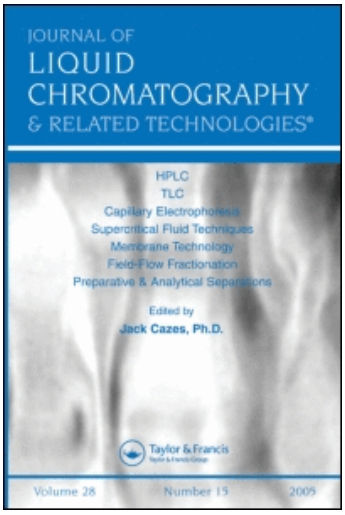


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

^{125}I-Labeled Peptide Mapping and High-Performance Liquid Chromatography I-Peptide Separation of Protein I of Four Strains of Neisseria Gonorrhoeae

Ralph C. Judd[ab]
[a] Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, Hamilton, Montana [b] Department of Microbiology, University of Montana, Missoula, Montana

To cite this Article Judd, Ralph C.(1983) '^{125}I-Labeled Peptide Mapping and High-Performance Liquid Chromatography I-Peptide Separation of Protein I of Four Strains of Neisseria Gonorrhoeae', Journal of Liquid Chromatography & Related Technologies, 6: 8, 1421 — 1439
To link to this Article: DOI: 10.1080/01483918308064862
URL: http://dx.doi.org/10.1080/01483918308064862

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(8), 1421-1439 (1983)

^{125}I-LABELED PEPTIDE MAPPING AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ^{125}I-PEPTIDE SEPARATION OF PROTEIN I OF FOUR STRAINS OF NEISSERIA GONORRHOEAE

Ralph C. Judd[1]
Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, Hamilton, Montana 59840-2999;
[1]Current address: Department of Microbiology University of Montana, Missoula, Montana 59812 (406)243-2873

ABSTRACT

^{125}I-labeled α-chymotryptic peptides of the principal outer membrane proteins (P.Is) of four strains of Neisseria gonorrhoeae were separated and visualized by two-dimensional (2-D) ^{125}I-peptide mapping and by high-performance liquid chromatography (HPLC) coupled with a Beckman Biogamma counter. In addition, ^{125}I-peptides were recovered from the HPLC separation and re-separated by the 2-D ^{125}I-peptide mapping system. The results indicated that the 2-D ^{125}I-peptide mapping procedure was best suited for comparative analyses of α-chymotryptic digests whereas the HPLC system, which is able to detect many more peptides than the 2-D system, is ideally suited for preparative separation of the ^{125}I-peptides. ^{125}I-peptides separated by HPLC could be recovered, rerun on the 2-D system, and the location of each peptide ascertained. The coupling of these two procedures allows for the isolation of specific ^{125}I-labeled peptides for further immunological and structural analyses of these outer membrane proteins.

INTRODUCTION

The technique of ^{125}I-labeled peptide mapping has proved to be a useful tool for evaluating the primary structural relationships of proteins. The coupling of this procedure with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7), which provides excellent protein separation, and in-gel radiolabeling of single protein bands (3, 12) has extended the applicability of

 0148-3919/83/0608-1421$3.50/0

^{125}I-labeled peptide mapping to the outer membrane proteins of several Neisseria species and strains (4, 5, 6, 12, 13, 16).

The separation of ^{125}I-peptide residues is accomplished by high voltage thin-layer electrophoresis (TLE) in the first dimension, followed by thin-layer chromatography (TLC) in the second dimension. Autoradiography then yields characteristic ^{125}I-peptide patterns (i.e., two-dimensional [2-D] ^{125}I-peptide maps). By comparing these patterns, structural relationships can be ascertained.

Until recently, the resolution and reproducibility of 2-D ^{125}I-peptide mapping have been restricted, due to batch variations in TLE sheets and the difficulty of maintaining precise temperature control during TLE (unpublished observations). The use of identical sheets and TLE chambers which maintain precise temperature control by immersing the thin-layer sheet in a cooling menstrum such as Varsol has greatly improved both the resolution and reproducibility of 2-D ^{125}I-peptide mapping (5, 6, 17). One drawback of this system is the inability to recover usable quantities of the separated ^{125}I-peptides for further experimentation.

In this study, I describe a system which compares an improved 2-D ^{125}I-peptide mapping procedure with a high-performance liquid chromatography (HPLC) ^{125}I-peptide separation using the major outer membrane proteins (P.Is) of four strains of Neisseria gonorrhoeae (GC). The results show both procedures yield similar information about the proteins' primary structure. In addition, ^{125}I-peptides separated by HPLC can be recovered, rerun using the 2-D ^{125}I-peptide mapping procedure, and identified by their migration characteristics, making it possible to isolate useful quantities of ^{125}I-peptides of outer membrane proteins for further investigations.

MATERIALS AND METHODS

Bacteria

Nonpiliated, transparent (11) GC strains JS1, JS2, JS3, and JS4 (5) were grown on clear typing medium and harvested as previously described (11).

SDS-PAGE

Unreduced lysates of whole GC were separated by SDS-PAGE using the discontinuous system of Laemmli (7) as previously described (5).

