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Structural Analysis of Chlamydial Major Outer Membrane Proteins

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The primary structure and surface exposure of the major outer membrane protein (MOMP) isolated from ^{14}C intrinsically or ^{125}I extrinsically radiolabeled *Chlamydia trachomatis* serotypes D/UW-3, G/UW-57, H/UW-4, I/UW-12, and L2/434 and the *Chlamydia psittaci* meningopneumonitis strain were analyzed by two different peptide-mapping techniques. Radiolabeled proteins were digested with either *Staphylococcus aureus* V8 protease, the patterns of peptide fragments produced being displayed by sodium dodecyl sulfate gel electrophoresis, or α-chymotrypsin, the peptides being analyzed after separation by high-voltage electrophoresis and thin-layer chromatography. The comparative structural data obtained from these two different techniques were remarkably similar. From these data, the following points could be made. (i) MOMPs are structurally heterogeneous between members of chlamydial species; the *C. psittaci* MOMP was clearly distinct from each of the *C. trachomatis* MOMPs. (ii) Considerable structural homology occurs among MOMPs from different *C. trachomatis* serotypes; however, distinct differences in the primary structure of each *C. trachomatis* MOMP were evident. (iii) These observed differences were most obvious in peptide maps of MOMPs isolated from chlamydiae that had been surface labeled by lactoperoxidase-mediated radioiodination. The surface-exposed portions of the MOMPs from serotypes L2 and D were very similar. In contrast, those from serotypes G, H, and I were quite different. These structural data are in agreement with the serospecificities described for these proteins.

A single protein predominates in chlamydial outer membranes (4). This protein, termed the major outer membrane protein (MOMP), is exposed on the surface of all *Chlamydia trachomatis* strains studied (4, 9, 16). The MOMP for a given strain has a characteristic apparent subunit molecular weight (aMW), but MOMPs vary in their aMWs among strains (5, 16). The abundance and surface exposure of these proteins suggest that they may play important roles in both host immune response to chlamydial infections and the pathogenesis of chlamydial disease.

Previous studies have shown that the MOMPs of *C. trachomatis* are serotyping antigens and are antigenically complex (5). Sodium dodecyl sulfate (SDS)-denatured MOMPs elicit formation of antibodies with species, subspecies, and serotype specificity as determined by microimmunofluorescence (micro-IF). The subspecies determinants separate the 15 *C. trachomatis* serotypes into two separate serogroups, B and C. Of additional interest are studies which show that anti-MOMP serum neutralizes *C. trachomatis* infectivity in vitro and that the specificity of this neutralization is serogroup specific (H. D. Caldwell and L. J. Perry, submitted for publication). These results suggest that *C. trachomatis* serotypes within a particular serogroup have common antigenic MOMP determinants on their exposed surfaces and that antibody against these determinants may be protective. A major goal of this work is to elucidate the structural features of the proteins that are responsible for various antigenic determinants.

In this study, we attempted to analyze both primary structural relationships and surface exposure of isolated MOMPs from selected chlamydial strains by the techniques of one-dimensional (1-D) peptide mapping in SDS-polyacrylamide gel electrophoresis (PAGE) and by two-dimensional (2-D) ^{125}I-labeled-peptide mapping. The data indicate that each MOMP has some unique primary structure which results in different portions of the molecule being exposed on the surface of the organism. In addition, the

results of the surface exposure analyses correlate well with the serospecificities described for these proteins.

MATERIALS AND METHODS

Organisms. The *C. trachomatis* strains L2/434, D/UW-3, G/UW-57, H/UW-4, and I/UW-12 and the *Chlamydia psittaci* meningopneumonitis (Mn) Cal-10 strain were used. Growth conditions and purification of elementary bodies (EBs) have been previously described (4).

Radiolabeling. Chlamydial EBs were surface radioiodinated with Na-^{125}I (ICN, Irvine, Calif.), using lactoperoxidase and hydrogen peroxide (H_2O_2) (15). *Chlamydia* strains were intrinsically radiolabeled with ^{14}C-amino acids as follows. For each strain used, six 150-cm^2 tissue culture flasks (Corning Glass Works, Corning, N.Y.) containing 2×10^7 HeLa 229 cells were infected with 1 ml of chlamydial inoculum (approximately 2×10^8 inclusion-forming units) for 1 h at 37°C. Monolayers were fed with 50 ml of minimal essential medium containing 10% fetal calf serum and incubated for 2 h at 37°C. The medium was removed, and each monolayer was refed with 50 ml of minimal essential medium containing 10% fetal calf serum, one-tenth the normal concentration of amino acids, and 1 μg of emetine-hydrochloride (Sigma Chemical Co., St. Louis, Mo.) per ml. After 10 h of incubation at 37°C, the medium was again removed and replaced with 50 ml of minimal essential medium containing 10% fetal calf serum, one-tenth the normal amino acid concentration, 1 μg of emetine per ml, and 50 μCi of uniformly labeled ^{14}C-amino acids (ICN, Irvine, Calif.). Infected HeLa monolayers were incubated further at 37°C for 30 h (strains L2 and Mn) or 60 h (strains D, G, H, and I), at which time chlamydiae were harvested and EBs were purified by centrifugation through discontinuous Renografin gradients (5).

