Identification and characterization of a Mycoplasma hyopneumoniae adhesin.

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Abstract
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Disciplines
Veterinary Microbiology and Immunobiology | Veterinary Pathology and Pathobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

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Identification and Characterization of a Mycoplasma hyopneumoniae Adhesin

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An adhesin of Mycoplasma hyopneumoniae was identified and characterized in this study. A monoclonal antibody (MAb), F2G5, and its F(ab')2 fragments inhibited the adherence of M. hyopneumoniae to porcine tracheal cilia, the natural targets to which the mycoplasma binds during infection. MAB F2G5 detected multiple bands, but predominantly recognized a 97-kDa (P97) protein of M. hyopneumoniae on immunoblots. Affinity chromatography, conducted with immobilized MAB F2G5, mainly purified P97. The purified proteins were able to bind to cilia and blocked the adherence of intact M. hyopneumoniae cells to cilia. Immunolabeling of mycoplasmas with MAB F2G5 under electron microscopy demonstrated that the proteins recognized by MAB F2G5 were located at the surface of the mycoplasma, predominantly on a surface fuzzy layer. These results indicate that P97 functions as an adhesin of M. hyopneumoniae. The N-terminal amino acid sequence of P97 did not have significant homology with any known bacterial or mycoplasmal adhesins, suggesting that P97 is a novel protein. The predominant proteins detected by MAB F2G5 in different strains varied in size, indicating that the antigen bearing the epitope for MAB F2G5 undergo intraspecies size variation. Antigenic variation of adhesins may be a pathogenic mechanism utilized by M. hyopneumoniae to evade the porcine immune system.

MATERIALS AND METHODS

Mycoplasmas and culture conditions. M. hyopneumoniae LI27 was originally derived from an experimentally infected pig. Strains 232 2A3 and 232 FA1 were cloned from strain 232. Strain J was originally obtained from the American Type Culture Collection and maintained in Fris mycoplasmal medium (9) in this laboratory. Strain 144L was isolated in the laboratory from the lung of an infected pig and filter-cloned three times. It was known that strains LI27, 232, and 144L were pathogenic for pigs, whereas strain J was nonvirulent (38). In this study, mycoplasmas were grown in Fris mycoplasmal medium supplemented with 20% acid-adjusted swine serum. The cultures were incubated in a waterbath shaker at 37°C for 24 to 48 h. The number of mycoplasmas was estimated by the determination of color-changing units (CCU). Mycoplasmas were harvested by centrifugation at 25,000 × g for 15 min. For adherence and adherence inhibition assays, the mycoplasmal pellets were resuspended to 1/10 of the original volume and further diluted with adherence buffer (RPMI 1640 containing 1% gelatin) to the appropriate CCU. For production of mycoplasmal proteins required for affinity chromatography, the pellets were further washed three times with phosphate-buffered saline (PBS, pH 7.2) and resuspended in PBS to 1/100 of the original volume.

MAbs and fragmentation. MAbs to various antigens of M. hyopneumoniae were supplied by Barbara Erickson (Veterinary Medical Research Institute, Iowa State University, Ames). For adherence inhibition, these MAbs in cell culture supernatants were concentrated about 12 times with ammonium sulfate. MAB F2G5, which inhibited the adherence of M. hyopneumoniae to porcine cilia, was purified from ascites with the ImmunoPure immunoglobulin M (IgM) purification kit (Pierce, Rockford, Ill.). The purified MAB F2G5 was resuspended in PBS and stored at -10°C for affinity chromatography and fragmentation. Fragmentation of MAB F2G5 was performed with immobilized pepsin as described before (2) with some modifications. This digestion method generates mainly F(ab')2 fragments from mouse IgM; essentially no (F(ab')2) can be produced (2). Briefly, 2 mg of MAB F2G5 was applied to a Centricron 100 microconcentrator (Amicon, Danvers, Mass.) and spun down at 4,000 × g for 40 min at 4°C. The retentate containing the MAB was washed twice with IgM F(ab')2 digestion buffer (10 mM sodium acetate, 150 mM NaCl, 0.05% NaN3 [pH 4.5]) and finally resuspended in 1 ml of digestion buffer. A small column packed with 2 ml of pepsin-agarose (Pierce) was equilibrated with 10 ml of the digestion buffer and warmed at 37°C for 5 min. Then 1 ml of MAB F2G5 was added to the column and incubated at 37°C for 2 h. The digest was eluted with 4 ml of the digestion buffer. The eluate was filtered with a Microsep 300 concentrator to remove nondigested IgM. The filtrate was further concentrated with a Centricron 30 microconcentrator. The retentate, mainly containing F(ab')2 fragments, was washed twice with PBS and...
resuspended in 0.5 ml of PBS. F(ab’), fragments of MAB 80.1, an antibody against a 64-kDa protein which did not inhibit the adherence of *M. hyopneumoniae* to porcine cilia, were prepared by a similar procedure.

