscopy. Bacteria (18-h cultures of *H. somnus* 8025 on blood agar plates)-mounted grids were incubated with MAbs and then incubated with goat anti-mouse immunoglobulin G conjugated to 10-nm colloidal gold spheres (E/Y Laboratories). The grids were examined with a transmission electron microscope (model JEM-100CXII; JEOL Ltd.).

Antibody absorption. To determine whether the antibodies recognize antigenic determinants that are expressed on the surface of an intact bacterium, we used the antibody absorption method of Loeb (15). *H. somnus* 8025 was cultured in 200 ml of broth medium in a shaking water bath for 5 h, harvested by centrifugation at 10,000 × *g* for 10 min, and washed once in phosphate-buffered saline. The MAb 27-1 immunoglobulin G fraction, which was prepared by ammonium sulfate precipitation from a hybridoma culture supernatant, and calf convalescent serum were diluted 1:100 in phosphate-buffered saline containing 1% (vol/vol) Teleostean gelatin (Sigma). The washed live bacterial pellet (1.5×10^{11} CFU) was gently suspended in 1 ml of antibody solution. The suspensions were incubated at 4°C for 30 min with gentle agitation. No change of the live bacterial cell numbers was confirmed after incubation. After bacterial cells were centrifuged, the supernatants of MAb 27-1 and convalescent serum were diluted to final concentrations of 1:1,600 and 1:800, respectively, and then examined for antibody reactivity in immunoblot analysis.

Amino-terminal sequencing. The OMP preparation of *H. somnus* 8025 was electrophoresed by SDS-PAGE. After SDS-PAGE, the proteins were electrophoretically transferred onto a polyvinylidene difluoride membrane (17), with modifications as described previously (23). The section of the membrane containing the 37-kDa heat-modifiable OMP was excised and applied to a gas-phase sequencer (model 477; Applied Biosystems). The N-terminal sequencing was performed by H. Hirano, National Institute of Agrobiological Resources, Tsukuba, Japan.

Protein determination. The protein concentration was measured by the bicinchoninic acid method (21) by using Micro BCA protein assay reagent (Pierce) with bovine serum albumin as a standard.

RESULTS

Identification of a heat-modifiable OMP. Figure 1 shows a gel stained with Coomassie brilliant blue and an immunoblot

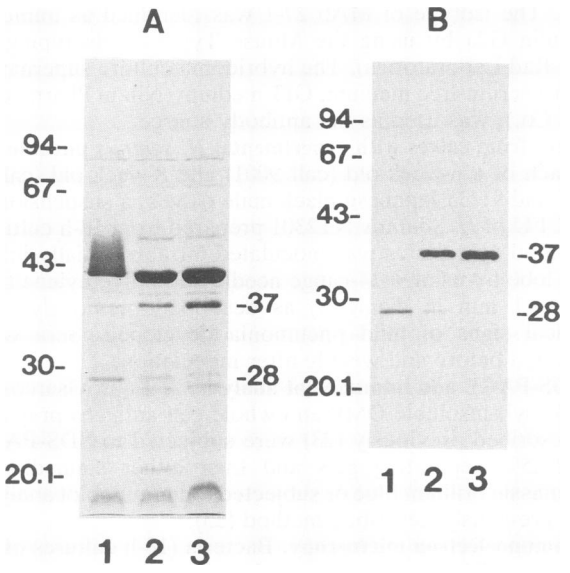

FIG. 1. Heat modifiability of a major OMP of *H. somnus*. Sarkosyl-insoluble OMP preparation from *H. somnus* 8025 was solubilized as follows before separation by SDS-PAGE: lanes 1, 60°C, 20 min; lanes 2, 100°C, 5 min; lanes 3, 100°C, 15 min. Approximately 2 μg of protein per lane was resolved. (A) Coomassie brilliant blue-stained gel. (B) Immunoblot analysis probed with MAb 27-1. MAb was diluted 1:20. Molecular mass standards (in kilodaltons) are noted on the left. Relative molecular mass estimates (in kilodaltons) are noted on the right.