Gel electrophoresis. SDS-PAGE was performed by the method of Laemmli (13) for discontinuous gel systems. Details for qualitative and preparative slab gel electrophoresis (5) have been described in detail elsewhere. For analysis of peptides generated by proteolysis, 18.5% acrylamide gels (30:0.8 [by weight] acrylamide-bisacrylamide) containing 6 M urea were used. The stacking gel was 5% acrylamide without urea. Gels were stained in 0.25% Coomassie blue–50% methanol–10% acetic acid.

Isolation of radiolabeled MOMPs. ^{14}C intrinsically or ^{125}I extrinsically radiolabeled EBs (approximately 2×10^{10} organisms) were suspended in 2 ml of sample buffer and boiled for 3 min. The solubilized suspensions were adjusted to contain approximately 2×10^6 cpm for ^{14}C-labeled organisms and 4.7×10^7 cpm for ^{125}I-labeled chlamydiae. One milliliter of these suspensions was then centrifuged for 5 min in a Beckman microfuge (Beckman Instruments, Inc., Fullerton, Calif.). The supernatants were loaded onto 12.5% slab gels and electrophoresed at 25 mA constant current for 3 to 3.5 h. Gels were briefly (2 min) stained with Coomassie blue, and the lightly stained MOMP band was excised from the gel. The excised radiolabeled MOMPs isolated for each strain were cut into fragments (2 by 1.5 by 1.5 mm) and stored at −30°C. These gel fragments were used as substrates for 1-D peptide mapping. The radioactivity of ^{125}I-labeled MOMP gel fragments was determined by counting slices in a Beckman 4000 gamma counter (Beckman). ^{14}C-labeled MOMP gel fragments were digested in 200 μl of 60% perchloric acid and 400 μl of 30% H_2O_2 for 4 h at 60°C as described by Mahin and Lofberg (14). To this was added 10 ml of Aquasol scintillation cocktail (New England Nuclear Corp., Boston, Mass.), and the radioactivity was counted in Beckman LS8100 liquid scintillation counter (Beckman). The amount of ^{14}C radioactivity in the MOMP gel fragments ranged between 1.2×10^4 to 1.6×10^4 cpm.

1-D peptide mapping. The procedure used for 1-D peptide mapping was essentially that described by Cleveland et al. (6) with some modification. Excised MOMP gel fragments were soaked in 200 μl of 0.125 M Tris-hydrochloride–2 mM EDTA–10% glycerol (pH 6.8) for 1 h. The gel fragments were then carefully placed into sample wells containing electrophoresis running buffer. Each gel fragment was overlaid with 5 μg of *S. aureus* V8 protease (Miles Laboratories, Elkhart, Ind.) (5 μl of 0.125 M Tris-hydrochloride–2 mM EDTA–10% glycerol containing 1 mg of V8 protease per ml). Gels were then electrophoresed at 17 mA constant current until the dye marker had migrated immediately above the separating gel. Electrophoresis was discontinued, and the gel apparatus was incubated at 37°C for 2 h. These conditions for proteolysis have been previously shown to result in complete digestion of the L2 MOMP (3). After incubation, electrophoresis was continued for 6 h at 17 mA. Gels were then fixed, stained with Coomassie blue, and analyzed by fluorography as described by Bonner and Lasky (2). Molecular-weight markers were: ovalbumin, 43,000 (43K); α-chymotrypsin, 25.7K; β-lactoglobulin, 18.4K; lysozyme, 14.3K; cytochrome *c*, 12.3K; bovine trypsin inhibitor, 6.2K; and insulin, 3K (Bethesda Research Laboratories, Inc., Bethesda, Md.).

2-D ^{125}I-labeled-peptide mapping analysis. The chlamydial MOMPs were surface radioiodinated, using lactoperoxidase as described above or by the chloramine-T (chloro-T) procedure for radioiodinating proteins in excised gel slices (7, 18).

Chloro-T iodination. The chlamydial MOMPs were separated by SDS-PAGE as described above. The MOMP bands, identified by Coomassie staining, were excised from the gel, soaked in 7% acetic acid for 1 h and then in distilled water for 1 h, and dried in a Speed-Vac (Savant Instruments, Inc., Hicksville, N.Y.). To the dehydrated gel slice was added 10 μl of chloro-T (1 mg/ml in Dulbecco phosphate buffer, pH 7.4) and 100 μCi of ^{125}I (as NaI; 50 μCi/μl). This was incubated at room temperature for 1 h. The reaction was terminated by the addition of 1 ml of sodium metabisulfite (1 mg/ml) at room temperature. After 15 min, the fluid was aspirated and the gel slice was transferred to a well of a Linbro 24-well tissue culture plate (Flow Laboratories, Inc., McLean, Va.) which contained 0.5 g of Dowex 1X-8 (20 to 50 mesh) anion exchange resin (BioRad Laboratories, Richmond, Calif.) and 2.5 ml of 15% methanol. The gel slice was removed after 24 h at room temperature and dried in a Speed-Vac.