**Adherence and adherence inhibition.** A microtiter plate adherence assay (MPAA) was conducted as described previously (36). Briefly, *M. hyopneumoniae* cells were grown to an OD of 0.5 and mycoplasma proteins (10 µg/ml) resuspended in adherence buffer were added to microtiter plates coated with sodium dodecyl sulfate (SDS)-solubilized porcine tracheal cilia and incubated at 37°C for 90 min. The nonattached mycoplasmas or proteins were washed off the plates with PBS. The mycoplasmas or mycoplasmal proteins attached to the plates were detected by sequential addition of rabbit anti-*M. hyopneumoniae* hyperimmune antibodies and goat anti-rabbit immunoglobulins conjugated with peroxidase. ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) was finally added, and the optical density at 405 nm (OD 405) was measured.

For inhibition assays, various MABs or F(ab’), fragments were preincubated with intact mycoplasmas at 37°C for 60 min. Two washes with PBS were conducted to remove unbound antibodies. The preincubated mycoplasmas were added to cilia-coated plates, and adherence was measured as described above. Percent inhibition was calculated as described previously (36). Adhesins purified by MAB F2G5 affinity chromatography were evaluated for inhibition of the adherence of intact mycoplasmal cells. For this purpose, cilia-coated wells of microtiter plates were first incubated with the purified adhesin at 37°C for 90 min. The adherence buffer was used as the noninhibitory control. After four washes with PBS, intact *M. hyopneumoniae* cells were added to the wells and incubated at 37°C for 90 min. Four washes with PBS were performed to remove nonadherent mycoplasmas. The attached mycoplasmas were subsequently incubated with a monospecific rabbit antiserum (R409) against a 64-kDa surface protein which did not inhibit the adherence of *M. hyopneumoniae* cells to cilia.

**Immunoelectron microscopy.** Immunolabeling of mycoplasmas with MABs was performed as described previously (1). Briefly, *M. hyopneumoniae* cells resuspended in PBS containing 1% gelatin were incubated with 1:10-diluted MAB F2G5 at 37°C for 1 h. Mycoplasmas incubated with cell culture medium were used as negative controls. The mycoplasmas were washed three times with PBS and then reacted with 1:10-diluted goat anti-mouse IgM (m-chain specific) conjugated with gold (10 nm; Sigma) at 37°C for 1 h. After three washes with PBS, the mycoplasmas were fixed with 3% glutaraldehyde-cacodylate buffer, dehydrated in acetone, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and observed under a Hitachi H500 electron microscope.

**Comparison of different strains for adherence activities.** Various strains of *M. hyopneumoniae* were cultured in Fries mycoplasmal medium, harvested by centrifugation, and washed with PBS as described above. The protein concentration of each strain was adjusted to 25 µg/ml for the adherence assay. The protein profiles of different strains were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and by immunoblotting with MAB F2G5.

**SDS-PAGE and immunoblotting.** SDS-PAGE was performed by the method of Laemmli (11), 10% separating gel and 5% stacking gel. Mycoplasma or mycoplasmal proteins were mixed with equal volumes of treatment buffer (0.125 M Tris-HCl [pH 6.8], 4% SDS, 20% glycerol, 10% mercaptoethanol) and heated at 100°C for 3 min prior to electrophoresis. The separated proteins were further reacted with 1:1,000-diluted goat anti-mouse immunoglobulins conjugated with peroxidase and washed three times with the transfer buffer [10 mM 3-(cyclohexylamino)-1-propanesulfonic acid, 10% methanol (pH 11)]. The proteins on the gel were electroblotted to a polyvinylidene difluoride (PVDF) Immobilon-P membrane (Millipore) at 50 mA for 60 min. After being rinsed with distilled water, the membrane was stained with 0.1% Coomassie brilliant blue R-250 for 5 min. Destaining was conducted with a solution of 50% methanol and 10% acetic acid. Finally, the PVDF membrane was thoroughly washed with distilled water and air dried. The membrane was submitted to the Protein Facility at Iowa State University, where P97 was excised from the membrane and subjected to automatic Edman degradation with an Applied Biosystems 477A liquid-pulse sequencer. For analysis of total amino acid composition, P97 was excised from the PVDF membrane and analyzed with an Applied Biosystems 420 PTC amino acid analyzer.

