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Characterization of the Strain-Specific and Common Surface Antigens of *Mycoplasma arginini*

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A combination of quantitative immunoelectrophoresis and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was used to determine location and molecular weights of surface membrane antigens of four strains of *Mycoplasma arginini*. Two major surface antigens were identified for *M. arginini* by absorption of antiserum with whole cells: one surface antigen was strain specific, electrophoretically fast, and prominently located on the surface, whereas the other surface antigen was common to the four strains and of intermediate electrophoretic mobility. Three of the four strains of *M. arginini* (G-230, 23243, and 27389) possessed immunologically strain-specific antigens which did not cross-react, whereas the leonis strain lacked an immunologically detectable unique surface antigen. A monospecific antiserum prepared against immune precipitates of the strain-specific antigen of strain G-230 detected three polypeptides of 74,000, 44,000, and 17,000 daltons in SDS-polyacrylamide gels of membrane preparations. All four strains shared the common surface antigen which appeared considerably more hydrophobic than the strain-specific surface antigen because it could only be demonstrated by charge-shift immunoelectrophoretic conditions (addition of deoxycholate to the nonionic detergent). Monospecific antiserum to the common antigen of strain G-230 reacted with all four *M. arginini* strains, but did not react with two other arginine-utilizing species, and recognized three polypeptides of 40,000, 29,000, and 20,000 daltons in membranes of strain G-230. Whereas the common surface antigen is a likely target for conventional serological reactions used for identification of the species *M. arginini*, strain-specific antigen cannot fulfill this role but must participate in other surface reactions.

The *Mycoplasmatales* have proven to be highly useful models for the study of membrane structure and function because of their simplicity of form: the cells are bounded by a unit membrane and have no internal membranes. In addition, the role of membrane components as antigens has been of interest because of the obvious importance of the surfaces of the organisms in the initial interaction with the animal host. Further definition of the surface membrane antigens of a *Mycoplasma* species has clear utility as a model for study of other membrane-bounded organisms.

Studies of the antigenic character of mycoplasmic membranes have provided the information that: membrane protein antigens of several species participate in a variety of serological reactions used to identify *Mycoplasma* species (4, 13, 18, 19, 22, 24), the external surfaces of *Acholeplasma laidlawii* and *Mycoplasma arginini* each bear at least one antigenic protein solubilized readily by nonionic detergents (1, 12, 17), and serological differences between strains of *M. hominis* and *M. arginini* are correlated with differences in the antigenic composition of their membranes in contrast to the similarity of cytoplasmic components (1, 14).

Strains of the species of *M. arginini* were selected for our studies because of their intraspecies heterogeneity (34) and the availability of antisera sufficiently powerful for the production of distinct profiles in two-dimensional immunoelectrophoresis. In our previous study (1), we characterized the antigens of strain G-230, resolved by this method, with respect to their membrane and cytoplasmic origin. This strain was shown to possess a unique surface membrane antigen. In addition, five other membrane antigens were resolved more clearly by two-dimensional immunoelectrophoresis in the presence of both Triton X-100 and sodium deoxycholate than with the nonionic detergent alone (2). In the present study, the surface antigens of four strains of *M. arginini* are compared and characterized with respect to molecular weight, disposition, and taxonomic specificity. We identify a strain-specific surface antigen and an *M. arginini* common surface antigen. This is the

first mycoplasmic species for which such antigens have been described.

MATERIALS AND METHODS

Organisms. Four strains representing *M. arginini* were used in this study. *M. arginini* strain G-230 was obtained from M. F. Barile (6), *M. arginini* strain leonis was obtained from W. Dowdle (35), and *M. arginini* strains 23243 and 27389 were obtained from the American Type Culture Collection. Additional arginine-utilizing strains used were *M. hominis* (ATCC 14027) and *M. gateae* strain SIAM, a gift from B. C. Cole.

Preparation of cellular antigens and antisera. Immunogens, serological test antigens, and antisera were prepared as described in a previous study (1). Briefly, immunogens were cultivated in soy peptone-fresh yeast dialysate broth (20) supplemented with 5 to 10% agamma rabbit serum. Serological test antigens were also grown in the same broth, but 10% agamma horse serum was used as the supplement. Rabbits were immunized with whole-cell preparations of each strain as described previously (21). Monospecific sera were prepared, using as immunogens two specific immunoprecipitates (membrane antigens a-b and c from strain G-230; 2) excised from two-dimensional immunoelectrophoresis gels (1, 10). Freund complete adjuvant was used for immunization for monospecific antiserum in contrast to the incomplete adjuvant used for production of antibody to whole cells.

Preparation of membranes. Cells were lysed with digitonin as described by Rottem and Razin (30). Cell suspensions (30 to 60 μg of protein per ml, at 37°C) were mixed with (40 μg/ml) digitonin, 40:1 (vol:vol), and incubated for 20 min at 37°C. Lysed cells were centrifuged at 34,000 × *g* to sediment the membranes. The membranes were washed three times and suspended in 0.25 M NaCl.

