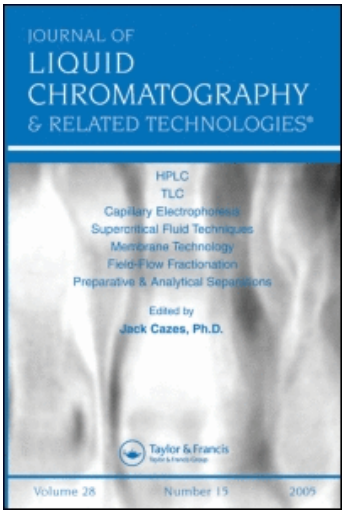

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IDENTIFICATION AND ISOLATION OF SURFACE-EXPOSED PORTIONS OF THE MAJOR OUTER MEMBRANE PROTEIN OF *CHLAMYDIA TRACHOMATIS* BY TWO-DIMENSIONAL PEPTIDE MAPPING AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

In this study, we use high-performance liquid chromatography (HPLC) to isolate surface-peptides of the Chlamydia trachomatis serotype G major outer membrane protein (G-MOMP). The HPLC elution profile was compared with that of peptides of the G-MOMP which was iodinated with chloro-T. Two-dimensional peptide mapping of chloro-T ^{125}I-labeled, surface-labeled peptides, and HPLC-isolated surface-peptides was then used to correlate HPLC-separated peptides with the 2-D maps of the G-MOMP. Results demonstrated that a 2-D-separated surface-peptide that had no apparent corresponding peptide in the 2-D chloro-T map was indeed present in the chloro-T labeled G-MOMP preparation, but at very low relative intensity, and that HPLC can be used to isolate surface-exposed regions of OM proteins for further immunological and structural studies.

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INTRODUCTION

The technique of two-dimensional (2-D) peptide mapping of surface-radioiodinated proteins has been used to study the structural properties of a variety of outer membrane (OM) proteins from Neisseria gonorrhoeae (3), Borrelia hermsii (1), and Chlamydia trachomatis (2). In each case, surface-peptide mapping data has correlated exactly with the antigenic properties of these proteins as defined by both polyclonal and monoclonal antibody (1,2,7,8,9). Therefore, surface-exposed regions of OM proteins, as identified by lactoperoxidase (LPO) surface-radioiodination or 1,3,4,6-tetrachloro-3α-6α-diphenylglycoluril (Iodogen, Pierce Chemical Co., Rockford, IL) surface-radioiodination (5), appear to represent immunodeterminant sites on the native protein that elicit serotyping antibodies.

Surface-peptide mapping has been used as an analytical tool to compare the primary structure of surface-exposed OM proteins. Unfortunately, the (2D) thin-layer electrophoresis (TLE) and thin-layer chromatography (TLC) mapping system (2-D mapping) does not lend itself to preparative applications. In addition, it is common to see peptides in the 2-D surface-peptide map that have no corresponding peptides in the ^{125}I-labeled using chloramine-T (chloro-T) peptide map of the same protein (2,3) radioiodinated. This adds a degree of confusion to the interpretation of results since the chloro-T peptide map should reflect the entire primary structure of the protein.

In this study, we use high-performance liquid chromatography (HPLC) to isolate surface-peptides of the Chlamydia trachomatis serotype G major outer membrane protein (G-MOMP). The HPLC elution profile was compared with that of peptides of the G-MOMP which was iodinated with chloro-T. Two-dimensional peptide mapping of chloro-T ^{125}I-labeled, surface-labeled peptides, and HPLC-isolated surface-peptides was then used to correlate HPLC-separated peptides with the 2-D maps of the G-MOMP. Results demonstrated that a 2-D-separated surface-peptide that had no apparent corresponding peptide in the 2-D chloro-T map was indeed

present in the chloro-T labeled G-MOMP preparation, but at very low relative intensity, and that HPLC can be used to isolate surface-exposed regions of OM proteins for further immunological and structural studies.

