Preface

NIH Director Elias Zerhouni, speaking at the annual meeting of the Association of American Medical Colleges in late 2002, addressed the need for the development of new and revolutionary research tools to understand complex biological systems that can then be applied to cure diseases. "Among revolutionary methods of research, we need to accelerate proteomics and large-scale technology for the post-genomic era," he noted, reminding the audience that sequencing the genome is essentially only "getting the parts list."

It was in this spirit of enthusiasm that the groundwork for this volume was begun earlier that year, by selecting well-recognized authors, who have contributed mightily to the field of proteomics, and identifying areas of interest and potential growth that could lead to a useful methods handbook.

Proteomics, as a word and as a discipline, is new to most of us and we expect that many will find this volume a useful dictionary for understanding the work of others as well as a map for setting out on our own research programs. From the start, it was our goal to produce a volume that would be valuable both to the neophyte and the seasoned worker. We encouraged the authors to include hints and tricks that might not be obvious in those original publications that did not describe the procedure in the detail reported here.

This editor, who marveled at the speed that the human genome was sequenced, realized that he was looking only at the blueprint for the Taj Mahal, not seeing a photo, much less actually having an experience in Agra. The beauty of the detail is the study of Proteomics.

The editor wants to thank the authors for interrupting their busy schedules to participate in this important project and the publisher for recognizing the need for placing it on a high priority timetable.

P. Michael Conn

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Protein Identification from 2-D Gels Using In Vitro Transcription Translation Products

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1. Introduction

The genomics revolution offers the opportunity to describe biological processes on the basis of global and quantitative gene expression patterns from cells or organisms representing different states. Although DNA microarrays are extremely powerful for measuring gene expression at the mRNA level ***(1)***, the nonpredictive correlation between mRNA and protein levels ***(2,3)*** and the discovery of post-translational mechanisms that either modify the structure and function of proteins or alter their half-life and rate of synthesis ***(4)*** indicate that direct measurement of protein expression is essential for a proper analysis of the biological processes.

Global analysis of proteins from cells and tissues is termed *proteomics*. Although a variety of alternative procedures are under study ***(5,6)***, at present the most popular approach for proteomics analysis requires the previous knowledge of the genome sequence of the organism under investigation and is based on the combination of two-dimensional gel electrophoresis (2-DGE) and mass spectrometry ***(6)***. According to this approach, proteins are separated by 2-DGE, stained, digested *in situ* with trypsin or other proteolytic enzymes, and finally subjected to matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. The MALDI-TOF analysis provides peptide mass fingerprints, which lead to protein identification once they are compared with the theoretical *in silico* fingerprints generated from the available genome sequence. Usually, 80–85% of the analyzed spots give a mass fingerprint, which is in most cases sufficient for protein identification. A limited number of spots (approx 5%) further requires tandem mass spectrometry (MS/MS) analysis for unambiguous characterization.

The major drawback of this approach is that it requires expensive and sophisticated instruments, which need to be operated by well-trained and specialized scientists. In addition, the method presents some limitations in sensitivity, not because of mass spectrometers (which could analyze samples in the low fmole range), but to sample preparation procedures, which are usually inefficient, making the analysis feasible only when protein quantities greater than 0.1–0.2 pmol are available ***(7)***. Finally, although the approach is relatively rapid, especially when robotic stations are utilized to handle spot

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picking and processing, full characterization of a complex protein mixture may require a few weeks of intensive work.

We have recently described an alternative method for the characterization of proteomes, in particular bacterial proteomes, which may offer some advantages over the current proteomic approaches ***(8)***. The method is based on in vitro transcription and translation of genes from the organism under investigation to obtain radioactive translation products. The proteins produced in vitro are then separated on 2-D gels, and the corresponding autoradiographs are superimposed by computer-assisted image acquisition and processing on the 2-D gel of the total proteins from the organism under investigation (sample gel). The matching of the radioactive products with spots of the sample gel allows immediate protein identification, demonstrating that the genes used in the transcription and translation experiments are in fact expressed in vivo.

Here, the method is applied to test rapidly the conservation of two membrane proteins among different clinical isolates of *Neisseria meningitidis* group B.