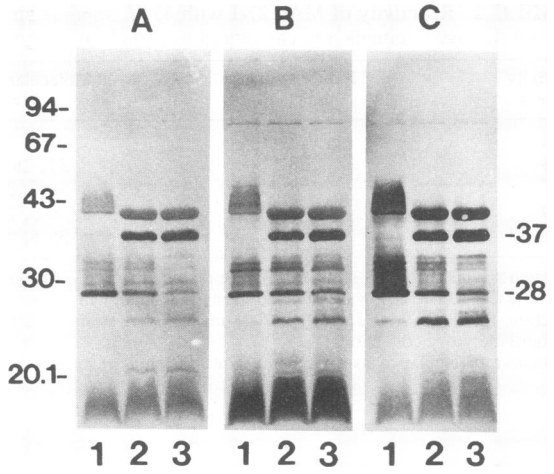

FIG. 2. Immunoblot analysis of the reactivity of sera from calves with experimental *H. somnus* pneumonia. Convalescent sera from calves 9001 (A), 9007 (B), and 9111 (C) were diluted 1:800. Sarkosyl-insoluble OMP preparation from *H. somnus* 8025 was used as the antigen. The OMP preparation was solubilized as follows: lanes 1, 60°C for 20 min; lanes 2, 100°C for 5 min; lanes 3, 100°C for 15 min. Approximately 2 μg of protein per lane was resolved. Molecular mass standards (in kilodaltons) are noted on the left. Relative molecular mass estimates (in kilodaltons) are noted on the right.

analysis probed with MAb 27-1 when the Sarkosyl-insoluble OMP preparation of *H. somnus* 8025 was solubilized under different solubilization temperatures. In the sample which was solubilized at 60°C, a protein band with a molecular mass of 28 kDa was apparently detected (lanes 1). When the samples were solubilized at 100°C, a progressive shift to a protein band with a molecular mass of 37 kDa was observed (lanes 2 and 3). These results indicate that the 37-kDa OMP of *H. somnus* is heat modifiable and that MAb 27-1 reactivity is specific to the heat-modifiable OMP.

Reactivity of sera from calves with experimental *H. somnus* pneumonia with the heat-modifiable OMP. To determine whether sera from calves with experimental *H. somnus* pneumonia contain antibodies to the heat-modifiable OMP, immunoblot analysis with the Sarkosyl-insoluble OMP preparation as the antigen was performed. No antibodies reactive with *H. somnus* OMPs were detected in preinfection sera (1:800) (data not shown). Convalescent sera (1:800), which were collected at 2 weeks (calf 9001) and 6 weeks (calves 9007 and 9111) after infection, reacted intensely at 28 kDa but not at 37 kDa when the OMP preparation was solubilized at 60°C (Fig. 2, lanes 1). In contrast, the convalescent sera reacted intensely at 37 kDa when the OMP preparation was solubilized at 100°C (Fig. 2, lanes 2 and 3). These results indicate that the heat-modifiable OMP is antigenic and elicits an intense antibody response in *H. somnus*-infected animals.

Surface exposure of the heat-modifiable OMP. To determine whether MAb 27-1 recognizes an epitope that is exposed on the surface of an intact *H. somnus* bacterium, immunoelectron microscopy was performed. MAb 27-1 showed no staining on the bacterial cells (data not shown). In control experiments, P3U1 tissue culture supernatant (as a negative control) and lipopolysaccharide-specific MAb 25-1 (as a positive control) were incubated in place of the test antibody and yielded negative and positive results, respectively (data not shown). To further elucidate the question of surface exposure, MAb 27-1 and convalescent serum were absorbed with log-phase whole cells. Absorption of MAb 27-1 did not remove the MAb reactivity with the heat-modifiable OMP (Fig. 3, lanes 1 and 2). In contrast, absorption of convalescent serum reduced the antibody reactivity with the heat-modifiable OMP (Fig. 3, lanes 3 and 4). These results indicate that the epitope recognized by MAb 27-1 is not exposed on the surface of the intact bacterium; however, a surface-exposed portion of the heat-modifiable OMP is expressed on the intact bacterium.

