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A 16-Kilodalton Lipoprotein of the Outer Membrane of *Serpulina (Treponema) hyodysenteriae*

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Serpulina (Treponema) hyodysenteriae **P18A and VS1 were extracted by using the detergent Triton X-114 and separated into detergent and aqueous phases. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western immunoblot analysis confirmed that a membrane-associated 16-kDa antigen was hydrophobic, since it was found in the detergent phase. A 45-kDa antigen partitioned into the aqueous phase, suggesting that it was hydrophilic and may be of periplasmic origin. When spirochetes were grown in the presence of [^{3}H]palmitic acid, a predominant 16-kDa antigen was labeled; from the results of immunoprecipitation experiments, this antigen appeared to be the same as that recognized by both polyclonal and monoclonal antisera to a previously described 16-kDa antigen. This antigen was proteinase K sensitive and was not a component of the lipopolysaccharide, which, although [^{3}H]palmitate labeled, was resistant to proteinase K digestion. The most probable explanation is that the 16-kDa antigen is a membrane-associated, surface-exposed, immunodominant lipoprotein.**

Swine dysentery is a disease that affects pigs in the postweaning period and is associated with a mucohemorrhagic diarrhea that results in rapid weight loss (16). The etiological agent of the disease is the anaerobic spirochete *Treponema hyodysenteriae* (17, 36), although the severity of the disease may be exaggerated by other bacterial species (15, 20, 26). It has been proposed that the names of this organism and *Treponema innocens*, a porcine nonpathogen should be reclassified under the new genus *Serpulina* (33, 34).

An immune response to the spirochete is generated in both natural and experimental infections with a pathogenic strain of *S. hyodysenteriae* (4, 12, 21); however, it is not always effective in conferring protection against subsequent challenges (28, 30).

It is the outer membrane of the organism which is exposed, during mucosal colonization of the pig colon, to the immune system of the host (19) and which hence provides a target for a protective immune response.

Immunogold-labeling experiments with a polyclonal antiserum raised against outer membranes of *S. hyodysenteriae* demonstrated antibody binding to the surface of the spirochete. The antiserum recognized a major antigen of 16 kDa, which was common to all strains of *S. hyodysenteriae*, and one of 45 kDa, which was restricted to two strains, P18A and MLC52/80 (32). The inhibition of growth of all *S. hyodysenteriae* strains was also demonstrated by using this antiserum (32).

Gram-negative bacteria possess lipid-modified, integral membrane polypeptides (18). In their processed form these proteolipids have three fatty acid residues covalently linked to an N-terminal cysteine (14). It would be expected that modifications of this kind should confer an amphiphilic character to the polypeptides in question (7). An indication of the hydrophobic nature of integral membrane proteins and proteolipids in particular is given by their tendency to enter the detergent phase during the temperature-dependent partitioning of the nonionic detergent, Triton X-114. Integral membrane proteolipids have also been found in the spirochetes *Treponema pallidum* (6, 7, 35) and *Borrelia burgdorferi* (3), and many appear to be immunodominant antigens.

This study was concerned with the characterization of a 16-kDa major antigen of *S. hyodysenteriae* because it appeared to be a potentially important pathogen-specific immunogen.

MATERIALS AND METHODS

Bacterial strains. *S. hyodysenteriae* P18A and VS1 were used in this study. The strains were described by Kent et al. (24) and Sellwood et al. (32). Spirochetes were grown in Trypticase soy broth (BBL), supplemented with 10% rabbit serum, under anaerobic conditions (23).

Immunological reagents. Gnotobiotic pig antisera were raised, either against the outer membrane, designated B50 (32), or against the purified endoflagella (C11) of *S. hyodysenteriae* P18A (24). Monoclonal antibody (MAb) F325 AC4 against the 16-kDa antigen of *S. hyodysenteriae* VS1 (37) was also used in this study.

Protein assay. Protein concentrations were determined by using the BCA protein assay reagent kit (Pierce). Bovine serum albumin standards were prepared from dilutions of 1 to 20 $\mu g \cdot ml^{-1}$, and the reagents were used as specified by the supplier.