**RESULTS**

**Adherence evaluation by MABs or MAB fragments.** Various MABs were evaluated for the ability to inhibit the adherence of *M. hyopneumoniae* to porcine cilia (Table 1). Two MABs, F2G5 and F1B6, inhibited adherence by up to 67% (Table 2). A similar result was obtained when the hyperimmune serum used for detection was replaced with antiserum R409 (a rabbit monospecific antisera to a 64-kDa surface protein of the mycoplasma), indicating that the two MABs indeed blocked the adherence of mycoplasmas rather than the detection process. As determined by immunoblotting, the two MABs had identical antigenic specificities, detected multiple bands, and reacted predominantly with P97 (Fig. 1). Several other MABs, such as R6C10, I3A6, and F3SD, which also detected multiple bands on immunoblots, did not inhibit the adherence (Table 1).

**Affinity chromatography.** From 1.2 mg of starting material, approximately 150 µg of protein was purified by MAB F2G5 affinity chromatography. SDS-PAGE showed that the predominant protein captured by immobilized F2G5 from LI27 was P97 (Fig. 2). Some faint bands were also present below P97. These minor proteins also reacted with MAB F2G5. On immunoblots, the purified proteins reacted specifically with MAB F2G5 (Fig. 2). Besides P97, a ladder of proteins was detected by F2G5 in close proximity to P72. MAB F2G5 did not detect P78 but did detect an 81-kDa protein in the purified materials (Fig. 2). Purified P97 was diluted to 3.7, 7.5, 15, and 30 µg/ml and evaluated for adherence activity in the MPAA. The results showed that P97 adhered strongly to cilia and that this binding was dose dependent. Non-specific binding of P97 to the control wells that were coated with gelatin or albumin was not detected. Purified P97 also produced a dose-dependent inhibition of the adherence of intact *M. hyopneumoniae* cells to cilia (Table 3).

**Tryptic digestion of purified adhesins.** Treatment of P97 purified by antibody affinity chromatography yielded a ladder immediately neutralized to pH 7.5 with 1 M Tris and concentrated to 1 ml with a Centricon 10. The purity of the proteins was determined by SDS-PAGE and immunoblotting; the adherence activity of the eluted proteins was evaluated in the MPAA; and the purified proteins were also used to block the adherence of intact *M. hyopneumoniae* cells to cilia.
TABLE 1. Adherence inhibition by various MAbs