Surface-radioiodinated MOMP bands, identified by autoradiography and Coomassie staining, were excised and treated in a fashion identical to that used for chloro-T iodination, except no ^{125}I was added.

Enzyme digestion. The radioactive dehydrated

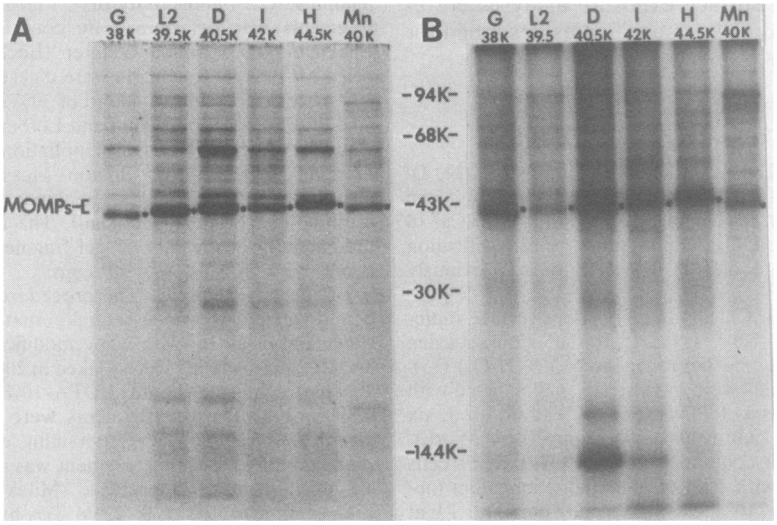

FIG. 1. SDS-PAGE polypeptide profiles of ^{125}I surface-radiolabeled EBs. (A) Coomassie brilliant blue-stained gel. (B) Accompanying autoradiograph of the same gel. The MOMPs for each serotype are identified by asterisks. Note that for each serotype, the MOMP is the most abundant protein and is also most intensely iodinated by lactoperoxidase-mediated iodination of viable chlamydiae.

MOMP bands were rehydrated in 250 μl of 0.05 M NH_4HCO_3 (pH 8.5). Twenty-five microliters of α-chymotrypsin (1 mg/ml in 0.01 N HCl; Calbiochem-Behring, La Jolla, Calif.) was added, and digestion was allowed to proceed for 4 h at 37°C. The supernatant, which contained peptides liberated from the gel slice, was aspirated and saved. The digestion process was repeated once more as described above, except the reaction was allowed to proceed for 16 h at 37°C. The supernatants were pooled, dehydrated in a Speed-Vac, and washed eight times with 250 μl of distilled water. The washed chloramine-T–^{125}I-labeled-peptide residues (chloro-T–^{125}I-peptides) were rehydrated to 10^5 cpm/μl in distilled water containing 1 mg each of L-leucine, L-tyrosine, and L-aspartate (Sigma) per ml. The washed lactoperoxidase surface-radioiodinated peptide residues (surface peptides) were rehydrated in the same solution at 2.5×10^4 cpm/μl.

2-D ^{125}I-labeled-peptide mapping. 2-D ^{125}I-labeled-peptide mapping was performed on Polygram Cel 300 thin-layer chromatography sheets (Brinkmann Instruments, Westbury, N.Y.). Three MOMP preparations were spotted near the middle of each sheet (2×10^5 cpm of the chloro-T–^{125}I-peptides or 5×10^4 cpm of the surface peptides). High-voltage thin-layer electrophoresis was carried out at 1,200 V for 30 min at 13°C in a Savant TLE 20 apparatus equipped with auxiliary cooling coils fabricated by our staff. The electrophoresis buffer was a pH 3.7 solution of water-acetic acid-pyridine (200:10:1). The sheets were removed after electrophoresis, air dried, and split into three pieces. Each piece, containing one MOMP preparation, was turned 90° and subjected to ascending thin-layer chromatography in *n*-butanol–pyridine–water–acetic acid (13:10:8:2). The radioemitting peptides were visualized by exposure to XAR-5 or XL-5 (Kodak) film at −76°C, using Cronex-Par Speed screens (E. I. du Pont de Nemours & Co., Wilmington, Del.). All peptide maps, in different combinations, were exposed on single pieces of film.

RESULTS

Molecular weights and surface radiolabeling of MOMPs. The *C. trachomatis* serotypes used in this study were selected on the basis of their serological classification by micro-IF, using antisera to isolated SDS-denatured MOMPs (5). Briefly, the L2, D, and G serotypes are serogroup B organisms, whereas H and I serotypes belong to serogroup C. Antisera against the *C. psittaci* Mn MOMP do not react with *C. trachomatis* serotypes by micro-IF and, therefore, cannot be classified within either serogroup.

The SDS-PAGE polypeptide profiles of surface-radioiodinated EBs from each serotype are shown in Fig. 1. Different aMWs of the MOMP were found for each serotype (Fig. 1A). The MOMPs with the lowest aMWs were found among the B serogroup serotypes G, L2, and D, and those with higher aMWs were found for the C serogroup H and I serotypes. For each serotype, the MOMP was the most heavily stained polypeptide and the most intensely radioemitting protein band found after autoradiography (Fig. 1B).