MATERIALS AND METHODS

Organism

Chlamydia trachomatis serotype G/UW-57 (G) were grown and elementary bodies (EBs) purified as previously described (2).

Gel Electrophoresis

The gel electrophoresis procedure of Laemmli (5) as modified by Caldwell and Judd (2) was used to separate MOMP of unlabeled and surface-radioiodinated EBs. The G-MOMP bands were excised, radiolabeled or sham-iodinated, and subjected to α-chymotrypsin cleavage.

Radiolabeling

Chlamydia EBs were surface-iodinated with $Na^{125}I$ (ICN, Irvine, CA) using lactoperoxidase (LPO) and hydrogen peroxide as previously described (2).

The G-MOMP bands from unlabeled EBs were excised from SDS-PAGE gels and exhaustively iodinated using the chloro-T procedure (4). Surface-labeled G-MOMP bands were also iodinated after excision from the SDS-PAGE gels using the chloro-T procedure with cold, 30 μM NaI. This resulted in a loss of about 10% of the radioactivity of the band.

2-D Peptide Mapping

Radiolabeled G-MOMP bands, both chloro-T labeled and surface-labeled, were digested with α-chymotrypsin (Calbiochem-Behring, LaJolla, CA) in 250 μl of 0.05 M NH_4HCO_3 as described in detail elsewhere (4). Washed chloro-T were rehydrated to 1 x 10^5 cpm/μl in distilled water containing 1 mg/ml each of L-leucine, L-tyrosine, and L-aspartate (Sigma Chemical Co., St. Louis, MO). Surface-peptides were resuspended at 2.5 x 10^4 cpm/μl in the same solution.

Two-dimensional peptide mapping was performed on Merck 0.1 mm thin-layer cellulose sheets. Three MOMP preparations were spotted near the middle of each sheet (2×10^5 cpm of chloro-T peptides or 5×10^4 cpm of surface-peptides). High voltage thin-layer electrophoresis (TLE) was carried out at 1200 constant V for 30.5 min at 13.5°C in a Savant TLE 20 apparatus. 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). Radioemitting-peptides were visualized by exposure to XAR-5 (Kodak) film at -70° using Cronex-par speed screens (EI Du Pont de Nemours and Co., Wilmington, DE).

HPLC Separation of Peptides

All HPLC separations were performed on a Waters System (Waters Associates, Milford, MA) equipped with dual M6000A pumps. A Waters μBondapac C_{18} reverse-phase column (3.9 mmID x 30 cm) was used to separate peptides.

The chloro-T peptides were rehydrated to 2×10^5 cpm in 200 μl water containing 1 mg/ml each of L-phenylalanine, L-tryptophane, and L-tyrosine. The surface-peptides were rehydrated to 5×10^4 cpm in 200 μl in the same solution. Two-hundred microliters of each peptide preparation was then injected by the WISP 710 injector at time 0. The dual pump system, pump A delivering filtered, distilled water -0.05% TFA and pump B delivering acetonitrile -0.05% TFA (AcN-TFA) (reagent grade, Sigma), then delivered the gradients used to separate the peptides. The peptides were separated using a 1 ml/min solvent flow of a 0-10% AcN-TFA linear gradient (06 program) over the first 6 min. From 6-45 min, a 10%-70% AcN-TFA gradient (09 program) was generated, followed by a 3 min 100% AcN-TFA wash. An LKB 7000 Ultrorac (LKB Instruments, Sweden) was started at injection. Two-tenths minute fractions were collected during the separation. These were then counted in a Beckman Biogamma Counter (Beckman Instruments, Palo Alto, CA).

Counts per minute were plotted vs. retention time as derived from fraction number. The elution of amino acid standards were monitored at 254 nm. No peptides could be detected in the sample by UV absorption.