Radioiodination and enzyme digestion

The appropriate Coomassie-stained P.I bands were excised and radioiodinated by the chloramine-T procedures of Elder (3) and Swanson (12). Radioiodinated protein bands were digested with α-chymotrypsin in 0.05 M NH_4HCO_3 buffer, pH 8.5, and the ^{125}I-labeled peptides washed eight times by dissolving the peptides in 250 µl in distilled water (5, 12) and drying in a Savant (Savant Insts., Hicksville, New York) Speed-vac.

2-D ^{125}I-peptide mapping.

The washed ^{125}I-peptides from each P.I were rehydrated at 1 x 10^5 counts per minute (CPM) per µl in distilled water containing 1 mg/ml each of L-leucine, L-aspartate, and L-tyrosine as markers. ^{125}I-peptides recovered from the HPLC separation of the JS1 P.I (see below) were dried in a Speed-Vac and washed eight times in distilled water. The dried residues were resuspended in 10 µl of distilled water containing the above amino acid markers.

Two microliters of the P.I ^{125}I-peptide preparations (2 x 10^5 CPM) were spotted onto a Polygram Cel 300, 20 x 20 cm thin-layer sheet (Brinkmann Instruments, Westbury, N.Y.). Three preparations, spotted 8 cm from the anodal edge of the sheet and 1 cm, 7.5 cm, and 14.5 cm from

the side of the sheet, were run on each sheet. All 10 µl of each ^{125}I-peptide preparation recovered from the HPLC separation of the JS1 PI were spotted either 4.5 cm or 11.5 cm from the anodal edge of the sheet and 1 cm, 7.5 cm, and 14.5 cm from the side of the sheet. Six preparations could thus be run on each sheet (a pattern similar to that seen on a playing card having six spots, viewed side-on [Hoyle]).

Each thin-layer sheet was electrophoresed at a constant 1200 V for 30.5 min in a Savant TLE 20 (Savant Instruments, Inc.) apparatus, which immerses the sheet in Varsol during electrophoresis. A Forma Scientific 2095 bath and circulator (Forma Scientific, Marietta, Ohio) maintained a constant 8.5°C circulating coolant which held the Varsol menstrum at a constant 13.5°C throughout the run. Auxiliary cooling coils, fashioned by the Rocky Mountain Laboratories staff, were inserted horizontally, about 1 cm below the surface of the Varsol. These coils were mandatory for precise temperature control. The electrophoresis buffer was a pH 3.7 solution of water-acetic acid-pyridine (200:10:1, v:v:v). Following electrophoresis, the plate was removed, air dried, and cut in thirds longitudinally, so that each piece contained either one preparation, if three were added to the sheet, or two preparations, if six ^{125}I-peptides recovered from the HPLC separation were added to each sheet. Each piece was turned 90°C and subjected to ascending TLC in a solution of n-butanol-pyridine-water-acetic acid (13:10:8:2, v:v:v:v). A moat was scored 2 mm from the top of each piece of thin-layer sheet prior to TLC separation. When the solvent front reached the moat, the sheets were removed, air dried, and sprayed with 0.25% ninhydrin in acetone to locate the amino acid markers. ^{125}I-peptides were visualized by placing the thin-layer sheets on Kodak XAR-5 film and exposing for 18 h at -76°C using Cronex Par-Speed (E. I. dupont de Nemours and Co., Wilmington,

Delaware) intensifying screens. Origins on the 2-D maps of ^{125}I-peptides recovered from the HPLC were marked with 1-5 x 10^3 CPM of ^{125}I for reference.

HPLC.

All HPLC separations were performed on a Waters System (Waters Associates, Inc., Milford, Massachusetts), equipped with dual M 6000A pumps, system controller, data module, 440 UV detector, and a WISP 710 sample injector. A Waters μ Bondapak C_{18} reverse-phase column (3.9 mm ID x 30 cm) was used to separate the ^{125}I-peptides.

Approximately 2.1 x 10^5 CPM of each of the P.Is ^{125}I-peptide preparations were dissolved in 210 μl of distilled water-0.05 trifluoroacetic acid (TFA) Aldrich Chemical Co., Inc., Milwaukee, Wisconsin) containing 0.3 mg/ml each of L-tyrosine, L-tryptophan, and L-phenylalanine (Sigma Chemical Co., St. Louis, Missouri) as markers. Two hundred microliters of this solution was injected by the WISP 710 injector at time 0. The dual pump system, with pump A delivering filtered, distilled water-0.05% TFA and pump B delivering acetonitrile (reagent grade, Sigma)-0.05% TFA, then delivered 1 ml/min of a 0 to 8% acetonitrile-0.05% TFA linear gradient (06 gradient program) in the first 10 min. From 10 to 55 min, an 8 to 72% acetonitrile-0.05% TFA elution gradient, using the 09 gradient program, was generated followed by a 5-min period of 72% acetonitrile-0.05% TFA (60 min total separation time). Many different gradient profiles were tested; this profile was chosen as being representative of type of separation which can be obtained using these procedures.

At the time of injection, an LKB 7000 Ultrorac (LKB Instruments, Sweden) was started. Three hundred 2/10-min fractions were collected in Biovials (Beckman Instruments Inc., Palo Alto, California). Each

fraction was transferred to a Beckman Biogamma Counter and counted. Twice the background (100 CPM) was subtracted from the CPM of each fraction. The CPM of each fraction was then plotted versus retention time (RT) as derived from the fraction number. The elution of the amino acid markers was monitored at 254 nm by the model 440 UV detector. No ^{125}I-peptides could be detected by the 440 UV detector. A model 450 detector was unable to detect ^{125}I-peptidic peaks at 210 nm but was able to very weakly respond to autodigestion products of α-chymotrypsin at beginning concentrations of 200 μg.