<table>
<thead>
<tr>
<th>MAb</th>
<th>Isotype</th>
<th>Specificity*</th>
<th>Adherence inhibition†</th>
</tr>
</thead>
<tbody>
<tr>
<td>80.1</td>
<td>IgM</td>
<td>64</td>
<td>–</td>
</tr>
<tr>
<td>A1B2</td>
<td>IgM</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>A2H10</td>
<td>IgG1</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>F1B6</td>
<td>IgM</td>
<td>Multiplec</td>
<td>+</td>
</tr>
<tr>
<td>F2B11</td>
<td>IgG2a</td>
<td>86</td>
<td>–</td>
</tr>
<tr>
<td>F2G5</td>
<td>IgM</td>
<td>Multiplec</td>
<td>+</td>
</tr>
<tr>
<td>F3A6</td>
<td>IgG2b</td>
<td>116</td>
<td>–</td>
</tr>
<tr>
<td>F3B8</td>
<td>IgG1</td>
<td>105, 95, 65, 55</td>
<td>–</td>
</tr>
<tr>
<td>F3D5</td>
<td>IgG1</td>
<td>105, 95, 65, 55</td>
<td>–</td>
</tr>
<tr>
<td>F3D6</td>
<td>IgG1</td>
<td>105, 95, 65, 55</td>
<td>–</td>
</tr>
<tr>
<td>H1D8</td>
<td>IgG1</td>
<td>116</td>
<td>–</td>
</tr>
<tr>
<td>E1D2</td>
<td>IgG1</td>
<td>86</td>
<td>–</td>
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<td>I3A6</td>
<td>IgG1</td>
<td>200, 145, 140, 116, 86</td>
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<tr>
<td>I4A10</td>
<td>IgG1</td>
<td>70</td>
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<td>I4A4</td>
<td>IgG1</td>
<td>100, 90, 80, 60, 50</td>
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<tr>
<td>I4B1</td>
<td>IgG1</td>
<td>41, 43</td>
<td>–</td>
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<tr>
<td>I4B10</td>
<td>IgG1</td>
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<td>I4B2</td>
<td>IgG1</td>
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<tr>
<td>I4E3</td>
<td>IgG1</td>
<td>116</td>
<td>–</td>
</tr>
<tr>
<td>R1E9</td>
<td>IgG3</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td>R2E8</td>
<td>IgG1</td>
<td>67</td>
<td>–</td>
</tr>
<tr>
<td>R3G7</td>
<td>IgG1</td>
<td>116</td>
<td>–</td>
</tr>
<tr>
<td>R4B2</td>
<td>IgG1</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td>R4B7</td>
<td>IgG2a</td>
<td>116, 105, 90, 80, 65, 60, 55</td>
<td>–</td>
</tr>
<tr>
<td>R5F4</td>
<td>IgG1</td>
<td>116</td>
<td>–</td>
</tr>
<tr>
<td>R6C10</td>
<td>IgG2a</td>
<td>116, 105, 90, 80, 65, 60, 55</td>
<td>–</td>
</tr>
<tr>
<td>R6E1</td>
<td>IgG1</td>
<td>116, 40</td>
<td>–</td>
</tr>
<tr>
<td>R6E4</td>
<td>IgG2a</td>
<td>116, 105, 90, 80, 65, 60, 55</td>
<td>–</td>
</tr>
<tr>
<td>R6G8</td>
<td>IgG1</td>
<td>46</td>
<td>–</td>
</tr>
</tbody>
</table>

* Sizes of antigens (in kilodaltons) detected with strain LI27 on immunoblot. Some MAbs detected antigens of varied sizes in different strains.
† +, produced dose-dependent inhibition.
‡ Reacted with a set of proteins with distinct sizes, but predominantly recognized a 97-kDa protein.

of peptides that reacted with F2G5 (Fig. 3). The minimum resolvable fragment of trypsin-digested adhesins was approximately 20.5 kDa. As estimated by SDS-PAGE, the size difference between the peptides was approximately a multiple of 0.5 kDa.

**Immunoelectron microscopy.** *M. hyopneumoniae* cells were reacted with F2G5 and goat anti-mouse IgM conjugated with gold particles to determine the distribution of the adhesins on mycoplasmas. MAB F2G5 stained the fuzzy surface structures of *M. hyopneumoniae* (Fig. 4). The gold labeling was distributed randomly on the surface of mycoplasmas; it was not polarized at a specific region, as demonstrated with the P1 adhesin of *M. pneumonaeae* (12).

*M. hyopneumoniae* cells reacted with cell culture medium and the conjugate were used as negative controls and did not display any labeling with the gold particles (Fig. 4).

**Strain variation in adherence.** Five strains of *M. hyopneumoniae* differed markedly in adherence activity when evaluated by the MPAA (Fig. 5). The adherence activity of strain J was substantially lower than that of other strains, whereas strain 144L had the highest adherence activity among the five strains. One of the major differences revealed by SDS-PAGE among the five strains was in the regions detected by MABF2G5 (Fig. 6). Immunoblotting with MAB F2G5 revealed that P97 was missing in strain 144L and strain J, with the concomitant appearance of a 95-kDa protein in strain J and a doublet (93 and 92 kDa) in strain 144L that reacted with MAB F2G5 (Fig. 6). Also, P72 was hardly detectable in strain J and disappeared in strain 144L, but both strains J and 144L had an extra band (approximately 69 kDa) that reacted with the MAB (Fig. 6).