Triton X-114 extraction. *S. hyodysenteriae* strains were extracted with the nonionic detergent Triton X-114 by the method of Cunningham et al. (11) with modifications relating to the more favorable culture properties of this organism compared with *T. pallidum*. Cultures (500 ml) were centrifuged at 2,800 × *g* for 15 min, and the organisms were washed three times in 0.05 M Tris-buffered saline (pH 8.0) (TBS). The cell pellet was resuspended in 100 ml of extraction buffer, which contained 0.1% (vol/vol) Triton X-114 (Boehringer-Mannheim), 10 mM Tris, and 5 mM EDTA (pH 7.5). The preparation was mixed gently at 4°C for 18 h and centrifuged at 50,000 × *g* for 1 h at 4°C.

The soluble fraction was removed and warmed to 37°C for 1 h to generate the two phases. The insoluble pellet was reextracted as above, and the remaining pellet was dissolved

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in 2 ml of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. Phase partitioning of the initial detergent-soluble fraction was forced by centrifugation at 50,000 × *g* for 1 h at 25°C. The lower detergent layer was reextracted five times with the addition of sufficient extraction buffer to retain the original volume. The pellet that formed following phase separation and centrifugation at 25°C was reextracted in 0.1% Triton X-114 as above.

The final detergent phase was precipitated with 10 volumes of acetone on ice for 18 h and then centrifuged at 1,000 × *g* for 1 h at 4°C. The acetone was discarded, and the protein pellet was resuspended in phosphate-buffered saline (PBS).

The upper, aqueous phase was reextracted five times with the addition of an appropriate amount of detergent each time to retain a concentration of approximately 0.1% (vol/vol). These detergent phases were discarded. The aqueous phase was treated in a similar way to the detergent phase, except that the material was first concentrated to approximately 5 ml by ultrafiltration through YM5 membrane filter (Amicon) with a 5-kDa cutoff.

SDS-PAGE and Western blotting. The polypeptides were separated by SDS-PAGE, using the method of Laemmli (25) modified as described by Kent et al. (24). Samples were dissolved by boiling for 10 min in sample buffer (62.5 mM Tris-HCl [pH 6.8], 2% [vol/vol] SDS, 2% [vol/vol] 2-mercaptoethanol, 1% [wt/vol] glycerol). Gels were run at a constant 125 V for 5 h. Either the gels were stained with PAGE-Blue 83 (BDH) or polypeptides were electrophoretically transferred to nitrocellulose by the method of Towbin et al. (38). Western immunoblotting was performed with polyclonal gnotobiotic pig antisera B50 and C11, as described by Kent et al. (24). Western blots with MAb culture supernatant fluids required the use of rabbit anti-mouse serum to improve ^{125}I-protein A (Amersham) capture. The rabbit antiserum was preabsorbed with *S. hyodysenteriae* VS1. Autoradiography was performed with X-ray film type RX (Fuji) and enhancing screens at −70°C.

[^{3}H]Palmitic acid incorporation. Mid-log-phase cultures (50 ml) of strain VS1 were incubated with [9,10(*n*)-^{3}H] palmitic acid (Amersham) at a concentration of 10 μCi · ml^{-1}. After incubation for 4, 12, and 24 h at 37°C, 25-ml portions of these cultures were centrifuged at 11,600 × *g* for 15 min and the pellet was washed three times in PBS. The cells were resuspended in 500 μl of PBS and combined with an equal volume of double-strength SDS-PAGE sample buffer.

The gel was dried, and autoradiography was performed as above after fluorographic enhancement with sodium salicylate (5).

Proteinase K treatment. [^{3}H]palmitic acid-labeled spirochetes (strain VS1) were centrifuged at 11,600 × *g* for 10 min. The bacterial pellet and cells prepared from an unlabeled culture of strain VS1 were resuspended in proteinase K buffer (150 mM Tris, 7 mM EDTA, 0.05% SDS). Proteinase K (2 mg · ml^{-1}; Boehringer Mannheim) was added, and the preparations were incubated at 50°C for 2 h. Samples were dissolved in an equal volume of double-strength SDS-PAGE sample buffer for electrophoresis. Identification of the ^{3}H-labeled bands and probing with MAb F325 AC4 were performed as above.