Absorption of antisera. For absorption of antiserum by intact cells, a 700-ml batch of cells was grown to late exponential phase. The cells were harvested by centrifugation at 8,000 × *g* for 15 min, washed once in 0.25 M NaCl, and resuspended to approximately 1 mg of cell protein per ml. Volumes ranging from 0.0 to 0.15 ml of cell suspension were added to 1.0 ml of antiserum. The total volume of each sample was adjusted to 1.15 ml with 0.25 M NaCl. Samples were kept on ice for 14 h. Cells were removed by centrifugation for 5 min at 12,000 × *g* in a Brinkman Eppendorf 3200 centrifuge, and the supernatant was used as the antiserum in two-dimensional immunoelectropherograms. A 10-μl portion of the supernatant from a control cell sample (incubated with diluent only) was run as antigen against 0.2 ml of unabsorbed antiserum to detect leakage of cytoplasmic antigens during absorption.

SDS-polyacrylamide gel electrophoresis. Proteins were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, using the discontinuous system described by Laemmli (23) with modifications. The slabs consisted of 5% stacking and 10% separating gels. Cell and membrane preparations were solubilized with Triton X-100 and sodium deoxycholate at 0.5% final concentration of each. Samples were then treated with the Laemmli solvent [SDS-2-mercaptoethanol-glycerol-tris(hydroxymethyl)aminomethane] and boiled for 2 min. Volumes of 20 to 25 μl containing approximately 1 μg of protein per μl were applied to the sample slots and electrophoresed for 2.5 h at 24 mA until the bromophenol blue marker had migrated 6 to 7 cm from the beginning of the separating gel. Slabs were stained for protein with Coomassie brilliant blue R-250 in water-methanol-trichloroacetic acid (5:1:1, vol/vol/wt) and destained in water-methanol-trichloroacetic acid (38:2:3, vol/vol/wt) (26). This staining procedure, followed by storage of the stained and decolorized gels in 7% acetic acid, minimized distortion due to gel shrinkage. Some sections of the gels were removed before staining for use as antigen strips for two-dimensional immunoelectrophoresis.

Preparative agarose electrophoresis. To correlate patterns obtained from two-dimensional immunoelectrophoresis with those of SDS-polyacrylamide gels, fractions were prepared from preparative agarose plates run similarly to the first phase of two-dimensional immunoelectrophoresis (16). Agarose (6 ml) in Veronal buffer at 0.05 M, pH 8.6, was applied to each of eight slides (5 by 7.5 cm). The antigen mixture was placed in slits, (0.2 by 10 mm), and the material was electrophoresed for 4 h at 3 to 4 V/cm as tested directly on the agarose. Appropriate agar sections were cut out (as determined from previously developed two-dimensional patterns), pooled for each fraction, and frozen. The fluid which exuded when the agarose pieces were thawed was analyzed by SDS-polyacrylamide gel electrophoresis.

Two-dimensional immunoelectrophoresis. Two-dimensional immunoelectrophoresis of samples separated in agarose gels was performed as described previously (1, 33). The support was 0.5% agarose in Veronal buffer at 0.05 M, pH 8.6, containing Triton X-100 at 0.5% and sodium deoxycholate at 0.1% except for some absorption studies of strains 23243 and leonis in which Triton X-100 was the only detergent. Electroendosmosis was equal to $-m_r$ of 0.09. The concentration of the Veronal running buffer was 0.1 M, pH was 8.6, and the conductivity of the agarose support was 2.8 mS at a buffer concentration of 0.05 M.

Two-dimensional immunoelectrophoresis using SDS-polyacrylamide gels as the first phase. Strips containing samples separated by SDS-polyacrylamide gel electrophoresis were excised and placed in Veronal buffer containing 0.5% Triton X-100 and 0.1% sodium deoxycholate for 20 to 30 min before placement on agarose-precoated slides (5 by 7.5 cm) at a distance of 0.5 cm from the long edge of the slide. The polyacrylamide gel strips were completely covered with 0.5% agarose in buffer containing the same detergent concentrations so that a strip of agarose of 2 to 3 mm remained between the polyacrylamide strip and the antiserum-containing agarose (also containing both detergents) poured onto the remainder of the slide. Electrophoresis into the antiserum bed was for 7 h at 3 V/cm as tested directly on the agarose. Embedding of the polyacrylamide strips in the nonionic detergent was required to complex the SDS so that it would not interfere with the precipitin reaction (7, 32). Deoxycholate was included to enhance migration of membrane proteins (2).

RESULTS

Comparison of cell and membrane polypeptide patterns. The patterns from SDS-polyacrylamide gel electrophoresis of the polypeptides from detergent-solubilized whole cells were quite similar for strains G-230, 23243, and 27389 (Fig. 1A). In contrast, the patterns derived from membrane preparations of strains G-230, 23243, and leonis were noticeably different from each other (Fig. 1B). For instance, although the patterns of bands for G-230 and leonis were similar in the region between 65,000 and 75,000 daltons, for strain 23243 the distribution of bands differed strikingly. In addition, polypeptides smaller than 30,000 daltons showed considerable variation among the three strains. On the other hand, the proteins larger than 95,000 daltons varied only slightly.