RESULTS

The P.Is of the four strains of GC used in this study are seen in the Coomassie-stained SDS-PAGE gel shown in Fig. 1. These bands were excised, radioiodinated, and digested with α-chymotrypsin. The results of 2-D ^{125}I-peptide mapping of these preparations are displayed in Fig. 2. Previous studies using this technique (5, 6, 12) have indicated that the JS1 and JS2 P.Is are members of one "homology" group, whereas the JS3 and JS4 P.Is are in a second group. These relationships are seen in the 2-D ^{125}I-peptide maps (Fig. 2). However, the improved resolution provided by the technique described here shows a greater heterogeneity among these proteins than has been previously demonstrated by 2-D mapping.

The same preparations and the same number of CPM used to generate the 2-D ^{125}I-peptide maps seen in Fig. 2 were subjected to HPLC separation. The results of this procedure are seen in Fig. 3, which shows the plots of the CPM versus RT (fraction number) of the ^{125}I-peptides of the four P.I molecules under study. The gradient profile is an approximate representation of the 09 gradient program used to elute the peptides from the μ Bondapak C_{18} column. The RTs of the amino acid markers, as calculated by the data module from UV adsorbence

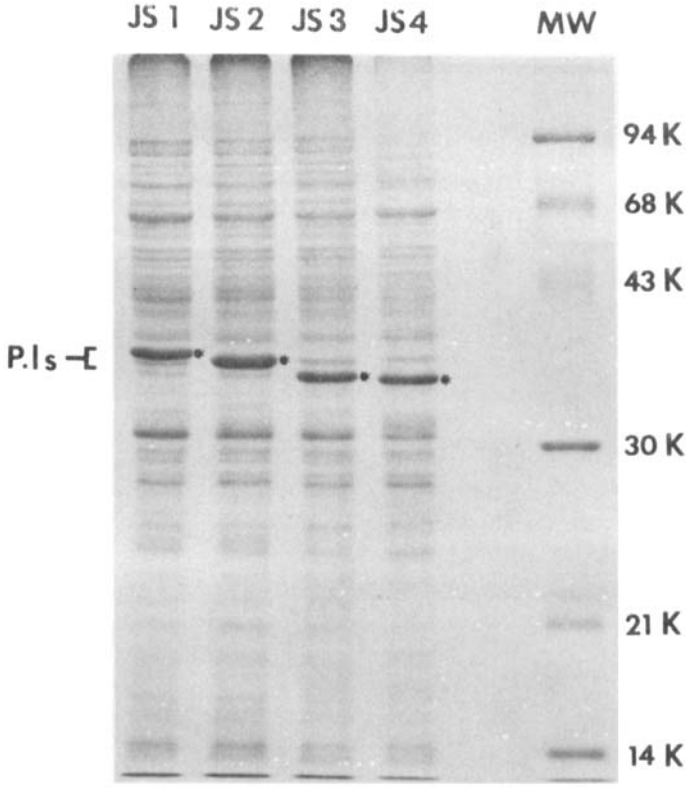


FIG. 1. Coomassie-stained SDS-PAGE gel of unreduced lysates of GC strains JS1, JS2, JS3, and JS4. P.I bands (asterisks) were excised and used in ^{125}I-labeled peptide mapping studies.

peaks, were 8.50 min (tyrosine), 9.65 (phenylalanine), and 13.90 (tryptophan) with no more than 5/100 min variation in all four runs for any marker.

The most striking feature of these separations is the large number of radioemitting fractions. Comparative interpretation is difficult due to the numerous small peaks which can be resolved by this very sensitive method. The JS1 and JS2 P.Is appear to be slightly more similar to one another than they are to the JS3 or JS4 P.Is, and vice versa,