LPS extraction of [^{3}H]palmitic acid-labeled whole cells. Lipopolysaccharide (LPS) was extracted from [^{3}H]palmitic acid-labeled cells of strain VS1 by the method of Westphal et al. (40). The bacteria were centrifuged at 11,600 × *g* for 15 min, and the pellet was resuspended in 500 μl of distilled water and warmed to 68°C. An equal volume of 88% (vol/vol) phenol, also warmed to 68°C, was then added to the cell suspension and mixed occasionally for 15 min. The preparation was cooled to 10°C, mixed, and centrifuged at 11,300 × *g* for 20 min at 4°C. The upper, aqueous phase was carefully removed without disturbing the fluffy interface. The lower, phenol phase was reextracted by rewarming the phase to 68°C and mixing with an equal volume of prewarmed water. The extraction procedure was repeated as above.

The combined aqueous phases were precipitated with 6 volumes of 90% ethanol at −20°C for 18 h. The precipitate was collected after centrifugation at 11,300 × *g* for 30 min at 4°C and extracted with 500 μl of acetone at −20°C for 18 h followed by centrifugation as above. The precipitate was extracted twice more with acetone, dissolved in 100 μl of proteinase K buffer, and divided into two aliquots. One portion was incubated with proteinase K at a concentration of 2 mg · ml^{-1} as above; the other remained untreated. The preparations were mixed with an equal volume of double-strength SDS-PAGE sample buffer for electrophoresis prior to autoradiography.

Immunoprecipitation. Formalin-fixed *Staphylococcus aureus* cells (Bethesda Research Laboratories) were washed three times in complete radioimmunoprecipitation assay (RIPA) buffer (1% [wt/vol] sodium deoxycholate, 1% [wt/vol] Triton X-100, 0.1% [wt/vol] SDS, and 1 mM phenylmethylsulfonyl fluoride) in 10 mM Tris-HCl [pH 7.4]. These cells were resuspended in an equivalent volume of rabbit anti-mouse immunoglobulin G serum (Nordic) diluted 1/20 in 10% [vol/vol] fetal calf serum. The preparation was incubated for 6 h at 4°C and washed in RIPA buffer before being divided into 50-μl aliquots. The cells were centrifuged, and the supernatant fluids were discarded. The cells were then resuspended for 18 h at 4°C in MAb ascites fluid diluted 1/10 to 1/1,000. They were washed in complete RIPA buffer three times as above and centrifuged. Each preparation of antibody-coated cells was resuspended in 50 μl of complete RIPA buffer and combined with an equal volume of solubilized antigen. The Triton X-114 detergent phase extract from strain VS1 (labeled with [^{3}H]palmitic acid) was dissolved in complete RIPA buffer by boiling (final protein concentration, 2 mg · ml^{-1}). The preparation was incubated for 18 h at 4°C, washed three times in complete RIPA buffer and twice in incomplete RIPA buffer (complete RIPA buffer but lacking phenylmethylsulfonyl fluoride). The washed cells were dissolved in 100 μl of SDS-PAGE sample buffer. Following SDS-PAGE the [^{3}H]palmitic acid-labeled preparation was enhanced with sodium salicylate and autoradiography was performed as above.

Immunogold labeling. A culture (2 ml) of *S. hyodysenteriae* VS1 was centrifuged for 15 min at 1,000 × *g* to minimize damage to the cells. After resuspension in PBS and a further centrifugation at 1,000 × *g*, the cells were treated with MAb F325 AC4 for 1 h at room temperature. They were then washed twice with PBS, as above, before treatment for 15 min with 10-nm colloidal gold particles linked to goat anti-mouse antibodies. The cells were washed in PBS, fixed in 3% glutaraldehyde followed by 1% osmium tetroxide, and embedded in Araldite. Ultrathin sections (70 nm) were cut and stained with uranyl acetate and lead citrate before being viewed in a Philips EM300 transmission electron microscope at an accelerating voltage of 80 kV.