1-D peptide mapping of MOMPs. The peptide maps of both ^{14}C intrinsically and ^{125}I extrinsically radiolabeled chlamydial MOMPs are shown in Fig. 2A. Fluorography of SDS-PAGE of ^{14}C-labeled whole-cell lysates revealed polypeptide profiles identical to those obtained by Coomassie staining (data not shown). Proteoly-

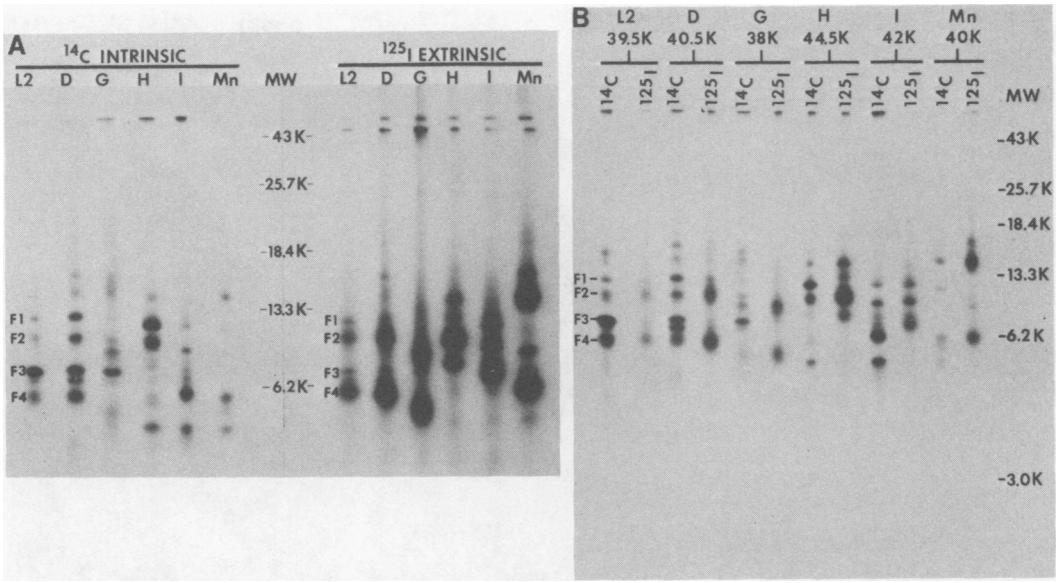

FIG. 2. (A) 1-D V8 protease peptide maps of ^{14}C intrinsically and ^{125}I extrinsically radiolabeled chlamydial MOMPs. Polyacrylamide gel fragments containing ^{14}C intrinsically or ^{125}I extrinsically labeled MOMPs were placed into stacking gel sample wells. ^{14}C intrinsically labeled MOMP gel fragments contained between 1.2×10^4 to 1.6×10^4 cpm. ^{125}I-labeled MOMP gel fragments ranged from 8×10^3 to 1×10^4 cpm. Five microliters of V8 protease (1 mg/ml) was gently layered over each gel fragment, and electrophoresis was carried out at 17 mA constant current until the dye marker had migrated immediately above the separating gel. Electrophoresis was then discontinued, and proteolysis was done in situ for 2 h at 37°C. Electrophoresis was continued for 6 h to separate the peptide fragments. Fluorography was done at −76°C for 96 h with Kodak XAR-5 X-ray film for ^{14}C maps. Autoradiography of ^{125}I maps was done at −76°C for 24 h with Kodak XAR-5 X-ray film, using a Lighting Plus intensifying screen. The ^{125}I-labeled-peptide maps were deliberately overexposed to accentuate the differences in the surface-labeled peptide units found for the MOMP of the G, H, I, and Mn serotypes. (B) Composite 1-D peptide maps of ^{14}C intrinsically and ^{125}I extrinsically radiolabeled chlamydial MOMPs. Intrinsically and extrinsically labeled MOMPs of each strain were electrophoresed after V8 proteolysis in alternate lanes. Note that for each MOMP, peptide fragments that were ^{125}I surface labeled have comigrating ^{14}C-labeled peptides. Fluorography was done at −76°C for 24 h with Kodak XAR-5 X-ray film.

sis of each MOMP with 5 μg of V8 protease for 2 h at 37°C generated relatively few peptides. As a point of reference, the most intensely radioemitting ^{14}C-labeled peptide fragments of the L2 MOMP were identified as F1 through F4. The aMWs of these fragments were: F1, 11K; F2, 10K; F3, 7.5K; and F4, 5.5K. Peptide fragments larger than F1 were sometimes observed for the L2 MOMP. These peptides are considered to be incomplete proteolysis products, which have been previously described (3).

The MOMPs of the L2 and D serotypes had nearly identical ^{14}C intrinsically labeled peptide maps, whereas the peptide profiles of G, H, I, and Mn MOMPs were distinct. Although the peptide profiles of these serotype MOMPs were different, comigrating peptides could be found among the various MOMPs.