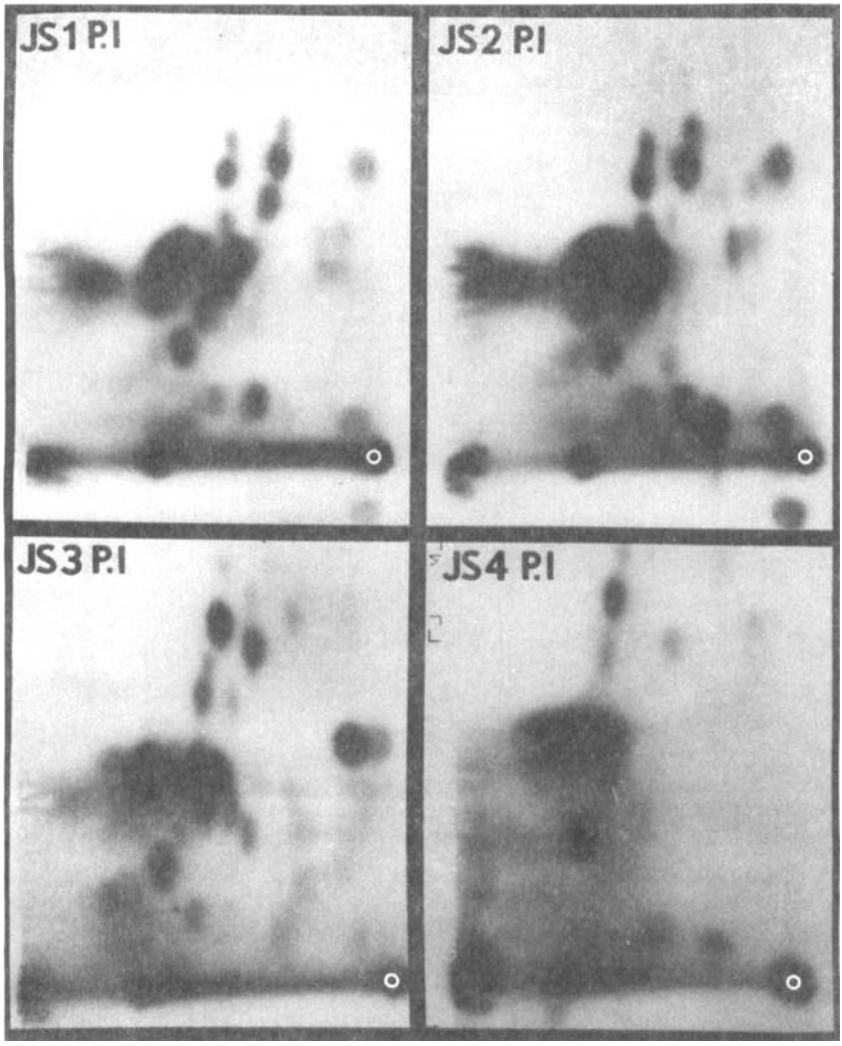


FIG. 2. 2-D ^{125}I-labeled peptide maps of α-chymotrypsin digested P.Is from GC strains JS1, JS2, JS3, and JS4. White circle marks the origin. Direction of TLE and TLC is as shown in Fig. 5.

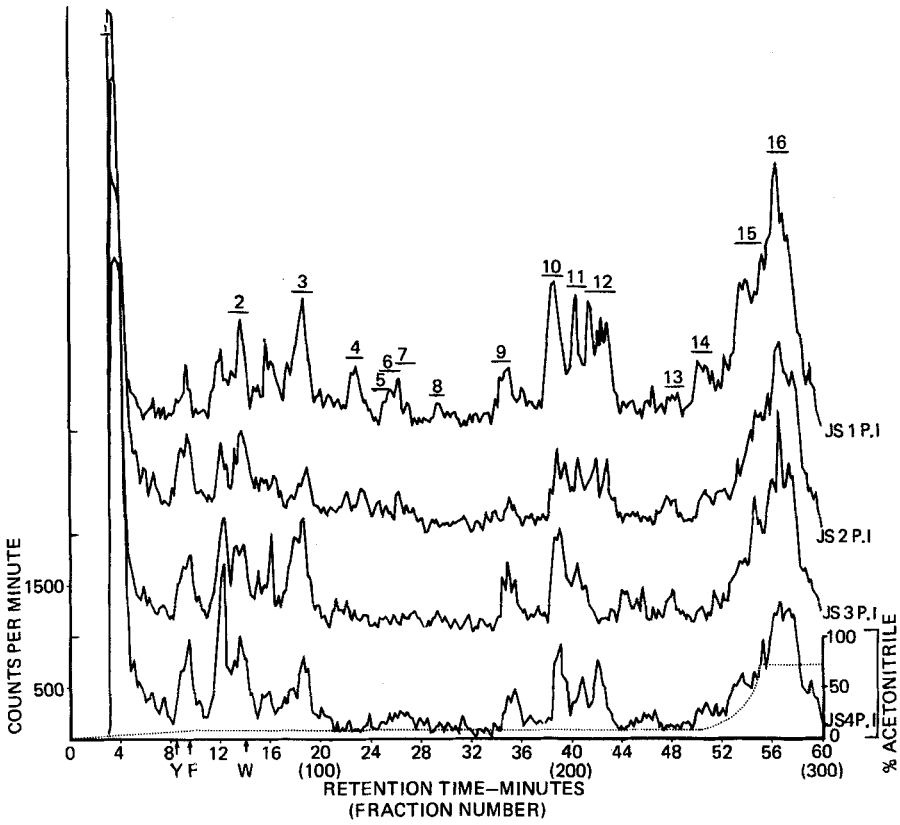


FIG. 3. HPLC profiles of α-chymotryptic ^{125}I-labeled peptides from P.Is of strains JS1, JS2, JS3, and JS4. Fractions of the JS1 P.I, designated by numbered bars over the JS1 P.I profile, were pooled for reseparation by 2-D ^{125}I-labeled peptide mapping. Y (tyrosine), F (phenylalanine), and W (tryptophan) designate amino acid markers RTs.

particularly in the 38- to 44-min region. Clearly, all the P.Is share several heavily emitting peaks having the same RT. Each also has a few strongly emitting and many weakly emitting peaks which are unique.

Complete separation of the peptides has not been accomplished using these elution parameters. Longer separation times and/or rechromatography of portions of these gradients using different

parameters can easily separate any peptide of interest. By altering the gradient profiles, the separation of peptides in any given region can be expanded; unfortunately, other regions are concomitantly compressed (data not shown and 10). The profiles shown here are presented as representative separations of ^{125}I-peptides of these proteins.