2. Materials and Methods

2.1. Reagents

1. Immobiline pH gradients (IPG) and IPG buffer, pH 3–10, nonlinear (NL) (Amersham Pharmacia Biotech, Piscataway, NJ).
2. Urea (enzyme grade, Life Technologies, Paisley, Scotland).
3. 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), dithiothreitol (DTT), thiourea, L-glutamine, cocarboxylase, trypsin inhibitor, trypsinogen, carbonic anhydrase, myoglobin, and diethyl pyrocarbonate (DEPC) (Sigma, St. Louis, MO).
4. Acrylamide and agarose (Bio-Rad, Hercules, CA).
5. Glucose and benzonase (Merck, Darmstadt, Germany).
6. Sodium thiosulfate and tributyl phosphine (TBP) (Fluka, Buchs, Switzerland).
7. Amidosulfobetaine-14 (ASB14) ***(9)*** (a generous gift from Prof. C. Scolastico, University of Milan, Italy).
8. All other reagents were analytical grade.

2.2. Preparation of Protein Standards

1. Selected open reading frames (ORFs) from *Chlamydia pneumoniae* CWL029, *Streptococcus agalactiae* 2603 V/R, and *Neisseria meningitidis* serogroup B strain MC58 were cloned into expression vectors to produce histidine tag and glutathione-S-transferase (GST) fused proteins ***(10)***.
2. Expression and purification of the recombinant proteins were as described by Montigiani et al. ***(10)***, except that an ultimate step of purification by preparative electrophoresis was added. Gel pore size, length, and running conditions were selected according to the instrument manual (491 model Prep Cell, Bio-Rad, Hercules, CA).
3. Purified proteins were precipitated with 20% trichloroacetic acid, and the pellet was washed with acetone and redissolved in the reswelling solution [7 *M* urea, 2 *M* thiourea, 2% (w/v) CHAPS, 2% (w/v) ASB14, 2% (v/v) IPG buffer, pH 3–10, NL, 2 m*M* TBP, 65 m*M* DTT] to a final concentration of 3 mg/mL.

2.3. Preparation of Template for Transcription/Translation Reactions

1. The genes of interest were polymerase chain reaction (PCR)-amplified from *Neisseria meningitidis* strain MC58 chromosomal DNA using Pwo DNA polymerase (Roche Diagnostics, Mannheim, Germany) and appropriate synthetic forward and reverse primers carrying the *Nde*I and *Xho*I (or *Hin*dIII) restriction sites, respectively.

2. The amplified fragments were digested with either *Nde*I/*Xho*I or *Nde*I/*Hin*dIII enzyme mixtures and ligated to plasmid pET-21b+ (Novagen, Madison, WI) previously digested with the same enzymes. In pET-21b+ a stop codon was introduced upstream from the nucleotide sequence coding for the hexa-histidine tag to avoid the addition of any extra amino acid to the protein of interest.
3. Plasmid preparations were made using the Qiagen kit (Qiagen, Hilden, Germany).

2.4. In Vitro Transcription/Translation Reactions (TTRs)

1. TTR was carried out using circular plasmids as template ***(11,12)***.
2. S30 extracts were prepared from *Escherichia coli* BL21 containing endogenous T7 RNA polymerase ***(13)*** as described by Pratt ***(14)***.
3. Aliquots (250 µL) of S30 preparations (12–15 mg/mL, final protein concentration) were stored at –80°C.
4. In vitro coupled transcription-translation was carried out as described ***(14)***, with minor modifications. The reaction was performed in a 10-µL final volume containing 20–25 µg/mL of DNA, 0.42 m*M* ^{14}C-labeled L-leucine and L-lysine (11.7 and 12. 2 Gbq/mmol, respectively; Amersham Pharmacia Biotech), 0.42 m*M* of each of the other 18 unlabeled amino acids, and 34% (v/v) of S30 extract. All other reagents needed for the reaction were as indicated by Pratt ***(14)***.
5. The reaction was allowed to proceed for 3 h at 37°C.
6. Prior to electrophoretic analysis, the proteins were precipitated with cold ethanol (90%, v/v).