Peptide maps of surface-labeled MOMPs showed that the surface-exposed portions of the proteins were structurally unique for each serotype, with the exception of L2 and D, which shared identical comigrating ^{125}I-labeled peptides. The ^{125}I autoradiogram shown in Fig. 2A was deliberately overexposed to accentuate the differences observed in surface-labeled-peptide maps for the MOMPs of serotypes G, H, I, and Mn. Under these conditions of autoradiography, H, I, and Mn MOMPs shared very weakly radioemitting ^{125}I-labeled peptides, with an aMW of approximately 4K. These peptides, although weakly surface iodinated, were easily identified in ^{14}C-labeled MOMP peptide profiles for these serotypes. ^{14}C- and ^{125}I-labeled peptide fragments were electrophoresed in adjacent sample wells so that intrinsically and extrinsically labeled bands from the same MOMP could be directly compared (Fig. 2B). With only a few exceptions, comigrating ^{14}C intrinsically labeled peptides could be identified for each ^{125}I-surface-labeled fragment.

Chloro-T–^{125}I-peptide mapping. The chloro-T–^{125}I-peptide maps of the MOMPs of *C. trachomatis* strains L2, D, G, H, and I and the

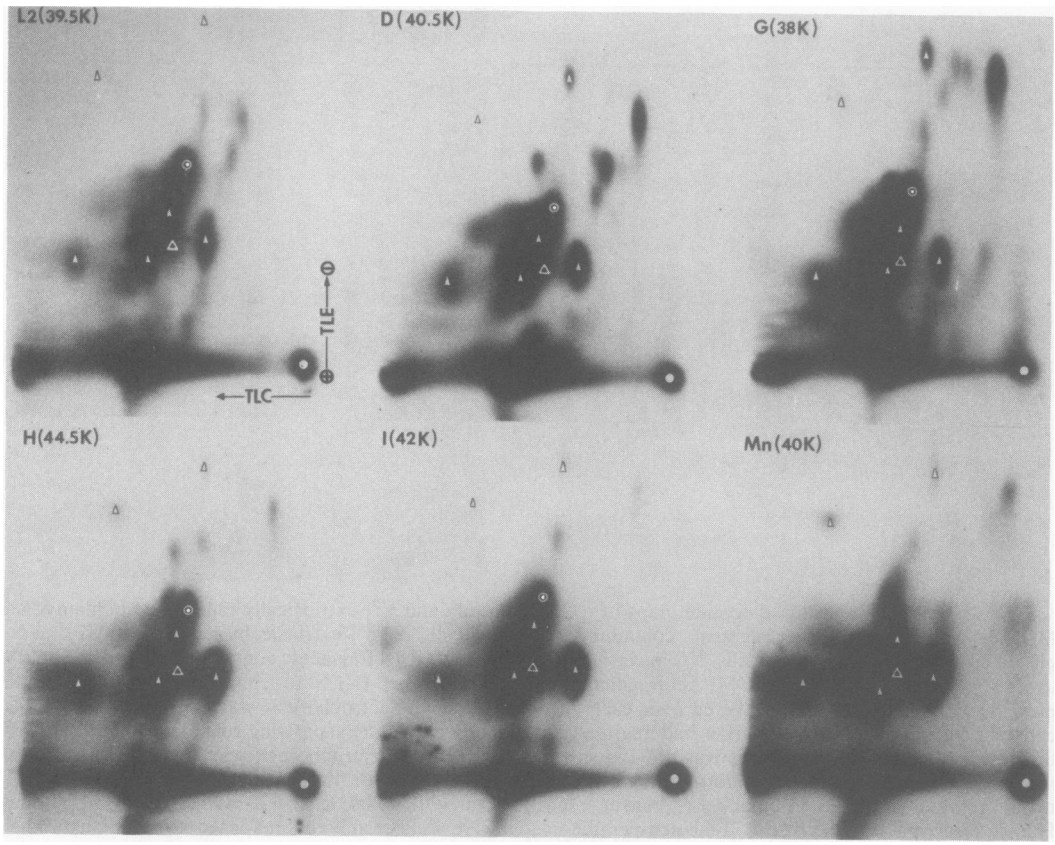

FIG. 3. 2-D α-chymotryptic chloro-T-^{125}I-peptide maps of the MOMPs of *C. trachomatis* strains L2, D, G, H, and I and the Mn strain of *C. psittaci*. The white arrowheads mark the chloro-T-^{125}I-peptides (some of which are not easily visualized in this reproduction) which appear to be common to all the MOMPs. The open triangles denote peptides which appear to be shared by all of the MOMPs in both the chloro-T-^{125}I-peptide and surface peptide maps. The circled dot marks a chloro-T-^{125}I-peptide that appears to be common to the *C. trachomatis* MOMPs. The origin is identified by a white circle.

MOMP of the *C. psittaci* Mn strain are shown in Fig. 3 and 5. Note that all 2-D maps, in various combinations, have been exposed on single pieces of film to facilitate direct comparisons. To visualize the weakly emitting peptides, some of the strongly emitting peptides were slightly overexposed. In addition, the peptide seen in the upper right of each chloro-T-^{125}I-peptide map (very weak in the L2 map) is felt to be artifactual, since it appears in chloro-T-^{125}I-peptide maps of unrelated proteins. The migration of the amino acid markers reflected the slight variations in migration seen in the various 2-D peptide maps.