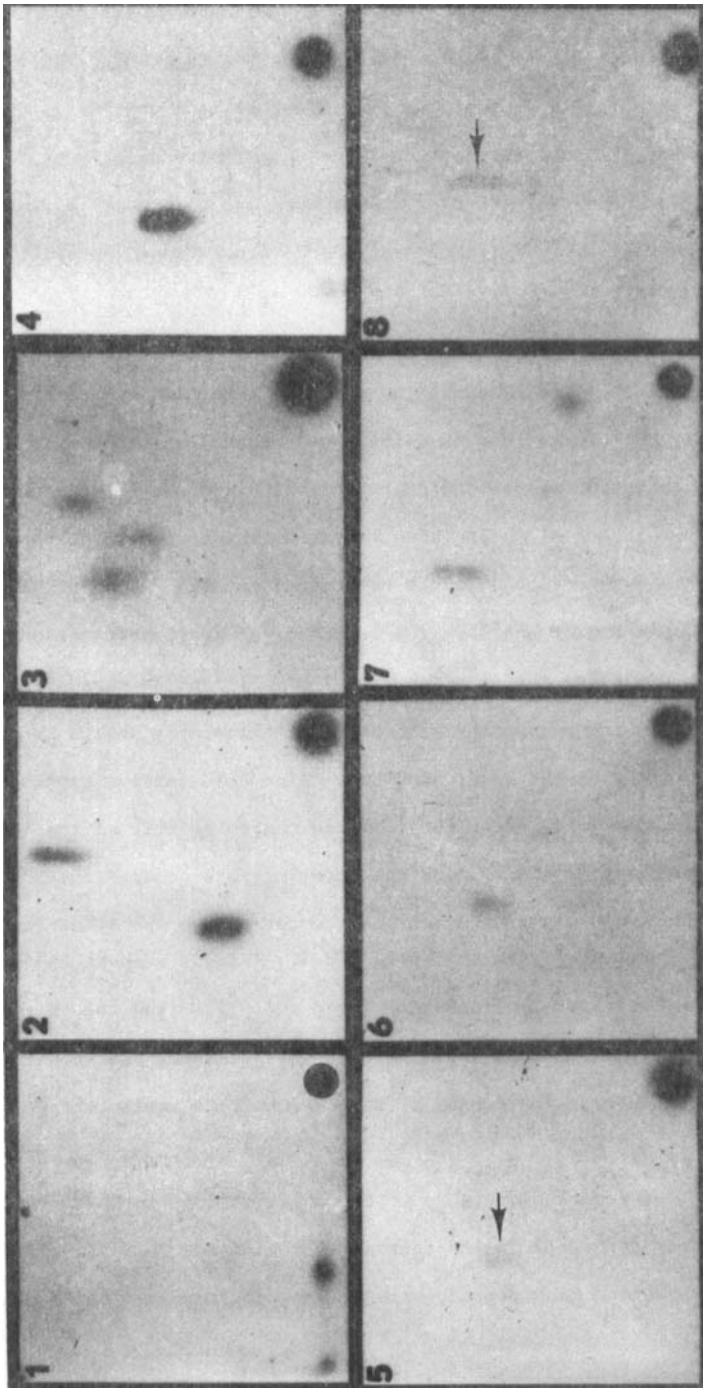
Fractions of the JS1 P.I HPLC separation, designated by numbered bars, were pooled, dried, washed, and subjected to 2-D ^{125}I-peptide mapping. [Note: all the fractions of one HPLC separation of the JS1 P.I were pooled, dried, washed, and subjected to 2-D mapping. All the peptides seen in the JS1 P.I 2-D map (Fig. 2 & 5) were recovered (data not shown)]. The results of these experiments are shown in Fig. 4. The number in the upper left of each ^{125}I-peptide map in this figure corresponds to the pooled fractions designated in Fig. 3. Every effort was made to quantitatively recover these peptides (only 10 to 15% of the original radioactivity was lost during handling). The relative intensities of the various peptides agree well with the height of the HPLC peaks from which they were recovered. The exception occurs with Peak 1, where a very large HPLC peak yielded two moderately emitting peptides (#1, Fig. 4). It appears that much of the material eluting with the HPLC solvent front migrates off the thin-layer sheet towards the anode since the anodal buffer became radioactive following the TLE separation. This suggests that this peak represents unbound ^{125}I.

It is clear from this figure that the HPLC separated ^{125}I-peptides can be recovered and returned to the 2-D ^{125}I-peptide mapping system for identification. The number of peptides recovered from each HPLC peak is consistent with the profile of the peak, e.g., Peak #10 is very broad, suggesting several peptides might be present, and this is confirmed in Fig. 4, #10, where four peptides are seen, etc. Also, all the P.Is have

an HPLC peak which has the same RT and approximate radioactivity as the dominant peak in the #2 pooled fractions (Fig. 3). The 2-D map of this peak (#2, Fig. 4 and 5) shows that this is a peptide which is present in about the same location and same approximate relative intensity in the 2-D maps of all the P.Is. This suggests that peaks having the same RT and radioactivity in the various PI separations contain the same peptides.