**N-terminal sequence and amino acid composition.** Expressed in the single-letter code, the N-terminal sequence of P97, beginning with alanine, was ADXKTDSDKDPSTLRAIDEQ. The X in position 3 indicates an ambiguous residue. This sequence was compared by the GCG program with all

**TABLE 2. Adherence inhibition by MAbs F2G5, F1B6, and F3A6**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>MAB</th>
<th>Mean % Inhibition‡ ± SD produced by MAB:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F3A6</td>
<td>F1B6</td>
</tr>
<tr>
<td>1:2</td>
<td>-11.9 ± 3.1</td>
<td>67.0 ± 5.1</td>
</tr>
<tr>
<td>1:10</td>
<td>2.5 ± 2.2</td>
<td>45.9 ± 3.4</td>
</tr>
<tr>
<td>1:50</td>
<td>-11.1 ± 6.5</td>
<td>-10.8 ± 6.1</td>
</tr>
<tr>
<td>1:250</td>
<td>-1.1 ± 2.4</td>
<td>-9.7 ± 8.0</td>
</tr>
</tbody>
</table>

* MAbs were concentrated approximately 12 times from the culture supernatant and further diluted in the adherence buffer.
† Mean of three independent experiments.
known protein and DNA sequences in the GenBank, EMBL, and Swiss-Prot databases. No homology between this sequence and the sequences of known bacterial or mycoplasmal adhesins was detected. Analysis with Peptidestructure in the GCG sequence analysis software package predicted that this piece of peptide from P97 was hydrophilic and surface exposed (data not shown). The total amino acid composition of P97 was also determined, and the molar ratios of various amino acids in P97 are shown in Table 4.

### DISCUSSION

It was found in this study that (i) MAb F2G5, which recognized a predominant 97-kDa protein in several strains of *M. hyopneumoniae*, and its F(ab')2 fragments inhibited the adherence of *M. hyopneumoniae* to cilia; (ii) that purified P97 adhered to cilia and blocked the adherence of intact *M. hyopneumoniae* cells; and (iii) MAb F2G5-reacting proteins were located on the surface of *M. hyopneumoniae*. These findings strongly indicate that P97 is an adhesin of *M. hyopneumoniae*. Previous studies indicated that P97 was expressed during in vivo infection and was one of the major immunogens of *M. hyopneumoniae* (33, 35). In fact, P97 elicited a very early immune response in the respiratory tract of swine, because IgA and IgM antibodies to P97 were detected in the airway wash samples of contact-exposed pigs 35 to 60 days earlier than antibodies to other major immunogens of the mycoplasma (33). Since P97 was only expressed by virulent *M. hyopneumoniae* and not by nonvirulent mycoplasmas, it had been proposed that P97 might be one of the virulence-associated antigens of *M. hyopneumoniae*, although the biological function of P97 was unknown at that time (35). The results from this study provided new evidence for the biological function of P97 in *M. hyopneumoniae* infection. These findings strongly indicate that P97 may be an important virulence factor of *M. hyopneumoniae*.

MAb F2G5 mainly stained the fuzzy structures on the surface of *M. hyopneumoniae*, suggesting that the fuzzy structures may be involved in the adherence of the mycoplasmas. It was reported previously that the fuzz on *M. hyopneumoniae* bridges the interaction between mycoplasmas and cilia (3, 22) and that the fuzzy structures were observed with a pathogenic strain but not with a strain that was nonadherent and nonpathogenic in pigs (22). This evidence, together with the information generated from this study, strongly suggested that the fuzzy structures on *M. hyopneumoniae* play an important role in adherence. The fuzz on the surface of streptococci, composed of M proteins and lipoteichoic acid, is known to mediate the adherence of streptococci to eucaryotic cells (6, 7, 25).

It is unlikely that the reactivity of MAb F2G5 to multiple proteins was caused by impurity of the MAb. The hybridoma cell line that secretes MAb F2G5 was subcloned by standard procedures, and the secreted antibodies were detected as a homogeneous IgM isotype. Also, MAb F1B6, which was produced by an independent hybridoma, had an antigenic specificity identical to that of F2G5. It is also unlikely that the multiple reactivity was caused by the degradation of P97 during the process of harvesting or SDS-PAGE, because inclusion of a proteinase inhibitor (phenylmethylsulfonyl fluoride) during the process did not change the reaction patterns of MAb F2G5. It is believed that the reactivity of MAb F2G5 to multiple proteins was indeed due to the presence of the epitope in multiple antigens. In fact, the expression of an epitope on multiple proteins is common to many mycoplasma species, such as *M. hyorhinis* (18, 19), *M. pulmonis* (28), *M. hominis* (16), *Ureaplasma urealyticum* (27), and *M. fermentans* (31).

MAb F2G5 reacted with multiple proteins in a single strain on immunoblots (Fig. 1) and recognized a different predominant protein in different strains (Fig. 6). Treatment of purified P97 with trypsin under controlled conditions yielded multiple peptide fragments that reacted with MAb F2G5 (Fig. 3). These data clearly showed the heterogeneity of the putative adhesin of *M. hyopneumoniae*. However, the relationships of these MAb F2G5-reacting proteins or peptides were not revealed by the data obtained from this study. It is our speculation that these proteins may be size variants of a surface protein encoded by a gene with repetitive sequences. Molecular cloning and sequencing of the gene for P97 will be required to verify this hypothesis.