All of the chlamydial MOMPs appeared to share four strongly emitting and two more weakly emitting peptides (arrowheads, Fig. 3). Note that, owing to the weak emission of these peptides relative to other peptides, it is difficult to adequately visualize these peptides in the reproduction of autoradiograms of the L2 and D MOMP ^{125}I-labeled-peptide maps. In addition, a fifth peptide (open triangle, Fig. 3–5) was not only shared by all MOMPs but was identified as being a common surface peptide as well (see below).

The MOMPs of the *C. trachomatis* strains all shared another dominant peptide (circle with dot, Fig. 3) not seen in the *C. psittaci* MOMP, suggesting that the *C. trachomatis* MOMPs are more closely related to one another than they are to the *C. psittaci* MOMP.

Each chlamydial MOMP had several unique peptides which tended to be weakly emitting and more hydrophilic and basic (19) than the common peptides. Each MOMP appeared to share at least one other ^{125}I-labeled peptide with one or more of the other MOMPs. No obvious groupings, beyond those described above, were apparent.

Surface peptide mapping. Surface peptide maps of the MOMPs under study are shown in

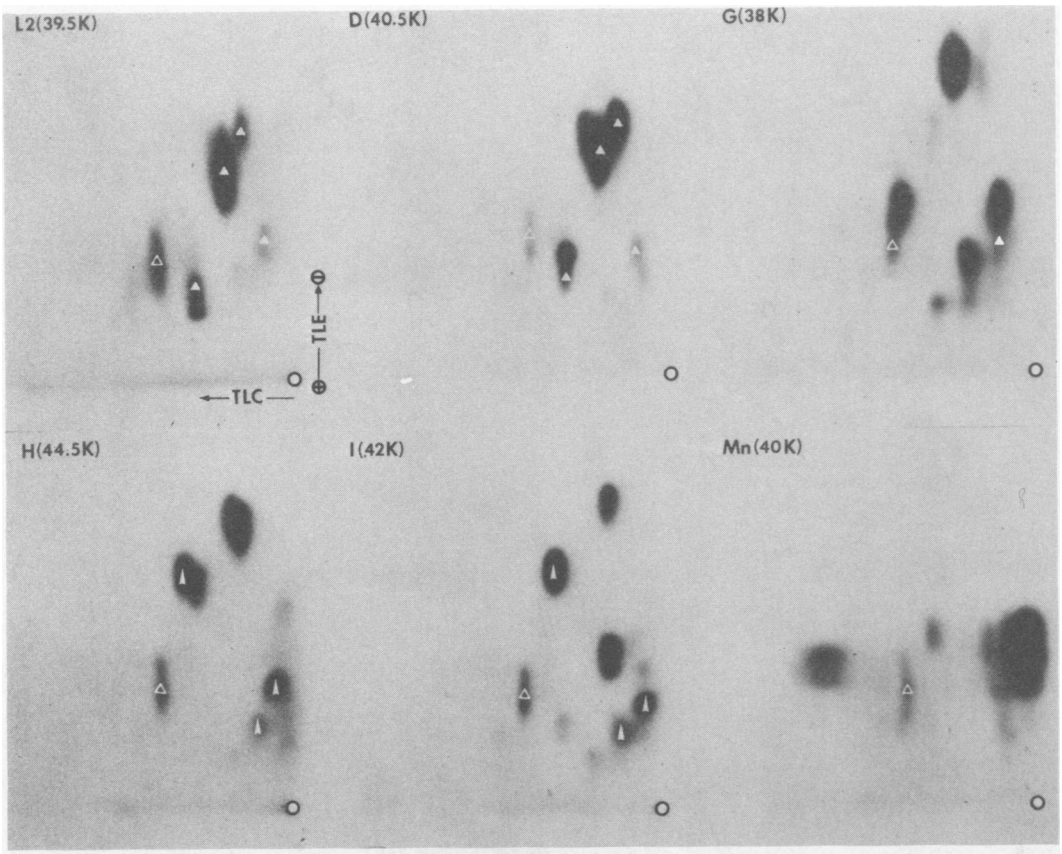

FIG. 4. 2-D α-chymotryptic surface peptide maps of the chlamydial MOMPs under study. The open triangle denotes a surface peptide that appears to be shared by all of the MOMPs. The white triangles denote surface peptides which seem to be shared by the L2, D, and G MOMPs. The white arrowheads mark peptides which appear to be common to the MOMPs of H and I. The origin is identified by a white circle.

Fig. 4 and 5. As expected, there were fewer peptides in these maps than were seen in the corresponding chloro-T–^{125}I-peptide maps. The surface peptides tended to be in the lower half of the thin-layer chromatography separation, suggesting that these are generally more hydrophilic peptides (19) than the heavily emitting common peptides seen in the chloro-T–^{125}I-peptide maps.

All of the MOMPs appeared to share one surface peptide (open triangle, Fig. 3–5). With this exception, the Mn surface peptide map was clearly very different from those of any of the *C. trachomatis* MOMPs.