The isolated peptides in Fig. 4 recovered from the peaks in Fig. 3 are identified by number on a 2-D ^{125}I-peptide map of the JS1 P.I in Fig. 5. Note: due to differences in the positions that each preparation was spotted onto the thin-layer sheets and differences in the batches of sheets used in the 2-D separation, the peptides seen in Fig. 4 cannot be directly related to the 2-D ^{125}I-peptide map of the JS1 P.I in Fig. 2 and 5). The locations of these peptides were established by comparing the peptides with 2-D ^{125}I-peptide maps of the JS1 P.I which were run on the same batch of thin-layer sheets in the same position on the sheet and whose amino acid markers migrated to the same location (data not shown). It is characteristic of the TLE separation that samples placed near the anode migrate considerably further than identical samples run near the center of the sheet, as the JS1 P.I ^{125}I-peptides were in Fig. 2 and 5. This is evident in #7 of Fig. 4, where the two peptides have moved quite far from the origin but are identified as peptides closer to the origin in Fig. 5. Preparations spotted near the center of sheets from the same batch are highly reproducible.

A remarkable number of peptides from all regions of the HPLC separation fall in the central region of the 2-D ^{125}I-peptide map, suggesting many peptides are present in this area but are not easily



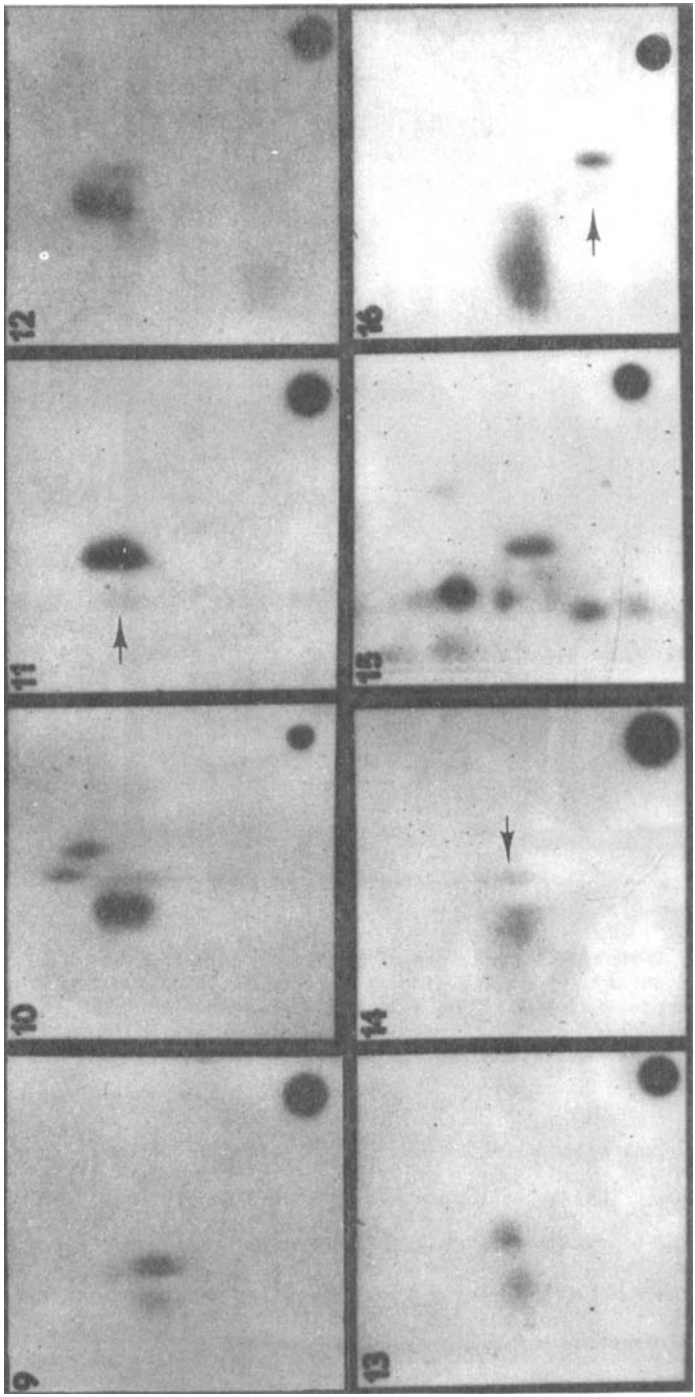


FIG. 4. 2-D ^{125}I-labeled peptide maps of pooled fractions from the HPLC separation of the α-chymotryptic ^{125}I-peptides of the JS1 P.I. Numbers correspond to the numbered bars over the JS1 P.I profile in Fig. 3. Arrows point to peptides which are very faint. Dark circles in the lower right corner of each 2-D map mark the origin. Direction of TLE and TLC is as shown in Fig. 5.