Conservation of the antigen recognized by MAb 27-1. To assess the degree of conservation of the antigen recognized by MAb 27-1 among *H. somnus* strains, 45 strains obtained from diseased animals and carriers were tested in immunoblot analysis. Whole-cell preparations were used in this study. MAb 27-1 reacted with all strains of *H. somnus* tested. The apparent molecular mass of the antigen varied among strains, and five reactivity patterns (A through E) were observed (Table 1 and Fig. 4).

Cross-reactivity of MAb 27-1 with other gram-negative bacteria. To assess the cross-reactivity of MAb 27-1 with other gram-negative bacteria, whole-cell preparations from a variety of gram-negative species were tested with MAb 27-1 in immunoblot analysis (Table 2). MAb 27-1 reacted with *Histophilus ovis*, *Haemophilus agni*, *H. influenzae*, *H. parainfluenzae*, *H. haemoglobinophilus*, *Actinobacillus seminis*, *E. coli*, and *Salmonella dublin*. The cross-reactive antigens of these gram-negative bacteria ranged in molecular mass from 26 to 38 kDa. The other gram-negative bacteria tested lacked the antigen recognized by MAb 27-1.

Relationship to OmpA protein. The electrophoretic mobility of the heat-modifiable OMP of *H. somnus* resembled that of the OmpA protein of *E. coli* K-12 (16). Furthermore, MAb 27-1 reacted with *E. coli* at a band with a molecular mass of

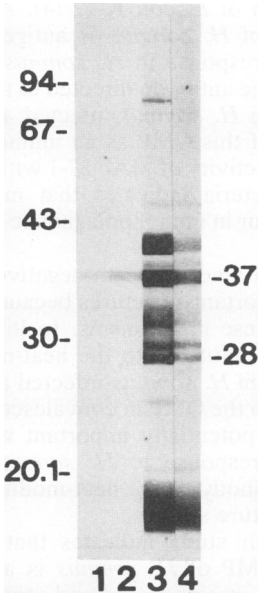

FIG. 3. Immunoblot analysis of the reactivity of MAb 27-1 and calf 9001 convalescent serum absorbed with log-phase whole cells of *H. somnus* 8025. Lane 1, unabsorbed MAb 27-1; lane 2, absorbed MAb 27-1; lane 3, unabsorbed calf 9001 convalescent serum; lane 4, absorbed calf 9001 convalescent serum. MAb and serum were diluted 1:1,600 and 1:800, respectively. Sarkosyl-insoluble OMP preparation from strain 8025 was used as the antigen. Approximately 2 μg of protein per lane was resolved. Molecular mass standards (in kilodaltons) are noted on the left. Relative molecular mass estimates (in kilodaltons) are noted on the right.

35 kDa, corresponding to the OmpA protein of *E. coli* (16). These results prompted examinations to clarify the relationship of the heat-modifiable OMP of *H. somnus* to the OmpA protein. Immunoblot analysis of whole-cell preparations from *E. coli* K-12 strains with MAb 27-1 revealed that MAb 27-1 reacted with *E. coli* C600 and P2899 which harbor OmpA protein (Fig. 5, lanes 1 and 3) but not with OmpA-

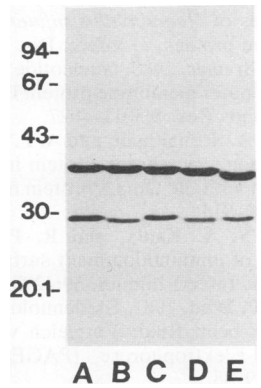

FIG. 4. Immunoblot analysis of whole-cell preparations from representative *H. somnus* strains with MAb 27-1. Five reactivity patterns were observed (lanes A through E). Lane A, strain 719; lane B, strain 8025; lane C, strain 15-PW; lane D, strain 11-2-1; lane E, strain 606. Approximately 5 μg of protein per lane was resolved. MAb 27-1 was diluted 1:20. Molecular mass standards (in kilodaltons) are noted on the left. Relative molecular mass estimates (in kilodaltons) of the bands associated with each reactive pattern were listed in Table 1.