Within the *C. trachomatis* group, the L2 and D MOMPs appeared to be very similar, sharing at least four surface peptides (triangles, Fig. 4) in addition to the common surface peptide. The G MOMP appeared to share only a single, very hydrophilic surface peptide (triangle, Fig. 4) with the L2 and D MOMPs, in addition to the common surface peptide, while having several unique surface peptides. The H and I MOMPs appear to share at least three surface peptides (arrowheads, Fig. 4) in addition to the common surface peptide; however, each had two heavily emitting peptides and several weakly emitting peptides which were unique. Each MOMP had at least one unique surface peptide, suggesting that each has some unique portions of the molecule exposed on the chlamydial surface.

The above relationships suggest that, based on the surface exposure, the L2 and D MOMPs compose the most closely related group. The G MOMP appears to be more closely related to the L2 and D MOMPs, by virtue of a single shared surface peptide in addition to the common surface peptide, than to either the H, I, or Mn MOMPs. The H and I MOMPs share several surface peptides, suggesting that they are related members of a different surface exposure group, whereas the Mn MOMP is clearly distinct.

Comparison of chloro-T–^{125}I-peptide maps and surface peptide maps. A comparison of the chloro-T–^{125}I-peptide maps and the surface pep-

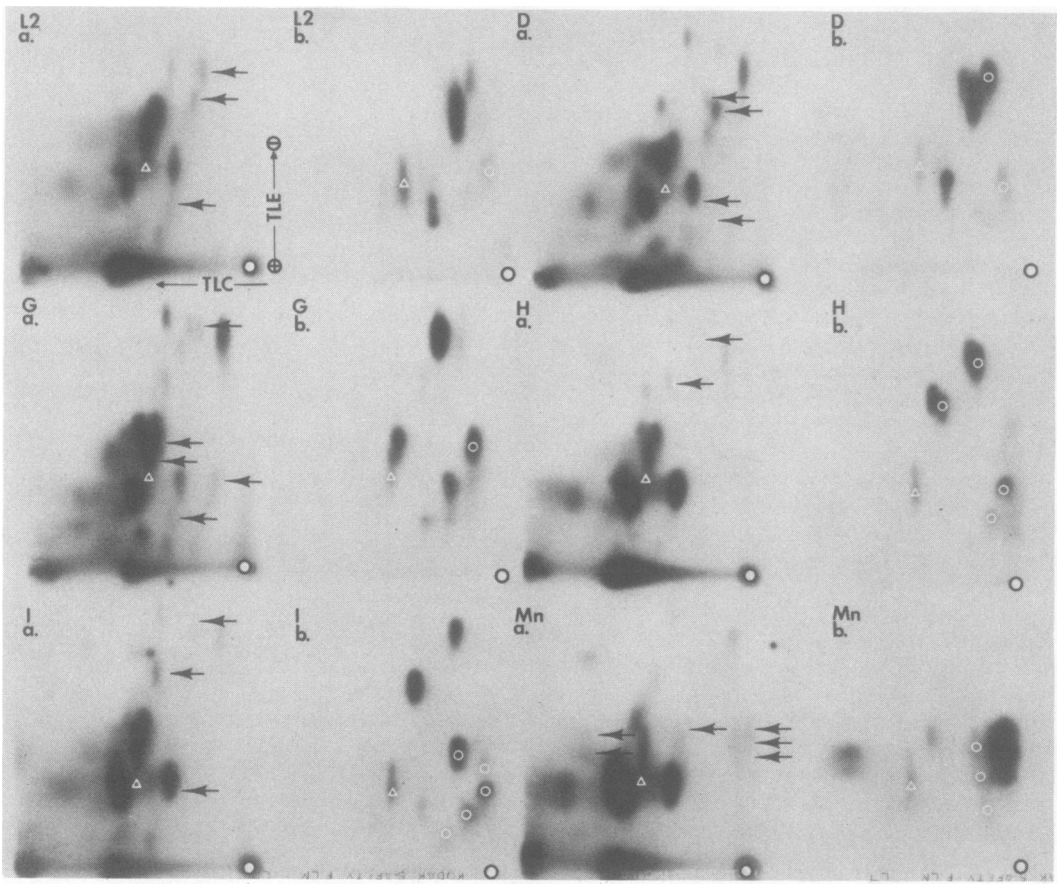

FIG. 5. Composite of chloro-T-^{125}I-peptide maps (a) and surface peptide maps (b) of the chlamydial MOMPs under study. The open triangle denotes the common chloro-T-^{125}I-peptide–surface peptide. The arrows identify chloro-T-^{125}I-peptides (some of which are not easily visualized in this reproduction) which align with peptides seen in the corresponding surface peptide maps. The open circles mark surface peptides which do not appear to have a corresponding chloro-T-^{125}I-peptide. TLE, High-voltage thin-layer electrophoresis; TLC, thin-layer chromatography.

tide maps of the chlamydial MOMPs is shown in Fig. 5. In this figure, the common surface peptide is identified by an open triangle. Black arrows indicate the chloro-T-^{125}I-peptides which align with peptides seen in the corresponding surface peptide maps. In the G MOMP, several of the strongly emitting common chloro-T-^{125}I-peptides (arrows and circles with dots, Fig. 3) appear to be surface peptides, but this seems to be the exception rather than the rule. In general, the more weakly emitting chloro-T-^{125}I-peptides align with the more intensely emitting surface peptides.