Fractions containing surface-peptides (see bars, Figure 3) were pooled, dried, washed in distilled water, and reseparated using the 2-D peptide mapping system. The origin of these 2-D maps (see Figure 4) were marked with ~1 x 10^3 cpm. By comparing these 2-D maps with the 2-D chloro-T and 2-D surface maps, each HPLC elution peak could be identified.

RESULTS

The C. trachomatis serotype G-MOMPs from both non-iodinated and LPO-surface-iodinated EBs, were separated in a 12.5% acrylamide gel. Figure 1 is an autoradiogram of the surface-labeled EBs of five serotypes of C. trachomatis (G, L_2, D, I, and H) and C. psittaci strain MN. Note that the C. trachomatis G-MOMP is heavily surface-labeled using this procedure.

The SDS-PAGE-separated MOMP bands of C. trachomatis G were excised from the gels and subjected to α-chymotrypsin digestion followed by chloro-T and surface 2-D peptide mapping. In Figure 2 the "a" panel is the 2-D peptide map of the G-MOMP which was exhaustively iodinated by the chloro-T procedure (i.e., a total primary structure map). Panel "b" is the surface 2-D peptide map of the same protein. The arrows identify surface-peptides in the primary structural map. The triangle marks a surface-peptide that is common to all chlamydia studied (2). Note a single heavily-emitting surface-peptide, open circle, Figure 2, which has no observable corresponding peptide in the primary structural map.

Both the chloro-T peptides and the surface-peptides seen in Figure 2 were separated by HPLC using a reverse-phase C_{18} column. The elution profiles of these separations are seen in Figure 3. Note that each surface-peptide peak had a corresponding peptide peak in the chloro-T labeled preparation. It can also be seen that this method can be used to separate and recover surface-peptides for further immunological and structural studies.