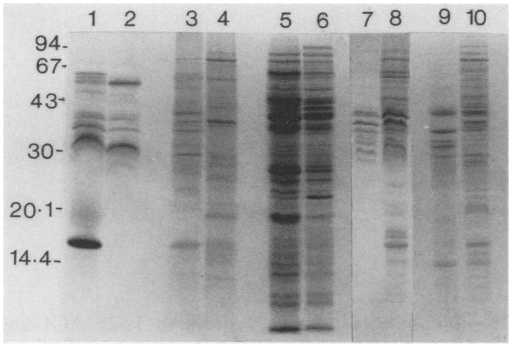

FIG. 1. Comparison by SDS-PAGE of the polypeptides of *S. hyodysenteriae* VS1 (lanes 1, 3, 5, 7, and 8) and P18A (lanes 2, 4, 6, 9, and 10) before and after extraction with Triton X-114. Lanes: 1 and 2, detergent-phase polypeptides; 3 and 4, aqueous-phase polypeptides; 5 and 6, insoluble pellets obtained after extraction with detergent at 4°C; 7 and 9, insoluble pellet obtained after phase partitioning at 37°C; 8 and 10, preparations of whole cells. Molecular size markers are indicated.

RESULTS

SDS-PAGE and Western blotting of Triton X-114 preparations. Phase partitioning of the Triton X-114 extracts from *S. hyodysenteriae* P18A and VS1 resulted in the separation of a small number of hydrophobic, detergent-phase polypeptides from a much larger number of aqueous-phase, hydrophilic polypeptides as shown by SDS-PAGE and PAGE-Blue 83 staining (Fig. 1).

The detergent phase of strain VS1 contained polypeptides of apparent molecular masses 58, 55, 52, 47, 45, 40, 37, 30, and 16 kDa. There were also a number of minor bands between 70 and 90 kDa. The extraction of strain P18A yielded a slightly different group of polypeptides. There was a strong band at 52 kDa, but the 55-kDa band was very weak and the 58-kDa band was absent. The 16-kDa band was greatly reduced and appeared to be absent by PAGE-Blue 83 staining but was visualized by immunoblotting (see below). Other bands in the region of 30 to 40 kDa were of similar size to those found in strain VS1.

The arcing of bands around the 30-kDa position could be diminished by reducing the amount of material loaded onto the gel. At reduced protein concentrations, however, the less abundant bands were not apparent.

Major differences between the polypeptide band patterns of the two strains of *S. hyodysenteriae* were observed in the aqueous phases. In particular, a 16-kDa band was also apparent in strain VS1. However, this was probably not the same polypeptide as that observed in the detergent phase because Western blots with the polyclonal pig antiserum B50 detected the 16-kDa antigen only in the Triton X-114 detergent phases of both P18A and VS1 (Fig. 2A, lanes 1 and 2). Sellwood et al. (32) observed that antiserum B50 also reacted with a band of 45 kDa found in strain P18A. This antigen was retained in the aqueous phase on Triton X-114 phase partitioning (Fig. 2A, lane 4). Western blotting with polyclonal antiserum C11 (24) against the endoflagella demonstrated that all the endoflagellar polypeptides were not solubilized by Triton X-114 and remained in the Triton X-114-insoluble fraction (Fig. 2B, lanes 5 and 6). When MAb F325 AC4 to the 16-kDa antigen was used, only the Triton X-114 detergent phases of both strains VS1 and P18A contained the antigen (Fig. 2C, lanes 1 and 2).

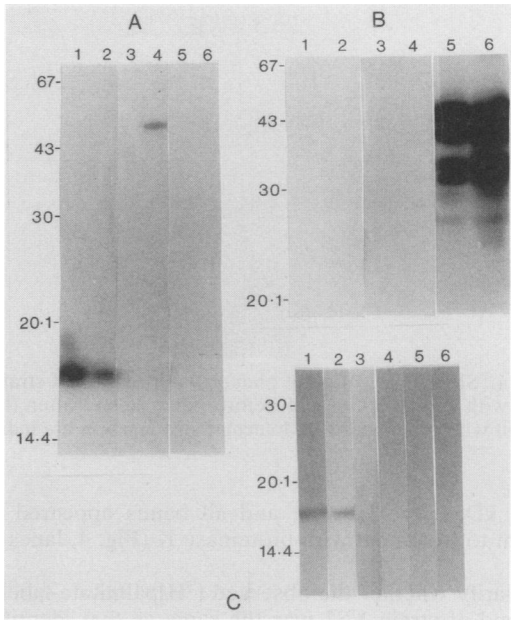

FIG. 2. Western blot analysis of detergent (lanes 1 and 2) and aqueous (lanes 3 and 4) phases and insoluble fractions (lanes 5 and 6) of *S. hyodysenteriae* VS1 (lanes 1, 3, and 5) and P18A (lanes 2, 4, and 6) by using (A) polyclonal, hyperimmune serum B50 to outer membranes, (B) antiserum C11 to purified endoflagella, and (C) MAb F325 AC4 to the 16-kDa antigen. Molecular size markers are indicated.