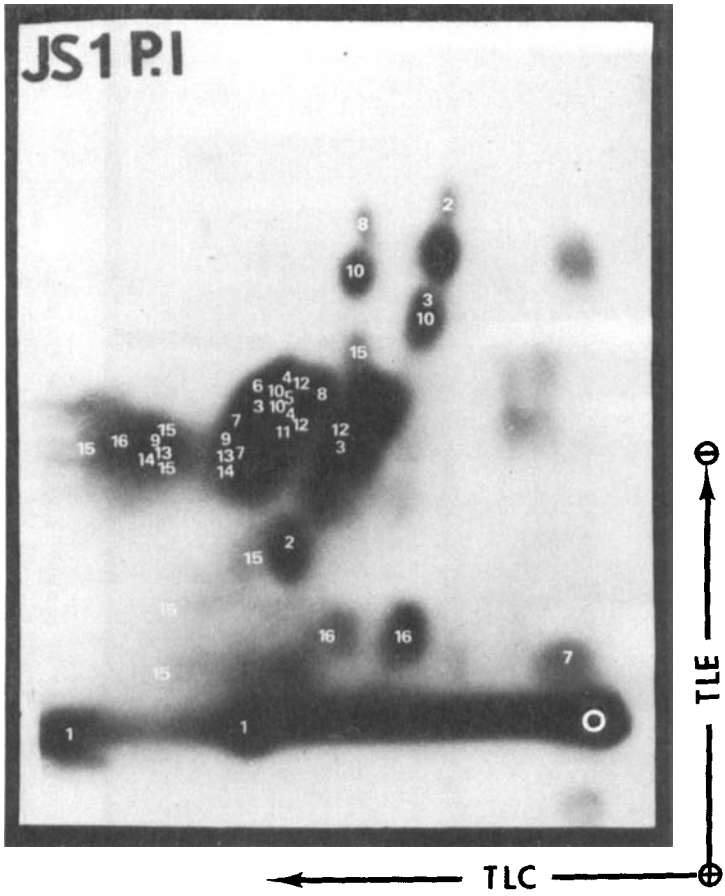


FIG. 5. 2-D ^{125}I-labeled peptide map of α-chymotrypsin digested JS1 P.I. Numbers identify location of isolated peptides (Fig. 4) recovered from HPLC separation (Fig. 3). See text for explanation of how locations were identified. White circle marks the origin. TLE, thin-layer electrophoresis; and TLC, thin-layer chromatography.

visualized. Several other 2-D ^{125}I-peptides also appear to have multiple components that are not seen due to overlapping. This, and the demonstration that ^{125}I-peptides from adjacent peaks (e.g., #2, #3, Fig. 3 and 4) can behave very differently in 2-D mapping, verifies that the HPLC and 2-D ^{125}I-peptide mapping procedures separate on different

physical parameters (8). It is not possible to accurately predict where a peptide will elute in the HPLC profile based on its migration in a 2-D map and vice versa.

DISCUSSION

In this study, two techniques of ^{125}I-peptide separation were compared. The P.Is of GC strains JS1, JS2, JS3, and JS4 were chosen for investigation because they have been well characterized by 2-D ^{125}I-peptide mapping (5, 6, 12). Both the 2-D ^{125}I-peptide mapping system and the HPLC separations gave similar information about the structural relationships of these proteins. However, due to the limitations and advantages of each technique, it appears that the two systems find their best use when used in tandem.

The 2-D ^{125}I-peptide mapping procedure for separation and visualization of α-chymotryptic digests has several advantages over the HPLC system that make it the preferable method for comparative analyses. Forty or more individual ^{125}I-peptide maps can be generated daily, allowing for multiple repeat runs of each preparation. With the improved cooling system described here and if strict attention is paid to precision, the 2-D system becomes highly reproducible and provides good resolution of the ^{125}I-labeled peptides.

Comparison of the 2-D system with the HPLC separations shows that the HPLC-Biogamma counter system is much more sensitive, detecting many very weakly emitting peptides, perhaps representing incomplete cleavage products and/or mono- versus diiodinated individual peptides (9). Whatever the reason for so many small peaks, this extensive "noise" makes comparative analysis of α-chymotryptic digests, on a fine scale, difficult. We are in the process of comparing 2-D maps with HPLC profiles of both gonococcal and chlamydial proteins digested with

Staphylococcal V-8 protease. Preliminary results indicate that V-8 protease yields many fewer peptides that are more easily resolved and comparable by HPLC. However, the HPLC is exquisitely sensitive to minor differences in the amount of dissolved gases, evaporation, oxidation, etc., in the elution buffers (especially the acetonitrile) which can result in very different elution profiles, even over a period of a few hours (unpublished observation and 10). This makes it difficult to run more than a few comparative separations at one time.

The demonstration that ^{125}I-peptides can be recovered from the HPLC separation, subjected to the 2-D ^{125}I-peptide mapping system, and their locations ascertained, indicates the HPLC is ideally suited for the preparative recovery of ^{125}I-labeled peptides for use in further analyses. In addition, the HPLC separation indicates several of the ^{125}I-peptides visualized by 2-D mapping may actually be multiple peptidic residues which, due to their proximity, are difficult to resolve in the 2-D system, showing that the HPLC separation coupled with the 2-D analysis can be used to dissect the heavily emitting regions seen in the 2-D ^{125}I-peptide maps.