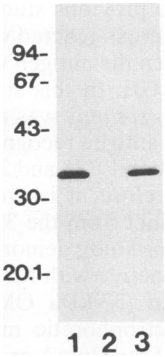

FIG. 5. Immunoblot analysis of whole-cell preparations from *E. coli* K-12 strains with MAb 27-1. Lane 1, OmpA-harboring strain C600; lane 2, OmpA-deficient strain P460; lane 3, *ompA* mutant but OmpA-harboring strain P2899. Approximately 5 μg of protein per lane was resolved. MAb 27-1 was diluted 1:20. Molecular mass standards (in kilodaltons) are noted on the left.

deficient *E. coli* P460 (Fig. 5, lane 2). Moreover, N-terminal amino acid analysis of the 37-kDa heat-modifiable OMP of *H. somnus* revealed that the 37-kDa OMP showed significant homology with the N-terminal sequences of the OmpA proteins from *E. coli* and *Actinobacillus actinomycetemcomitans* (Fig. 6). The first 12 amino acid residues of the 37-kDa heat-modifiable OMP of *H. somnus* showed a 67% overlap with the *E. coli* OmpA sequence (3) and a 100% overlap with the *A. actinomycetemcomitans* 29-kDa OMP sequence (28). We therefore conclude that the heat-modifiable OMP of *H. somnus* is structurally related to the OmpA proteins of other gram-negative bacteria.

DISCUSSION

In the present study, we have developed and used an MAb to characterize a major, 37-kDa heat-modifiable OMP of *H. somnus*. Several lines of evidence from comparisons of results from the present study of the 37-kDa OMP with published results of the 40-kDa OMP of *H. somnus* (5, 24) indicate that the 37-kDa OMP is an entity obviously distinct from the 40-kDa OMP. First, the molecular mass range of cross-reactive antigens of other gram-negative bacteria with MAb 27-1 is apparently different from that with antibody to the 40-kDa OMP. Second, heat treatment altered the mobility of the 37-kDa OMP on SDS-PAGE but not that of the 40-kDa OMP. Third, the N-terminal amino acid sequence of the 37-kDa OMP is different from the predicted N-terminal part of the 40-kDa OMP. Taken together, these observations indicate that the 37-kDa heat-modifiable OMP is an entity distinct from the 40-kDa OMP.

The presence of the 39-kDa OMP of *H. somnus*, which is antigenically distinct from the 40-kDa OMP of *H. somnus*,

OmpA	**A P K D N T W Y T G A K**
37 kDa OMP	**A P Q A N T F Y A G A K**
29 kDa OMP	**A P Q A N T F Y A G A K**

FIG. 6. Comparison of the N-terminal amino acid sequence of the 37-kDa heat-modifiable OMP of *H. somnus* with those of the OmpA protein of *E. coli* and the 29-kDa heat-modifiable OMP of *A. actinomycetemcomitans*.

has been shown in the previous study (5). An MAb against the 39-kDa OMP only cross-reacted with a broad band (38 to 40 kDa) in *H. agni* when the antigen was treated at 100°C for 5 min before SDS-PAGE. In this study, MAb 27-1 against the 37-kDa OMP of *H. somnus* was also cross-reactive with *H. agni*; however, the antigen recognized by MAb 27-1 in *H. agni* appeared as two bands (35 and 26 kDa) when treated at 100°C for 5 min. Therefore, it is probable that the 37-kDa OMP is an entity distinct from the 39-kDa OMP.