Identification of membrane antigens. Two-dimensional immunoelectrophoresis of the membrane preparations using the SDS-polyacrylamide strips for the first dimension resulted in the resolution of 11, 8, and 14 antigens for strains G-230, 23243, and leonis, respectively (Fig. 2). (In comparison, standard two-dimensional immunoelectrophoresis profiles of the same preparations with the first-phase separation in agarose contained 11, 11, and 14 peaks, respectively.) The most prominent peak in each profile, as determined by area and staining intensity, was at 76,000 (strain G-230), 72,000 (strain 23243), and 79,000 (strain leonis) daltons. Polypeptides smaller than 45,000 daltons did not produce distinct peaks in reactions with antiserum prepared to whole organisms. Interestingly, the density of staining of the bands on polyacrylamide gels did not necessarily correlate with the staining intensity, with the height, or even with the presence of peaks in the second dimension.

Demonstration of major surface antigens. When 23243 membranes were tested against anti-23243 serum absorbed with increasing concentrations of intact cells, the areas of both the prominent, electrophoretically fast peak (relative mobility versus bovine albumin of 1.1, arrow, Fig. 3A) and the less distinct, slower peak (0.65) increased, indicative of exposure on the exterior surface of the membrane (1, 17). A similar reaction of 27389 cells with absorbed anti-27389 serum also resulted in a demonstration of fast, externally exposed antigen (arrow, Fig. 3B). The use of such procedures with strain leonis has repeatedly failed to show a comparable fast outer membrane antigen even with addition of sodium deoxycholate to the agarose mixture to improve resolution. However, a low, densely staining band was visible in homologous reactions but not in heterologoous reactions and it may represent a relatively hydrophobic version of such an antigen (not shown). This band disappeared under charge-shift conditions.

Uniqueness of major surface antigens. When membranes or cell preparations of each of the strains were tested against any of the heterologous antisera, the fast surface antigens were

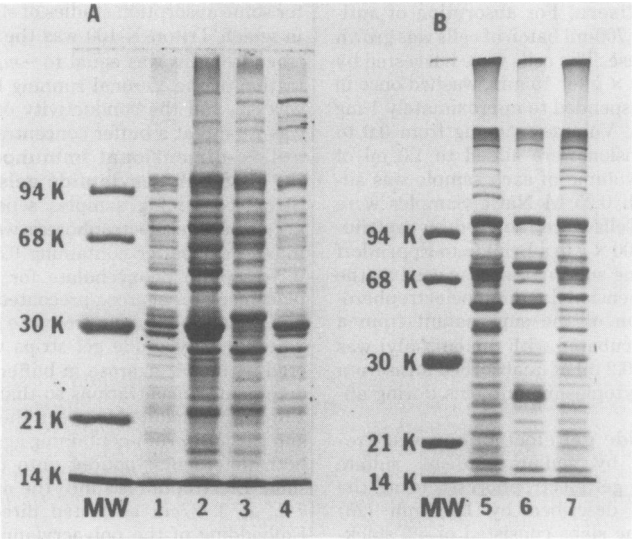

FIG. 1. *Comparative SDS-polyacrylamide gel electrophoresis of (A) whole-cell and (B) membrane preparations of M. arginini. Wells 1 and 5 represent strain G-230; wells 2 and 6 represent strain 23243; wells 3 and 7 represent strain leonis; well 4 represents strain 27389. Twenty-five micrograms of protein of each was applied to the gel. After electrophoresis, the gel was stained with Coomassie brilliant blue R-250.*