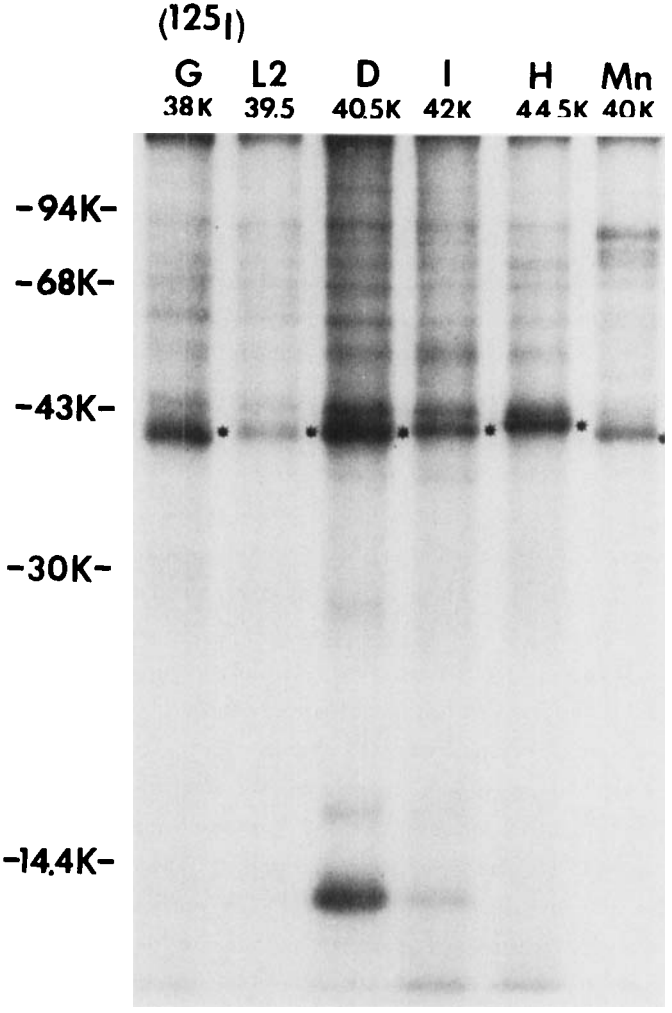

Figure 1. Whole lysates of LPO surface-iodinated EBs of C. trachomatis serotypes G, L2, D, I, H, and a C. psittaci strain MN separated in a 12.5% SDS-PAGE gel. Surface-labeled proteins were visualized by autoradiography. The major outer-membrane proteins (MOMPs) are identified by asterisks with their apparent molecular weights given in thousands of daltons (K). The G-MOMP was chosen for further study because of its high specific activity and the presence of a single surface-peptide that was not seen in its ^{125}I-labeled peptide map (see previously published figures in Ref. 2 and Fig. 2 below). Molecular weight markers were Bio Rad Low Molecular Weight Marker Kit.

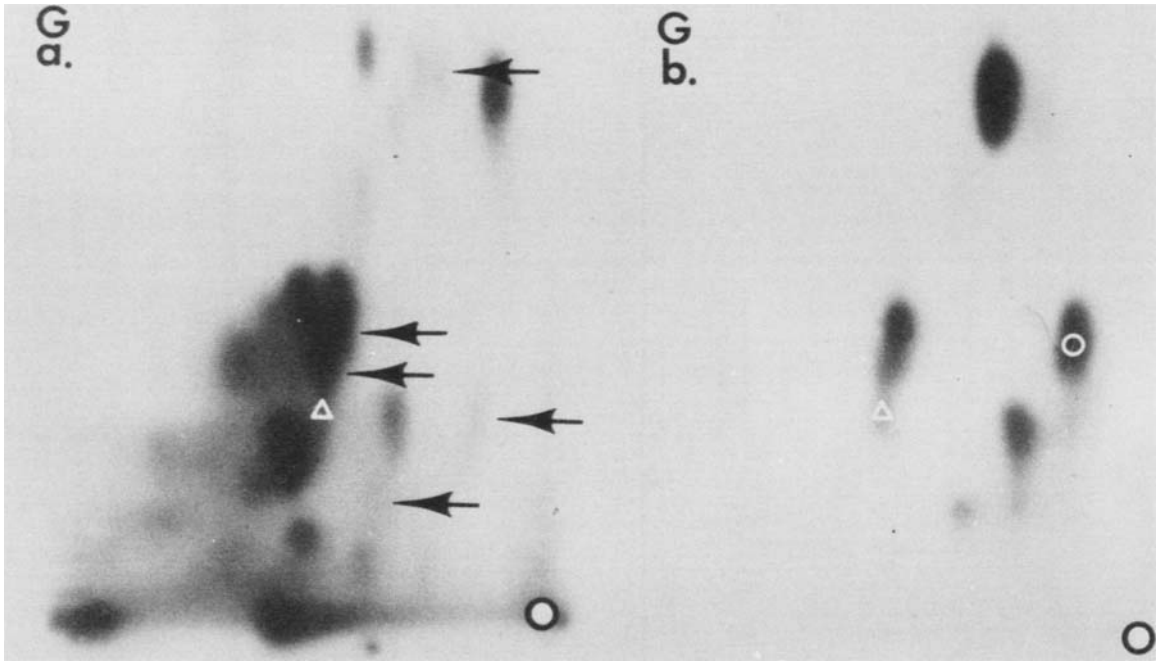

Figure 2. This is a comparison of 2-D chloro-T peptide map (a) and 2-D surface-peptide map (b) of the C. trachomatis serotype G- MOMP. The arrows point to chloro-T peptides which correspond to the surface-peptides as determined by overlaying the two maps. Note that a single surface-peptide, designated by an open circle, has no corresponding peptide in the chloro-T map. The origin of each map is in the lower right corner (black circle). Triangle designates a surface-peptide that is common to all chlamydia studied. Thin-layer electrophoresis was toward the top and thin-layer chromatography to the left (see Fig. 4).