In previous studies I demonstrated that surface-exposed portions of gonococcal outer membrane proteins I and III (6) could be identified and compared by 2-D mapping. Recent work by Tam, et al. (15), and Sandstrom (personal communication and MS submitted) has demonstrated that the differences observed by surface-mapping of P.Is correlate exactly with their P.I serotyping scheme (i.e. JS1 and JS2 being W11 and JS3 and JS4 being W1 - see references 6 and 15) using monoclonal reagents. In addition, Swanson, et al. (14) has confirmed that P.IIIs from all strains studied react identically with monoclonal antibody, confirming predictions based on the identical surface-peptide maps of P.IIIs. The

serological correlation with surface-peptide mapping of chlamyelial major outer membrane proteins (1) using polyclonal antisera and borrelial outer membrane proteins (Judd and Barbour, MS submitted) confirms that surface-peptides represent immunoreactive sites of native outer membrane proteins. With the technologies described in this paper, it will now be possible to not only identify and compare surface-peptides, but to physically isolate these peptides for further immunogenic-antigenic studies.

The coupling these techniques, then, provides a tool for acquiring defined peptides for use in antigenic, sequencing, or topographical studies of membrane proteins (15). The HPLC may also be helpful in comparing (and identifying) surface-labeled ^{125}I-peptides with chloramine-T iodinated peptides. There has been several reports of difficulty in relating surface peptides with chloramine-T peptides (1, 4, 6), possibly due to the greatly differing relative intensities of labeling by these procedures (1, 6). The sensitivity of the HPLC system described here may help resolve this problem. The HPLC may also be of value in comparing the α-chymotryptic peptides of ^{125}I-labeled proteins with peptides of intrinsically ^{14}C-labeled proteins, which are not efficiently visualized by 2-D peptide mapping (unpublished observation), in order to establish if the ^{125}I-labeling truly reflects the total primary structure of these proteins.

ACKNOWLEDGMENTS

I thank John Swanson for his continued guidance and support, Susan Smaus for her invaluable assistance in the preparation of this manuscript, and Chuck Taylor and Bob Evans for their fine photographic work. I am grateful to Joan Knapp for serotyping the JS1-4 GC strains. I am also indebted to Jim Williams, Ted Hackstadt, (both of Rocky

Mountain Laboratories), and Vince Fishetti (Rockefeller University) for their advice on HPLC, and a special thanks to Tom Kerr and the staff of Rocky Mountain Laboratories for their assistance and critical evaluation of this manuscript.

REFERENCES

1. Caldwell, H. D., and R. C. Judd, Structural Analysis of Chlamydial Proteins, Infect. Immun. In press (IAI #638), 1982.

2. Darveau, A., N. G. Seidah, M. Chretien, and J. Leconte, Peptide mapping of ^{125}I-labeled membrane protein of influenza viruses by reverse-phase high-performance liquid chromatography, J. Virological Methods, 4:77-85, 1982.

3. Elder, J. H., R. A. Pickett II, J. Hampton, and R. A. Lerner, Radioiodination of proteins in single polyacrylamide gel slices, J. Biol. Chem., 252:6510-6515, 1977.

4. Heckels, J. E., Structural comparison of Neisseria gonorrhoeae outer membrane proteins, J. Bacteriol., 145:736-742, 1981.

5. Judd, R. C., ^{125}I-labeled peptide mapping of Protein III isolated from four strains of Neisseria gonorrhoeae, Infect. Immun., 37:622-631, 1982.

6. Judd, R. C., Surface-peptide mapping of Protein I and Protein III of four strains of Neisseria gonorrhoeae, Infect. Immun., 37:632-641, 1982.

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

8. Meek, J. L., Prediction of peptide retention times in high-pressure liquid chromatography on the basis of amino acid composition, Proc. Natl. Acad. Sci. U.S.A., 77:1632-1636, 1980.

9. Seidah, N. G., M. Dennis, P. Cowal, J. Rochement, and M. Chretien, A rapid high-performance liquid chromatography purification of iodinated polypeptide hormones, Anal. Biochem., 109:185-191,1980.

10. Snyder, L. R., and J. S. Kirkland, Introduction to modern liquid chromatography, 2nd Ed., John Wiley and Sons, Inc., New York, 1979, p. 662-718 and 752-823.

11. Swanson, J., Studies on gonococcus infection. XII. Colony color and opacity variants of gonococci, Infect. Immun., 19:320-331,1978.

12. Swanson, J., Studies on gonococcus infection. XVIII. ^{125}I-labeled peptide mapping of the major protein of the gonococcal cell wall outer membrane, Infect. Immun., 23:799-810, 1979.

13. Swanson, J., ^{125}I-labeled peptide mapping of some heat-modifiable proteins of the gonococcal outer membrane, Infect. Immun., 28:54-64, 1980.

14. Swanson, J. S., L. M. Mayer, and M. R. Tam, Antigenicity of Neisseria gonorrhoeae Outer Membrane Proteins III Detected by Immunoprecipitation and Western Blot Transfer with a Monoclonal Antibody, Infect. Immun., In press (IAI #415), 1982.

15. Tam, M. R., T. M. Buchanan, E. G. Sandstrom, K. K. Holmes, J. S. Knapp, A. W. Diadak, and R. C. Nowinski, Serological Classification of Neisseria gonorrhoeae with Monoclonal Antibodies, Infect. Immun., 36:1042-1053, 1982.

16. Tsai, C. M., C. E. Frasch, and L. F. Mocca, Five structural classes of outer membrane proteins in Neisseria meningitidis, J. Bacteriol., 146:69-78, 1981.

17. Whittaker, R. G., and B. A. Moss, Comparative peptide mapping on the nanomole level, Anal. Biochem., 110:56-60, 1981.