Intrinsic [^{3}H]palmitic acid labeling and proteinase K treatments. There was strong labeling of a band of 16 kDa after a 24-h incubation of a culture of strain VS1 when [^{3}H]palmitic acid was included in the growth medium (Fig. 3). Fatty acid incorporation was also observed in a doublet of around 35 kDa and also in a band of approximately 60 kDa.

It is known that LPS components of *S. hyodysenteriae* migrate in SDS-PAGE gels in the region of 14 to 30 kDa, and therefore to establish whether the LPS component of *S. hyodysenteriae* was labeled with [^{3}H]palmitic acid, we prepared a hot phenol-water extract from the [^{3}H]palmitate-labeled cells. Several components of approximately 15, 18,

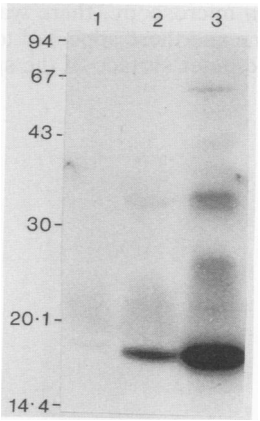

FIG. 3. SDS-PAGE analysis of *S. hyodysenteriae* VS1 labeled in vivo with [^{3}H]palmitic acid for 4 h (lane 1), 12 h (lane 2), and 24 h (lane 3). Molecular size markers are indicated.

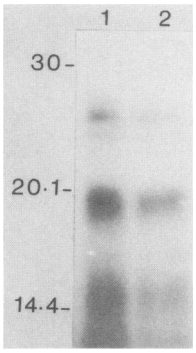

FIG. 4. SDS-PAGE of a hot phenol-water extract of strain VS1 labeled with [^{3}H]palmitic acid before (lane 1) and after (lane 2) treatment with proteinase K. Molecular size markers are indicated.

and 26 kDa were labeled, and all bands appeared to be resistant to treatment with proteinase K (Fig. 4, lanes 1 and 2).

To clarify whether the observed [^{3}H]palmitate-labeled 16 kDa band of strain VS1 was the same as that identified in Western blotting experiments, we used MAb F325 AC4 raised against the 16-kDa antigen of strain VS1. Treatment of whole cells with proteinase K removed the 16-kDa band of strain VS1 visualized both by PAGE-Blue 83 staining and by Western blotting with the B50 antiserum (data not shown) and MAb F325 AC4 (Fig. 5). Moreover, when MAb F325 AC4 was used in immunoprecipitation tests, the [^{3}H]palmitate-labeled material of 16 kDa was precipitated and was sensitive to proteinase K treatment (Fig. 6, lanes 1 and 2). If MAb F325 AC4 was omitted from the test, there was no immunoprecipitation of [^{3}H]palmitate-labeled material (Fig. 6, lanes 3 and 4). The most probable explanation for these observations is that there are two components of approximately 16 kDa which are labeled with [^{3}H]palmitic acid: an LPS component that is resistant to proteinase K digestion and a polypeptide of 16 kDa that is sensitive to proteinase K.

Immunogold-labeling studies. Because the outer membrane of the spirochetes is known to be very fragile, it was essential to reduce the possibility of damage during the immunogold-labeling procedures, and the low centrifugation speed adopted contributed to this. When sections of whole spirochetes from an early-log-phase culture of VS1 were labeled by using MAb F325 AC4 and gold particles and viewed by electron microscopy, there was little damage to the outer membrane and there appeared to be gold particles associated with the outer surface of the spirochete (Fig. 7).

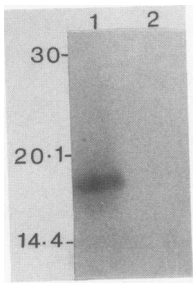

FIG. 5. SDS-PAGE of whole cells of strain VS1, immunoblotted with MAb F325 AC4, untreated (lane 1) and proteinase K treated (lane 2). Molecular size markers are indicated.