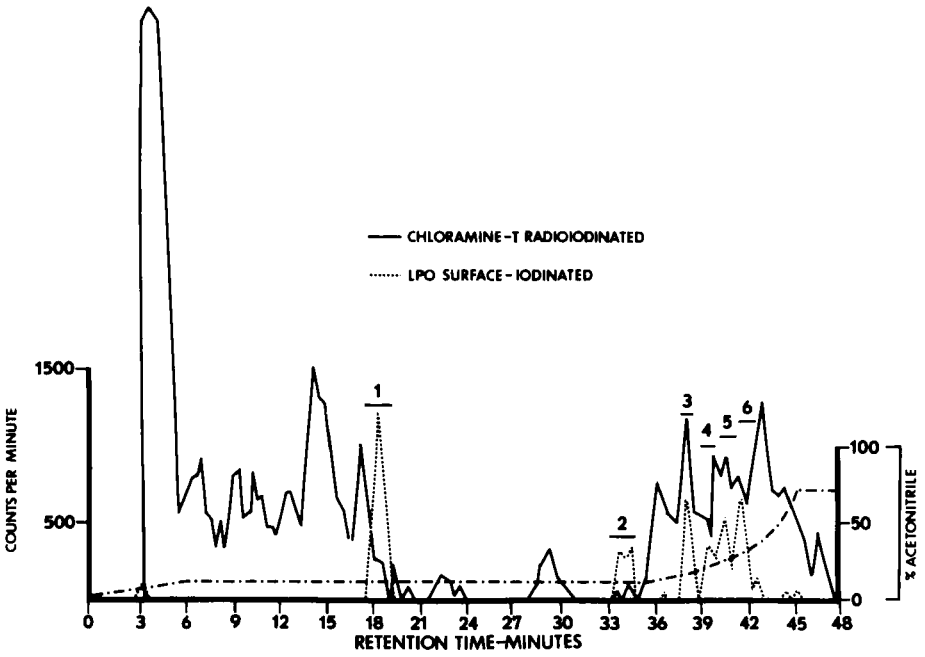

Figure 3. Chloramine-T peptides (solid line) and surface-peptides (broken line) of a G-MOMP were separated by HPLC and the elution profiles overlaid. Note that for every surface-peptide peak there is a corresponding chloro-T peptide peak. Surface-peptide fractions under the numbered bars were pooled, washed, and then returned to the 2-D mapping system.

To verify that each surface-peptide peak was indeed a peptide and to identify each of the surface-peptides, fractions under the numbered bars were pooled, dried, washed and reseparated in the 2-D peptide mapping system. Figure 4 shows the original 2-D chloro-T (chloro-T) map, 2-D surface map (surface), and the 2-D separations (1-6) of the HPLC-isolated surface-peptides of the G-MOMP. Numbers correspond to pooled peaks in Figure 3. Arrows point to weakly-emitting peptides. Each HPLC-separated surface-peptide can thus be identified in the surface-peptide map and the

chloro-T peptide map. Note that the relative intensities of emission of the peptides in the chloro-T and surface-peptides separated by HPLC correspond with the autoradiographic data in the 2-D peptide maps. That is, the heavily-emitting surface-peptide under bar #1, Figure 3, co-elutes at about 18 min with a weakly-emitting chloro-T peptide. The 2-D maps confirm this relationship and that of each surface and chloro-T peptide.

From these data it is clear that surface-exposed portions of OM proteins can be identified, compared, and isolated for further studies by a combination of SDS-PAGE, 2-D peptide mapping, and HPLC separation.

DISCUSSION

In this study we describe a method of identifying and isolating the surface-exposed portions of the MOMP of C. trachomatis serotype G. Previous studies on a variety of bacterial outer-membrane proteins (1,2,3) have demonstrated that the relationships of surface-exposure as determined by 2-D surface-peptide mapping correspond exactly with the immunological relationships using both polyclonal and monoclonal antibody (1,2,7,8,9). Therefore, peptides identified as being on the bacterial surface by these procedures appear to represent immunologically important epitopes on these molecules.