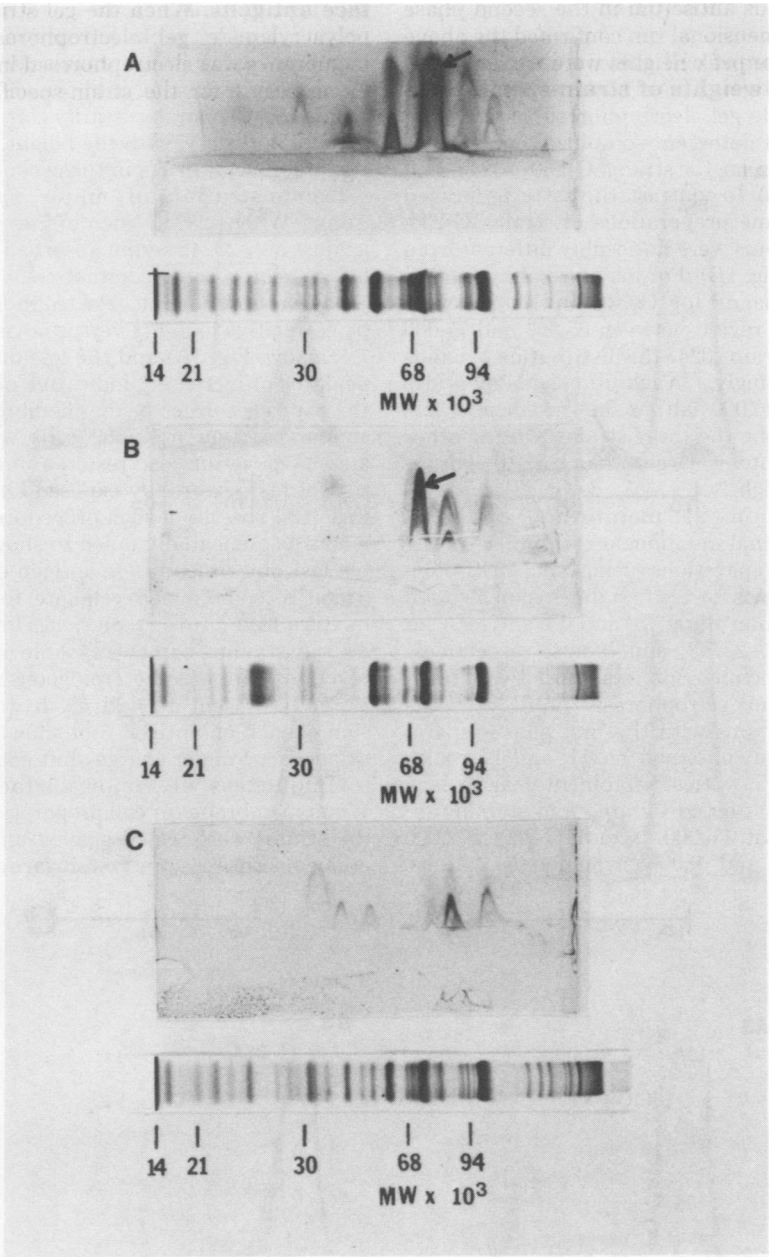

FIG. 2. *Two-dimensional immunoelectrophoresis of membranes of M. arginini. First-phase separation was carried out by SDS-polyacrylamide gel electrophoresis. (A) Strain G-230; (B) strain 23243; (C) strain leonis. Samples containing 20 μg of total membrane protein were subjected to electrophoresis in the polyacrylamide gel and were developed against antisera to whole cells of M. arginini. Antibody beds contained 10% serum for anti-G-230 and anti-leonis and 20% for anti-23243. A control stained polyacrylamide strip is shown at the base of the figure with molecular weights indicated below. Register is slightly imperfect because of shrinkage of the polyacrylamide. Anode is at the left and top of the figure.*

found to be unique to the strain. Antiserum against strain 27389 cells did not react with the antigen a-b of strain G-230 or the major surface antigen of strain 23243. The cross-reacting antigens seen were of relative mobilities of 0.05 to 0.5. In addition, suppression (5) of the antigen profiles of membranes of strains 23243 and leonis with the use of anti-G-230 serum in addition to

the homologous antiserum in the second phase of the two-dimensional run confirmed the above results because peak heights were unchanged.

Molecular weights of strain-specific surface antigens. When the gel strip from SDS-polyacrylamide gel electrophoresis of G-230 membranes was electrophoresed into antiserum monospecific for the strain-specific antigen of