It was shown previously that different strains of *M. hyopneu- moniae* vary in pathogenicity for pigs (38). Strains Li27, 144L, 232 2A3, and 232 FA1 were pathogenic, while strain J was incapable of inducing pneumonia in pigs (33, 38). Also, no mycoplasmas were isolated from the lungs of pigs inoculated

### Table 3. Inhibition of *M. hyopneumoniae* adherence by purified adhesins

<table>
<thead>
<tr>
<th>Mycoplasmas (CCU)</th>
<th>Mean adherence ± SD with indicated concn (μg/ml) of purified adhesins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>5 × 10^8</td>
<td>0.55 ± 0.03</td>
</tr>
<tr>
<td>2 × 10^8</td>
<td>0.40 ± 0.01</td>
</tr>
<tr>
<td>8 × 10^7</td>
<td>0.21 ± 0.01</td>
</tr>
</tbody>
</table>

a Detected with a monospecific serum (R409) that did not react with P97. Data are expressed as the mean OD of duplicate experiments.

b Significantly different from the controls without adhesins, as determined by analysis of variance (*P < 0.05*).
with strain J, indicating that this strain had lost the ability to colonize the porcine respiratory epithelium (38). As shown in Fig. 5, adherence to cilia differed among the five strains. It appeared that the low adherence activity of strain J was correlated with its nonpathogenicity in pigs. However, this interpretation should be made cautiously because these strains may also vary in virulence traits other than adherence activity. Size shifting of P97 was demonstrated in strains 144L and J (Fig. 6).

The effect of size variation of P97 on the adherence ability of the mycoplasma remains to be determined in future studies. P97 is unlikely to be a lipoprotein because P97 was not extracted with Triton X-114 (data not shown). It was also shown previously that *M. hyopneumoniae* only had four lipoproteins, with molecular masses ranging from 44 to 65 kDa (30), although a large number of lipid-modified proteins have been found in other mycoplasma species (29, 32) and bacteria.
Several mycoplasmal adhesins, such as P1 and P30 of \textit{M. pneumoniae} and P140 of \textit{M. genitalium}, have considerable homology at both the protein and DNA levels (4, 5, 17). However, the N-terminal sequence of P97 did not have any homology with known mycoplasmal adhesins. This result suggested that P97 of \textit{M. hyopneumoniae} might be distinct from other mycoplasmal adhesins. In addition, this sequence may be useful for cloning the P97 gene in future studies. The absence of cysteine and tryptophan residues in Table 4 does not necessarily mean that there are no tryptophan or cysteine residues in P97, because these two residues are labile to the acid hydrolysis step required for amino acid analysis (26). Also, the regular procedure of amino acid analysis was not calibrated to detect a lipid-modified cysteine. Since asparagine and glutamine were deaminated to the corresponding carboxylic acids (Asp and Glu, respectively) as a consequence of the acid hydrolysis step (26), the values for Asp and Glu (Table 4) represented a mixture of asparagine and aspartic acid and a mixture of glutamine and glutamic acid, respectively. It will not be possible to know the relative amount of each in the mixtures until the full amino acid sequence of P97 has been determined.

P97 may not represent the only adhesin of \textit{M. hyopneumoniae}, since MAb F2G5 and purified P97 did not completely inhibit the adherence of \textit{M. hyopneumoniae} to cilia. This is not surprising, since pathogenic bacteria and mycoplasmas usually have more than one adhesin. For example, \textit{M. pneumoniae} has two defined adhesins, P1 and P30 (17); a recent study suggested that another surface protein (P90) was also involved in the adherence of \textit{M. pneumoniae} (8). \textit{Bordetella pertussis} possesses four potential adhesins: filamentous hemagglutinin, pertussis toxin, pertactin, and fimbriae (24). These adhesins mediate the adherence of \textit{B. pertussis} either to respiratory ciliated cells or to macrophages (24). The interactions between the multiple adhesins of an organism and the receptors on host cells provide for increased adherence specificity and strength. It is expected that more adhesins, other than those recognized by MAb F2G5, of \textit{M. hyopneumoniae} may be discovered in future studies.

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