The addition of HPLC separation of surface-peptides makes it possible to isolate these peptides for further structural and immunological studies. It will be far easier to sequence these peptides than to sequence the entire protein, increasing the feasibility of synthetic peptide vaccines. In addition, peptides isolated by HPLC can be used immediately in tests to establish their immunogenicity and antigenicity.

An interesting outcome of this study is the resolution of a nagging problem: the appearance of surface-peptides which have no obvious corresponding peptides in the 2-D chloro-T peptide map of the same protein. The demonstration that the 2-D chloro-T preparation did contain a peptide corresponding to the "missing"

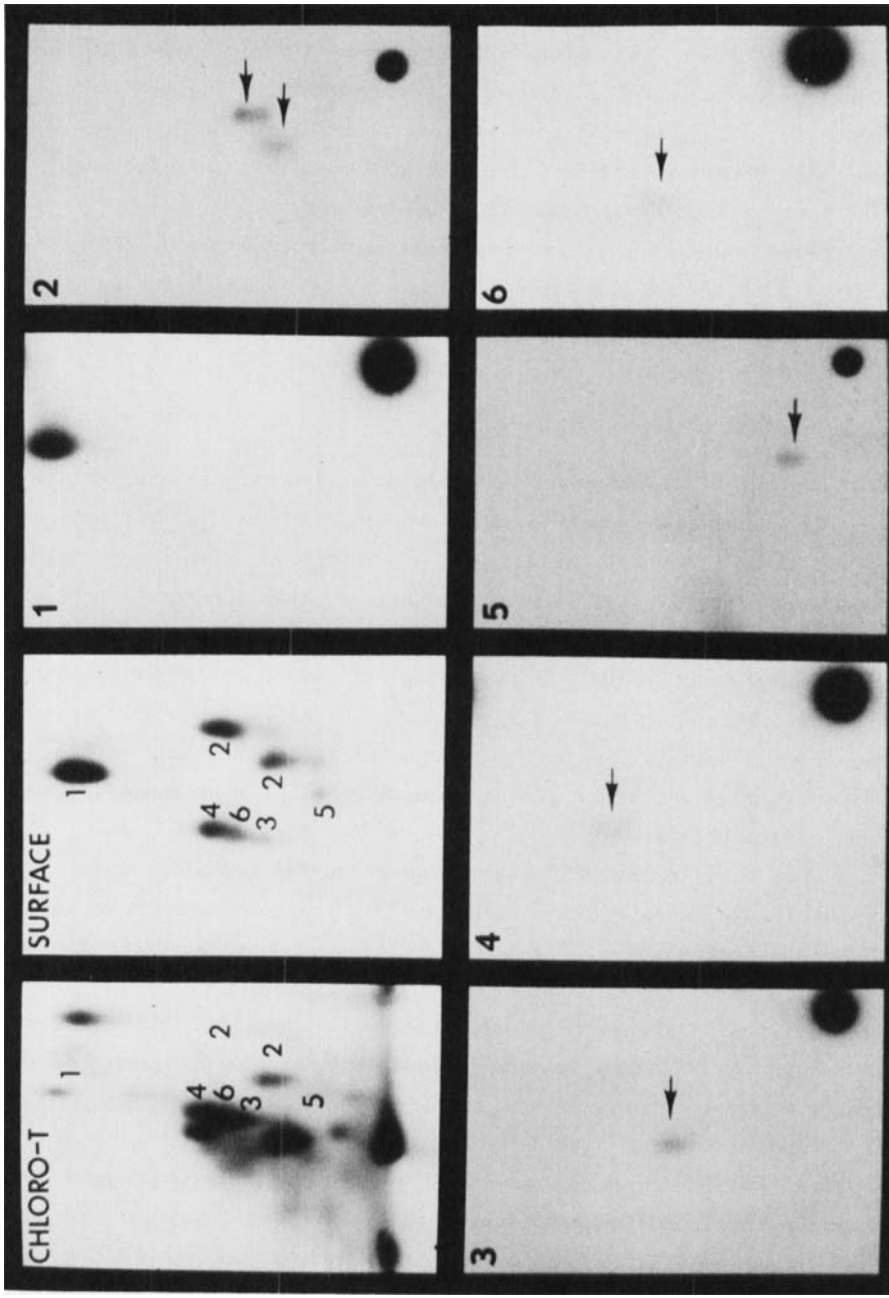

Figure 4. This is a composite of the 2-D chloro-T peptide map (chloro-T), the 2-D surface-peptide map (surface) and the 2-D reseparation of HPLC-separated surface-peptides of the C. trachomatis G-MOMP. Numbers correspond to numbered peaks in the HPLC separation, Fig. 3. Arrows point to weakly-emitting peptides. Each HPLC surface-peptide peak is identified by number in the 2-D chloro-T and 2-D surface map as determined by overlaying the 2-D maps. Note that one of the peaks in fraction 2 corresponds in position with the "missing" surface-peptide.

surface-peptide indicates the relative intensity of labeling varies greatly between chloro-T iodination of the SDS-PAGE isolated G-MOMP and surface-iodination of amino acids in the tyrosine rich peptides by the more efficient chloro-T procedure, accentuating the differences in intensity of emission of heavily vs. weakly iodinated peptides. It is also possible that the SDS-denatured MOMP assumes a configuration which internalizes those portions of the molecule which are normally exposed to the aqueous environment when the protein is intercolated into the native outer membrane.