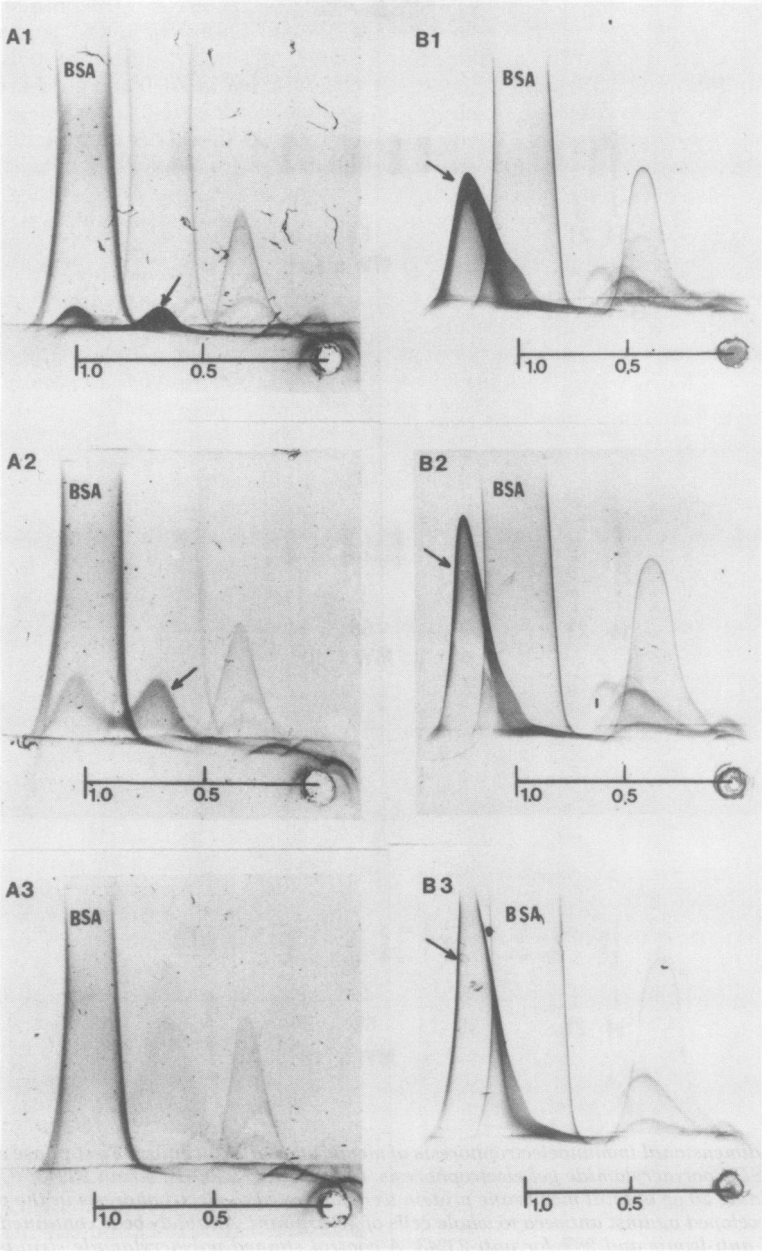

FIG. 3. *Two-dimensional immunoelectrophoresis profiles of M. arginini cells against homologous unabsorbed antisera and antisera absorbed with homologous intact cells. Antigens: (A) strain 23243; (B) strain 27389. Antisera: (1) unabsorbed; (2) absorbed with 0.04 to 0.08 mg of cell protein; (3) absorbed with 0.08 to 0.16 mg of cell protein (twice the concentration used in A2 and B2 above). Antigen samples contained 10 μg of cell protein. The bovine serum albumin (BSA)-anti-BSA peak was included as a marker for relative electrophoretic mobility. Arrows indicate surface antigens. Anode is at left and top of figure.*

that strain, peaks formed for polypeptides with molecular weights of 74,000, 44,000, and 17,000 (Fig. 4). The material with the largest molecular weight formed the most prominent peak. In addition, the three polypeptides showed partial antigenic identity with spurs formed between the peaks.

When material recovered from preparative agarose electrophoresis of 23243 membranes was analyzed by SDS-polyacrylamide gel electrophoresis, a single band in the region of approximately 70,000 daltons developed from fractions containing the major outer antigen (Fig. 5). The strain-specific surface antigen trailed across the entire agarose profile (Fig. 5A), and the 70,000-dalton band was present in each of the fractions. The agarose fraction into which the other membrane antigens migrated (fraction 1) showed several additional bands. Note that homologous whole-cell antiserum recognized a major peak in the 23243 polyacrylamide gel pattern in the approximate 70,000-dalton area (Fig. 2B, arrow), a peak similar to that recognized by the G-230 antiserum in the G-230 polyacrylamide gel pattern (Fig. 2A, arrow). In contrast, only a small peak was observed in the leonis profile even though a similar cluster of polypeptides was observed in the 70,000-dalton area as was observed in strain G-230 (compare Fig. 2A with Fig. 2C). Preliminary experiments analyzing preparative agarose fractions of cells of 27389 suggest the possibility that its strain-specific antigen also may have a molecular weight of approximately 70,000.

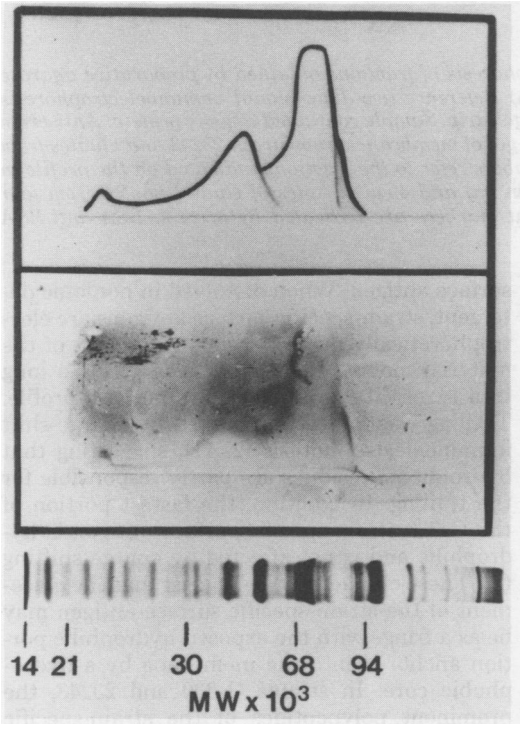

FIG. 4. *Two-dimensional immunoelectrophoresis of membranes of M. arginini G-230 against monospecific antiserum to the variable surface antigen (a-b). First-phase separation by SDS-polyacrylamide gel electrophoresis. The membrane sample containing 20 μg of total membrane protein was subjected to electrophoresis in the polyacrylamide gel and developed against the monospecific antiserum at a concentration of 0.4 ml per ml of agarose. A control stained polyacrylamide strip is shown at the base of the figure with molecular weights indicated below. Register is slightly imperfect because of shrinkage of gel.*

Common surface antigen. In addition to the strain-specific surface antigen of strain G-230, a second antigen with exposure to the outside of the membrane was demonstrated by reaction of membranes against antiserum absorbed with intact cells (Fig. 6). This antigen, previously designated c (2; now termed common surface antigen) and resolved only with the addition of both Triton X-100 and sodium deoxycholate, was increased in height as the amount of absorbing cells was increased. (The strain-specific surface antigen was also enhanced on these slides.) Monospecific antiserum prepared to immunoprecipitin peaks of the common surface antigen reacted with three polypeptides (40,000, 29,000, and 20,000 daltons; Fig. 7). The appearance of superimposed peaks at 44,000 daltons was probably an artifact resulting from uneven migration of the antigen from the polyacrylamide strip into the agarose bed. Interestingly, we always found that polypeptides were trapped in the polyacrylamide strips after immunoelectrophoresis because the gels still stained with Coomassie blue, albeit fainter than controls gels. The taxonomic specificity of antiserum to the common surface antigen was determined by cross-reaction with cells of the 23243, 27389, and leonis strains of *M. arginini* as well as with *M. hominis* and *M. gateae*: each *M. arginini* strain showed two peaks with relative mobilities of approximately 0.5 and 0.7, but *M. hominis* and *M. gateae* were negative. Antiserum from the same rabbits obtained after further boosts with immunoprecipitin material produced two additional weakly reactive peaks (mobilities of 0.3 to 0.4) with the G-230 antigen. However, antiserum from the first post-immunization bleeding reacted only with the antigen at 0.7 mobility.