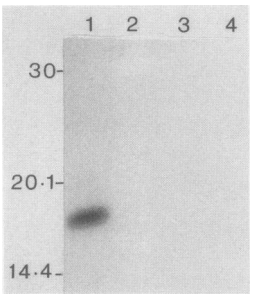

FIG. 6. Immunoprecipitation with MAb F325 AC4 and SDS-PAGE analysis of [^{3}H]palmitate-labeled antigens of *S. hyodysenteriae* VS1 (lane 1). Proteinase K treatment of the immunoprecipitate is shown in lane 2. Control treatments with no MAb are shown in lanes 3 and 4. Molecular size markers are indicated.

Where damage had occurred, there was no labeling of other cell components. These results suggested that the polypeptide antigen is exposed on the outer membrane of the spirochete.

DISCUSSION

The use of SDS for the preparation of outer membranes of *S. hyodysenteriae* appeared to cause solubilization of endoflagellar polypeptides because the B50 antiserum used by Sellwood et al. (32) raised against outer membranes also recognized, on extended exposure of autoradiographs, these polypeptides (31a). However, in this study the nonionic detergent Triton X-114 selectively solubilized polypeptides that were not of endoflagellar origin.

Above the cloud point of Triton X-114 (20°C), the detergent becomes immiscible with water and can be forced to form a separate phase by centrifugation. The upper, aqueous

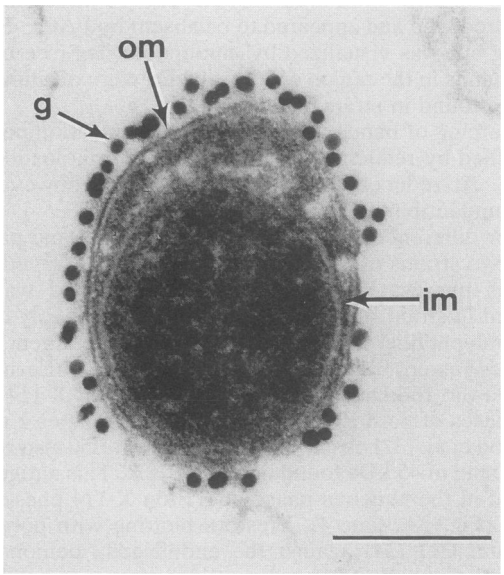

FIG. 7. Immunogold labeling of the 16-kDa antigen of a transverse section of *S. hyodysenteriae* VS1 with MAb F325 AC4. The labeling was carried out prior to embedding, sectioning, and staining with uranyl acetate and lead citrate. Arrows indicate the 10-nm gold particles (g), the outer membrane (om), and the inner cytoplasmic membrane (im). Bar, 0.1 μm.

phase contained a large number of bands. These polypeptides would tend to be hydrophilic and were probably either from the periplasm or released through the rupture of cytoplasmic cylinders.

The hydrophobic components would tend to enter the lower, detergent phase. The amphiphilic properties of polypeptides in the lipid-rich environment of the both the outer and cytoplasmic membranes would enable them to associate more readily with hydrophobic detergent micelles. Compared with the other phases of the extract, relatively few polypeptides entered the detergent phase. However, differences were evident between the two *S. hyodysenteriae* strains examined. The 16-kDa band of strain VS1 was very prominent by PAGE-Blue 83 staining but appeared absent from strain P18A, as observed by Sellwood et al. (32). A number of similar extractions were made with these two strains, and expression of the polypeptide appeared variable, particularly with strain P18A. However, the 16-kDa polypeptide was identified in all P18A cultures by Western blotting with B50 serum (32). Therefore, what appears to be variable expression of this polypeptide may be related to the stage during the growth cycle at which the cells were harvested and/or to regulatory mechanisms associated with the growth medium.

Western blotting experiments with the polyclonal pig serum C11, raised against purified endoflagella, demonstrated that the endoflagellar polypeptides, including one of approximately 44 kDa, were not solubilized by Triton X-114 at a concentration of 0.1% but were confined to the insoluble material. However, higher concentrations of Triton X-114 (0.5%) also solubilized endoflagellar polypeptides (data not shown). The use of this detergent at a concentration of 0.1% therefore provided a useful approach to obtaining preparations devoid of any endoflagellar polypeptides.