It is now clear that the procedures of 2-D surface-peptide mapping and HPLC separation of surface-peptides can be combined to identify, compare, and isolate surface-exposed portions of outer-membrane proteins. Since these peptides appear to represent immunological epitopes, we can now begin to apply these techniques to give us valuable information about the nature of outer-membrane proteins immunogenicity and antigenicity. Such studies may lead to safe and effective peptide vaccines for bacterial diseases and will surely help us to understand the immunobiology of gram-negative bacterial pathogens.

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REFERENCES

1. Barbour, A. G., O. Barrera, and R. C. Judd. 1983. Structural analysis of the variable major proteins of Borrelia hermsii. Jr. Exp. Med. In press (Dec.)

2. Caldwell, H. D., and R. C. Judd. 1982. Structural analysis of chlamydial proteins. Infect. Immun. 38:960-938.

3. Judd, R. C. 1982. Surface peptide mapping of Protein 1 and Protein 111 of four strains of Neisseria gonorrhoeae. Infect. Immun. 37:632-641.

4. Judd, R. C. 1983. ^{125}I-labeled peptide mapping and high-performance liquid chromatography ^{125}I-peptide separation of Protein 1 of four strains of Neisseria gonorrhoeae. Jr. Liq. Chromat. 6:1421-1439.

5. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.

6. Markwell, M. A. K., and C. F. Fox. 1978. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1, 3, 4, 6-tetrachloro-3α, 6α-diphenylglcoluril. Biochem. 112:278-281.

7. Sandstrom, E. G., K. G. Chen, and T. M. Buchanan. 1982. Serology of Neisseria gonorrhoeae: Coagglutination serogroups W1 and W11/111 correspond to different outer membrane Protein 1 molecules. Infect. Immun. 38:462-470.

8. Stevens, R. S., M. R. Tam, C. C. Kuo, and R. C. Nowinski. 1982. Monoclonal antibodies to Chlamydia trachomatis: antibody specificities and antigen characterization. J. Immunol. 128:1083-1089.

9. Swanson, J. S., L. M. Mayer, and M. R. Tam. 1982. Antigenicity of Neisseria gonorrhoeae outer membrane proteins III detected by immunoprecipitation and western blot transfer with a monoclonal antibody. Infect. Immun. 38:668-672.