Controls. Since the conditions used in this study were the same as in previous studies of the *M. arginini* model, the same controls apply (1, 33). Briefly, the rabbit antisera did not detect horse serum antigens in any of the cell or mem-

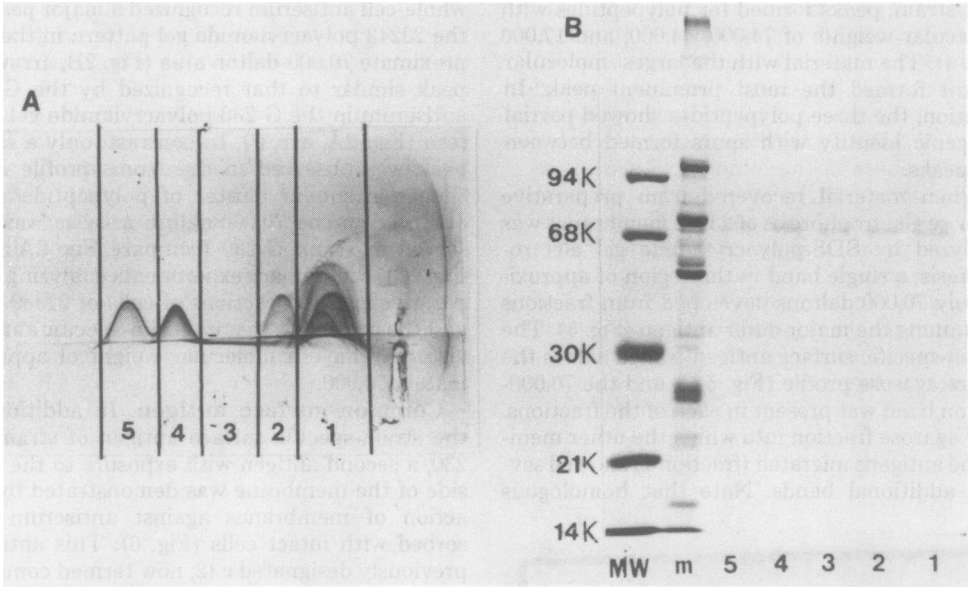

FIG. 5. *Analysis by SDS-polyacrylamide gel electrophoresis of fractions obtained by preparative agarose electrophoresis of membranes of M. arginini 23243. (A) Reference two-dimensional immunoelectrophoresis profile of membranes developed from a control agarose gel strip. Sample contained 80 μg of protein. Antiserum (10%) against 23243 cells. (B) SDS-polyacrylamide slab gel of membranes from strain 23243 and eluates from preparative agarose electrophoresis fractions. The numbers refer to the fractions indicated on the profile in (A). Sample wells contained 20 μg of membrane protein (m) and 40-μl samples of eluates pooled from four preparative slides for each fraction. Molecular weight markers are indicated by arrows. BSA-anti-BSA marker is omitted.*

brane antigen preparations, nor did antibody to mycobacteria (obtained by immunizing with complete Freund adjuvant alone) cross-react with the antigens detected by the monospecific antisera. Although the serological test antigens were known to contain horse serum antigens, none of the antigens recognized on the two-dimensional immunoelectrophoresis profiles was of horse serum origin because the immunogens had been propagated in dialysate broth supplemented with agamma rabbit serum (21). This conclusion was verified by the fact that none of the peaks produced was suppressed by the addition of anti-horse serum antibody. However, some of the bands present on the polyacrylamide gels were probably of horse serum origin, but none of the polypeptides recognized by mycoplasmic antiserum was of horse serum origin because, as above, the antiserum used did not recognize horse serum components.

DISCUSSION

Our study shows a remarkable degree of heterogeneity among strains of *M. arginini*. Major strain-specific surface antigens were found in three strains (G-230, 23243, and 27389). The leonis strain was different, lacking a comparable surface antigen. When dissolved in nonionic detergent, strain-specific surface antigens are electrophoretically heterogeneous with most of the material moving rapidly but also giving a long trail across the immunoelectrophoresis profile. Trailing was largely eliminated by charge-shift immunoelectrophoresis (2, 11), suggesting that hydrophobic residues are partly responsible for the trailing. In addition, the fastest portion of the G-230 strain-specific surface antigen is hydrophilic and is not affected by charge shifting (2). These characteristics suggest that the placement of the strain-specific surface antigen may be as a fringe with the exposed hydrophilic portion anchored into the membrane by a hydrophobic core. In strains G-230 and 23243, the prominent polypeptides of the strain-specific surface antigens had molecular weights of 74,000 and 70,000, respectively. Two additional polypeptides were identified for the G-230 antigen of approximately 44,000 and 17,000 daltons, but we do not presently know whether these are degradation products of the 74,000-dalton material or whether, together with the 74,000-dalton piece, they comprise a larger molecule of 135,000 daltons. In the analysis of the agarose gel electrophoresis fractions of 23243 membranes by SDS-polyacrylamide gel electrophoresis, the