The 45-kDa antigen of strain P18A, which was identified in SDS-prepared outer membranes and was recognized by serum B50 (32), could easily be confused with the 44-kDa endoflagellar polypeptide. This 45-kDa antigen was solubilized but was located in the aqueous phase after phase partitioning. Therefore, it appears that P18A probably has two antigens of approximately this size. One is a structural component of the endoflagella (24), and the other may be a periplasmic polypeptide or may result from disruption of the cytoplasmic membrane during the extraction with detergent, because it was present in the detergent-extractable preparations but not in the insoluble material which contained the endoflagella. However, there have been reports that membrane-associated proteins that have highly charged regions may still partition into the aqueous phase of Triton X-114 (9, 27).

Chatfield et al. (8) also used a nonionic detergent (Triton X-100) to solubilize outer membrane polypeptides. Probably because of the disruption caused by the method of preparation of the outer membranes (using a pressure cell), many polypeptides in the range from 24 to 45 kDa were evident. However, these polypeptides were insoluble in the detergent and were probably of endoflagellar origin. Also identified was a detergent-soluble polypeptide of 16 kDa, which may have been the same polypeptide as that described here.

The 16-kDa antigen which was recognized by serum B50 and was found in all strains of *S. hyodysenteriae* examined (32) was located in the detergent phase. This observation demonstrated that the antigen was amphiphilic and therefore may be membrane located. Studies with MAb F325 AC4 to the 16-kDa antigen of strain VS1 also suggested that the antigen resided in the outer membrane (37), and the immunogold-labeling studies of sections of spirochetes presented here suggested that it was exposed on the surface of the spirochete. Western blotting experiments with the MAb also demonstrated that the 16-kDa antigen was proteinase K sensitive and was therefore a polypeptide. These observations also suggest that this polypeptide antigen is distinct from the LPS, which appeared, on SDS-PAGE, to have components of similar size (13, 39) which were also proteinase K resistant (39).

Labeling experiments with [^{3}H]palmitic acid were carried out to establish whether *S. hyodysenteriae*, in common with the other spirochetes *T. pallidum* and *B. burgdorferi*, has lipid-modified polypeptides in its outer membrane. A number of bands were labeled, but labeling was particularly intense as a broad band of approximately 16 kDa. Preparation of LPS from [^{3}H]palmitate-labeled cells clearly demonstrated that the LPS component, which was of similar molecular size to the 16-kDa lipoprotein, was also [^{3}H]palmitate labeled but, unlike the lipoprotein, was proteinase K resistant. These results help to explain the previously described differences in the nature of antigens of this size (13, 22, 32, 39).

Immunoprecipitation experiments confirmed that [^{3}H]palmitic acid was incorporated into the same 16-kDa antigen as that recognized by MAb F325 AC4 in Western blotting experiments. This antigen, which was also sensitive to proteinase K, was therefore probably a lipid-containing polypeptide. These results, together with the surface labeling, agglutination, and growth-inhibitory properties of both the B50 polyclonal serum and this MAb (32, 37), suggest that both sera recognize the same lipid-containing polypeptide.

Therefore, it appears that *S. hyodysenteriae* has at least one integral membrane lipoprotein, which is associated with the outer membrane of the spirochete. A number of integral membrane lipoproteins have been identified in *T. pallidum* (6, 7, 29, 31, 35), but these lipoproteins are probably located within the cytoplasmic membrane and are not thought to be exposed on the surface of the spirochete (10). On the other hand *B. burgdorferi* does appear to possess at least two surface lipoproteins, OspA and OspB (1, 2). The 16-kDa lipoprotein of *S. hyodysenteriae* also appears to be surface exposed. It may sometimes be a component of the LPS-endotoxin preparations often used in vaccination studies (39) and could contribute to the serotype-specific, protective immune response induced. The only evidence, however, that antibodies to the 16-kDa lipoprotein antigen are bactericidal is provided by the growth inhibition studies of Sellwood et al. (32) and Thomas and Sellwood (37). At present the role and primary structure of this antigen are unclear. However, its pathogen specificity, its apparent surface exposure, and the fact that convalescent-phase swine sera appear to have antibodies to it also suggest that it may have some value as a component in the control of swine dysentery by vaccination.

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