MAb 27-1 used in this study demonstrated that the 28-kDa OMP of *H. somnus* reactive with MAb 27-1 was altered to a slower migrating form (37-kDa OMP) on heating before SDS-PAGE. The electrophoretic mobility on SDS-PAGE closely parallels that of the OmpA protein of *E. coli* (16). The results of this study indicate that the heat-modifiable OMP of *H. somnus* exhibits immunological cross-reactivity and N-terminal sequence homology with the OmpA protein of *E. coli* K-12. The OmpA protein serves as a receptor for several bacteriophages (7, 25) and is thought to be required for maintenance of the outer membrane integrity (22). Whether the heat-modifiable OMP of *H. somnus* serves similar functions remains to be determined. Furthermore, a recent study demonstrated that the OmpA protein is a factor in determining resistance to complement-mediated serum killing in a virulent strain of *E. coli* K-1 (26). Whether the heat-modifiable OMP of *H. somnus* contributes to pathogenesis of *H. somnus* infections is of great interest.

From the point of view of vaccine development, identifying common surface antigens among strains is important because immunization with a single common antigen might induce protection from disease due to many strains. Characterization of the conservation of the antigen reactive with MAb 27-1 among the wide range of *H. somnus* strains from diseased animals and carriers showed that this antigen was present in all *H. somnus* strains tested. Characterization of the surface exposure of the antigen revealed that the epitope recognized by MAb 27-1 was not surface exposed on an intact bacterium; however, a decrease in antibody to the antigen after absorption of convalescent serum with intact bacteria indicates that the antigen is surface exposed. Therefore, further studies of the conservation of a surface-exposed epitope(s) on the antigen will be required to elucidate the potential utility of the 37-kDa OMP of *H. somnus* as a vaccine antigen.

In this study, five distinct reactivity patterns in immunoblot analysis with MAb 27-1 were observed among *H. somnus* strains tested. Subtyping systems for certain gram-negative bacteria based on the differences of SDS-PAGE profiles of OMPs and whole-cell proteins have been useful in epidemiologic studies (2, 19). However, the OMPs including the major heat-modifiable OMP, P5, of nontypeable *H. influenzae* are likely to vary in size when serial cultures of the same strain from the same patient with chronic bronchitis are examined (10, 11). Whether the heat-modifiable OMP of *H. somnus* changes in size after animal passage or in vitro passage is of very great interest. If the size of the protein of the same strain of *H. somnus* is stable, a subtyping system for *H. somnus* strains based on the differences of the reactivity patterns with MAb 27-1 may be useful in the epidemiologic study of *H. somnus* infections.

MAb 27-1 directed to the heat-modifiable OMP of *H. somnus* cross-reacted with a variety of gram-negative bacteria in this study. This is consistent with the previous observation that the outer membranes of numerous species of gram-negative bacteria, both enteric and nonenteric, contain a major heat-modifiable OMP structurally similar to the OmpA protein of *E. coli* K-12 (4). Although the heat-modifiable OMP of *H. somnus* is antigenic and elicits an intense antibody response in *H. somnus*-infected animals, the specificity of the antibody directed to the heat-modifiable OMP in sera from *H. somnus*-infected animals is a major problem for use of this OMP as an immunodiagnostic antigen. The cross-reactivity of MAb 27-1 with antigens of other gram-negative bacteria indicates that many false-positive reactions may occur in immunodiagnostic tests based on this antigen.

The outer membranes of gram-negative bacteria are immunologically important structures because of their accessibility to host defense mechanisms. In this study, we have demonstrated that antibody to the heat-modifiable OMP is present in sera from *H. somnus*-infected animals. The presence of antibody to the OMP in convalescent serum suggests that this OMP is potentially important with regard to the bovine antibody response to *H. somnus*. The biological activity of the antibody to the heat-modifiable OMP should be evaluated in future studies.

In summary, our study indicates that a major, 37-kDa heat-modifiable OMP of *H. somnus* is a common antigen among *H. somnus* strains tested and structurally related to the OmpA protein of *E. coli*. Furthermore, antibody to the heat-modifiable OMP is present in convalescent sera from *H. somnus*-infected animals. Further studies of the heat-modifiable OMP will focus on determination of the function of the protein, further elucidation of the antigenic structure of this outer membrane antigen, and assessment of the biological activity of the antibody to this antigen. This will lead to an understanding of the role of this OMP in pathogenesis and its potential utility as a vaccine antigen.

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