The majority of surface peptides could be identified in the chloro-T-^{125}I-peptide maps. However, each surface peptide map had one or more peptides (designated by an open circle) which could not be identified on the corresponding chloro-T-^{125}I-peptide map. This phenomenon has been previously reported (10, 12). Results of high-performance liquid chromatography peptide mapping indicate that these peptides are present in chloro-T-^{125}I-peptide maps at very low relative intensities (data not shown).

DISCUSSION

In this study, we present the results of primary structural and surface exposure analyses of six chlamydial MOMPs. Three methods of radiolabeling MOMPs were used: (i) intrinsic ^{14}C-amino acid labeling, (ii) lactoperoxidase-mediated surface radioiodination of viable chlamydiae EBs, and (iii) chloro-T radioiodination of MOMPs excised from polyacrylamide gels. Two enzymes of differing specificity, *S. aureus* V8 protease, which cleaves on the carboxyl-terminal side of glutamic acid residues (11), and α-chymotrypsin, which preferentially cleaves peptide bonds of L-tyrosine, L-phenylalanine,

and L-tryptophane (1), were used for proteolysis. And finally, two methods of peptide separation were employed. The 1-D SDS-PAGE system separates on the basis of aMW and is ideally suited for analysis of the large peptide fragments generated by V8 protease proteolysis. The 2-D system separates peptides on the basis of both charge and hydrophobicity-hydrophilicity, providing the high resolution necessary to separate the larger number of peptides generated by α-chymotrypsin. The comparative structural data generated by this variety of technologies yielded remarkably similar results from which the following points can be made. (i) The primary structure of MOMPs differs among chlamydial species. The *C. psittaci* Mn MOMP was clearly distinct from each of the *C. trachomatis* MOMPs studied. (ii) MOMPs of different *C. trachomatis* serotypes share a large amount of primary structural homology, as shown by 2-D chloro-T-^{125}I-peptide mapping (Fig. 3). This similarity in primary structure was not as evident in 1-D maps of ^{14}C intrinsically labeled MOMPs. These results may reflect the greater resolving power of the α-chymotrypsin 2-D system, in which, owing to the larger number of peptide fragments generated, structural similarities and differences may become more apparent. (iii) Although they are similar, the primary structure of each *C. trachomatis* MOMP has distinct differences, as evidenced by both 1-D and 2-D peptide maps. These observed differences in primary structure were most obvious in peptide maps of MOMPs isolated from surface-radioiodinated chlamydiae. By both mapping techniques, the MOMPs of the L2 and D serotypes appeared to have very similar portions of the protein exposed on the surfaces of these organisms. By 1-D mapping, the surface portions of G, H, and I MOMPs appeared to be distinct. However, by 2-D mapping, the G MOMP shares one surface peptide with both L2 and D MOMPs, and the H and I MOMPs share three surface-exposed peptides.

The structural findings reported here correlate extremely well with previous published data regarding the antigenic properties of these macromolecules. Particularly, the correlation between surface peptide mapping and the serological classification of *C. trachomatis* by micro-IF as described by Wang and Grayston (8, 20) is striking. By using micro-IF with nonhyperimmune mouse antisera raised against viable chlamydiae, these investigators have shown that the L2 and D serotypes are highly cross-reactive, whereas the G, H, and I serotypes are more distinct antigenically. Caldwell and Schachter (5) have described similar serological relationships with hyperimmune rabbit antisera raised against isolated SDS-denatured MOMPs. The latter investigators have suggested that the MOMP of chlamydiae is a complex antigen and is the predominant serotyping antigen of *C. trachomatis* organisms. Although purely speculative, it does not seem unreasonable to suggest that the unique MOMP surface peptide fragments described in this study might reflect the serotype-specific antigenic determinants measured by micro-IF. Similarly, those surface-exposed peptides common to only certain MOMPs, such as those shared by L2, D, and G serotypes and serotypes H and I (Fig. 4), may be subspecies-specific antigens. Antibody against these determinants may differentiate *C. trachomatis* serotype into the B and C serogroups.

As mentioned above, the chlamydial MOMP is very complex antigenically. Studies by Caldwell and Schachter (5) have shown that polyclonal antibodies raised against SDS-denatured MOMPs have species and subspecies specificities in addition to type specificity. Using monoclonal antibodies and Western blot analysis, Stevens et al. (17) have also shown that the *C. trachomatis* MOMP possesses epitopes of species and subspecies specificity. The epitopes which elicit these antibody specificities may reside in those peptides which account for the large amount of structural homology observed among chloro-T-labeled MOMPs by 2-D peptide mapping.

Obviously, the antigenic characterization of the peptides identified in this study is necessary to prove the hypothesis we have made by correlation alone. The 1-D peptide mapping technique is well suited for these types of studies, since separated peptides are of a reasonable size to maintain antigenicity and can be electrophoretically transferred (Western blot) to a solid-phase matrix and then reacted with antibody. These sorts of studies are currently being done in this laboratory.

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