quantity of protein in each sample was near the sensitivity limit of the procedure so that smaller subunits may not have been present in quantity sufficient for detection. The immunological uniqueness of these antigens may represent genetically stable strain differences reflecting major differences in the amino acid sequences of these proteins. On the other hand, these proteins may contain not only regions of considerable variability participating in the high-affinity precipitin reactions, but also regions of homology, a structure similar to that which has been demonstrated for the trypanosomal variable surface antigens by the use of methods sensitive enough to detect weaker cross-reactions (9). Continuous antigenic change, as is true for the trypanosomes (8), has not been observed for *M. arginini*; thus, the comparison is relevant for structural considerations only.

Preparation of monospecific antiserum to another surface antigen, c (common surface antigen), permitted us to demonstrate that this antigen was present in all strains of *M. arginini* tested but was not present in solubilized whole-cell preparations of two other arginine-utilizing species. This antiserum recognized three polypeptides in polyacrylamide gels of the G-230 strain (40,000, 29,000, and 20,000 daltons). These three peptides may represent monomeric units of a multimeric protein, or, alternatively, some

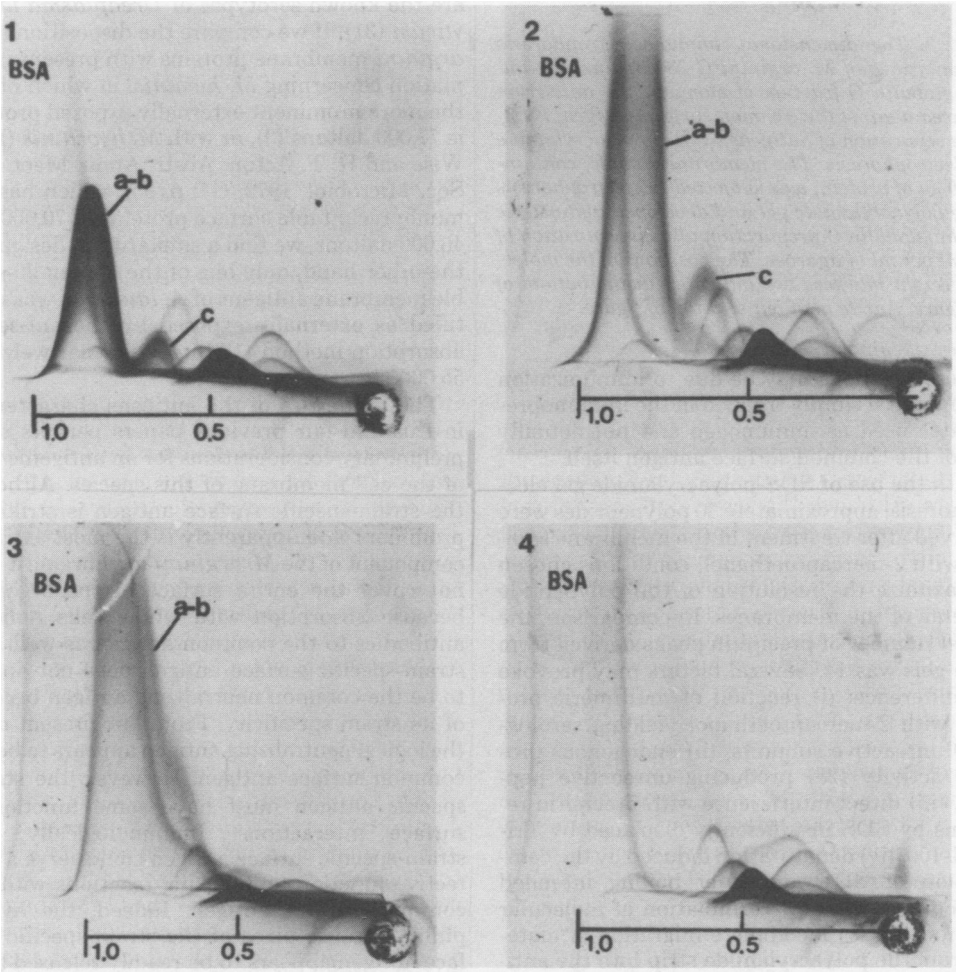

FIG. 6. *Two-dimensional immunoelectrophoresis profiles of membranes of M. arginini strain G-230 against unabsorbed antisera and antisera absorbed by intact cells. Antigen samples contained 20 μg of protein. Antisera: (1) unabsorbed; (2) absorbed with 0.15 mg of cell protein; (3) absorbed with 0.2 mg of cell protein; (4) absorbed with 0.3 mg of cell protein. Concentration of each antiserum was 0.1 ml per ml of agarose. The BSA-anti-BSA peak was included as a marker for relative electrophoretic mobility. Anode is at left and top of figure.*

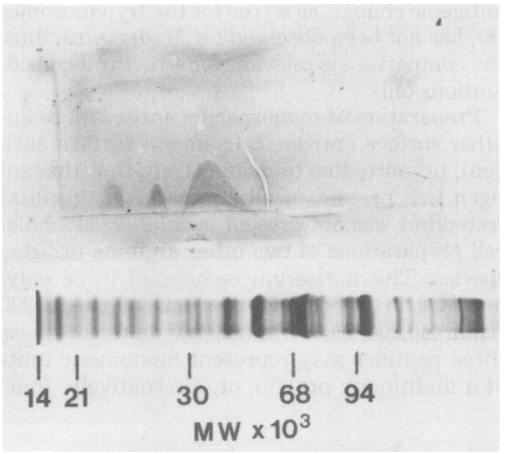

FIG. 7. *Two-dimensional immunoelectrophoresis of membranes of M. arginini G-230 against the immunoglobulin G fraction of monospecific antiserum prepared against the common surface antigen. First-phase separation of antigens by SDS-polyacrylamide gel electrophoresis. The membrane sample, containing 20 μg of protein, was subjected to electrophoresis in the polyacrylamide gel and developed against the immunoglobulin G preparation at a concentration of 0.25 ml per ml of agarose. The positions of the molecular weight markers are indicated at the bottom of the figure. Anode is at left and top of figure.*

of these reactions may be due to immunization with material simply trapped in the immunoprecipitates used as immunogen and not actually part of the common surface antigen itself.

With the use of SDS-polyacrylamide gel electrophoresis, approximately 30 polypeptides were observed after treatment of the membrane samples with 2-mercaptoethanol, conditions chosen to maximize the resolution of the polypeptide contents of the membranes. In comparison, the largest number of precipitin peaks derived from these gels was 14. Several factors may provoke this difference: (i) reaction of multimeric proteins with 2-mercaptoethanol yielding serologically unreactive subunits, (ii) endogenous protease activity (28) producing unreactive peptides, (iii) direct interference with precipitin reactions by SDS insufficiently displaced by Triton X-100, (iv) denaturation induced by the combination of SDS and brief boiling intended to produce optimal determination of molecular weights, and (v) incomplete migration of material from the polyacrylamide strip into the antiserum-containing agarose gel. Nevertheless, approximately half of the peptides showed antigenic activity.

Our comparison of polyacrylamide gels was quite interesting when viewed taxonomically. The degree of heterogeneity between membranes of *M. arginini* strains was as large as that observed between different species in quite different serotaxonomic groups (22) such as *M. gallisepticum*, *M. neurolyticum*, and *M. mycoides* (27, 29). Whole-cell preparations showed greater similarities than membrane preparations, a result similar to that obtained for *M. hominis* strains (14, 15). The apparent differences in polyacrylamide gel patterns of the *M. arginini* membranes are not necessarily a reflection of their phylogenetic differences (as expressed in amino acid sequences) but are probably due to small differences in molecular weights of their peptides. The *M. arginini* strains are more strikingly different from each other by polyacrylamide gel electrophoresis than are the known serotypes of *Ureaplasma urealyticum* (31). If we compare the disposition of *M. arginini* membrane proteins with present information concerning *M. hominis*, in which one of the more prominent externally exposed proteins is 77,000 daltons (3), or with *M. hyorhinis* (K. S. Wise and R. T. Acton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, G7, p. 83), which has immunoprecipitable surface proteins of 70,000 and 46,000 daltons, we find a similarity in design. On the other hand, only one of the Tween-20-soluble membrane antigens of *A. laidlawii* was identified as externally exposed by the antiserum absorption method (17); its molecular weight is 55,000 (18).

The properties of the antigens characterized in this and our previous papers permits some preliminary considerations for an antigenic map of the cell membrane of this species. Although the strain-specific surface antigen is strikingly prominent and apparently is the most extrinsic component of the *M. arginini* membrane, it does not cover the entire surface of the organism because absorption with whole cells removes antibodies to the common antigen as well. The strain-specific surface antigen does not appear to be the common neutralizing antigen because of its strain specificity. From our present data, the logical neutralizing antigen appears to be the common surface antigen. However, the strain-specific antigen must have some function in surface interactions. Immunologically, this strain-specific surface antigen could serve a protective function by blocking reactions with the common surface antigen. Indeed, the hydrophilic extrinsic piece of the strain-specific surface antigen appears to be readily released from the membrane (2). Furthermore, the common surface antigen is more hydrophobic (2) and thus would be less accessible for reaction with a multivalent antiserum: antibodies reacting with the strain-specific antigen could have a blocking or anti-complementary effect. If the leonis strain

proves to lack a strain-specific surface antigen, its surface-related activities may be different from those of the other three strains. The remaining membrane antigens, which have not been characterized in detail as yet, are intrinsic to the membrane (as defined by their solubility only in detergent and by their charge-shift reactions) and perhaps have transport or enzymatic functions, and some of these were clearly shared by all four strains. Clearly, further analysis of this model system can give us a detailed antigenic map with broad applicability to other membrane bounded organisms and cells: mammalian cells, protozoa, as well as other species in the *Mycoplasmatales*.

ACKNOWLEDGMENT

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