2.5. 2-D Electrophoresis and Autoradiography

1. *Neisseria meningitidis* membrane proteins were prepared as described by Molloy et al. ***(15)***. Proteins (125 µg) and TTRs (up to 20 µg) were mixed with 1 µg of each reference protein **(Table 1)** and brought to a final volume of 125 µL with reswelling solution.
2. The proteins were absorbed overnight onto an Immobiline DryStrip (7 cm, NL pH 3–10 gradient) using the Immobiline Dry-Strip Reswelling Tray (Amersham Pharmacia Biotech).
3. Proteins were then separated by 2-D electrophoresis. The first dimension was run using an IPGphor Isoelectric Focusing Unit (Amersham Pharmacia Biotech), applying sequentially 150 V for 35 min, 500 V for 35 min, 1000 V for 30 min, 2600 V for 10 min, 3500 V for 15 min, 4200 V for 15 min, and then 5000 V to reach 10 kVh. For the second dimension, the strips were equilibrated as described ***(16)***, and the proteins were separated on linear 9–16% polyacrylamide sodium dodecyl sulfate (SDS) gels (1.5 mm thick, 6 cm high) using the Mini Protean II Cell from Bio-Rad. Gels were stained with colloidal Coomassie ***(17)***.
4. Prior to autoradiography, gels were soaked with 125 m*M* salicylic acid in 40% methanol and dried on filter paper (Bio-Rad). Gels were autoradiographed overnight on BioMax MR-2 film (Eastman Kodak, Rochester, NY).

2.6. Mass Spectrometry Analysis

1. Protein spots were excised from the gel, washed with Milli-Q water and acetonitrile, and dried in a SpeedVac apparatus (Savant, Holbrook, NY) ***(18)***.
2. Gel pieces were rehydrated by adding 7–10 µL of 50 m*M* ammonium bicarbonate and 5 m*M* $CaCl_2$ containing 0.012 µg/µL sequencing grade trypsin (Roche Diagnostic, Mannheim, Germany).
3. Tryptic digestions were carried out at 37°C for 18 h, and peptides were eluted out by 30-min sonication in a sonicator bath after addition of 50% acetonitrile, 5% TFA (50 µL).
4. Peptide extraction was repeated once, extract solutions were pooled, and the volume was reduced to 10 µL in a SpeedVac apparatus.

Table 1
Standard Proteins Used for Gel Alignment

Name	Origin		MW (Kd)	pI
CPn0195	*Chlamydia pneumoniae*	recombinant GST fusion	84,0	6,78
CPn0197	*Chlamydia pneumoniae*	recombinant GST fusion	75,0	6,29
CPn0278	*Chlamydia pneumoniae*	recombinant GST fusion	55,0	6,67
CPn0324	*Chlamydia pneumoniae*	recombinant GST fusion	70,1	5,22
CPn0336	*Chlamydia pneumoniae*	recombinant GST fusion	60,2	6,21
CPn0420	*Chlamydia pneumoniae*	recombinant GST fusion	62,8	5,65
CPn1064	*Chlamydia pneumoniae*	recombinant GST fusion	32,0	5,00
GST	*Schistosoma japonicum*		27,4	6,40
CPn0278	*Chlamydia pneumoniae*	recombinant His Tag	29,6	7,01
CPn0764	*Chlamydia pneumoniae*	recombinant His Tag	47,9	7,28
CPn0399	*Chlamydia pneumoniae*	recombinant His Tag	23,3	8,07
CPn1034	*Chlamydia pneumoniae*	recombinant His Tag	29,0	6,05
CPn0113	*Chlamydia pneumoniae*	recombinant His Tag	41,0	5,92
CPn1067	*Chlamydia pneumoniae*	recombinant His Tag	21,8	6,26
CPn0321	*Chlamydia pneumoniae*	recombinant His Tag	40,9	5,57
CPn0273	*Chlamydia pneumoniae*	recombinant His Tag	22,9	6,63
CPn0297	*Chlamydia pneumoniae*	recombinant His Tag	34,7	5,67
CPn0624	*Chlamydia pneumoniae*	recombinant His Tag	37,7	6,6
SAg0681	*Group B Streptococcus*	recombinant GST fusion	65,7	8,62
SAg0079	*Group B Streptococcus*	recombinant His Tag	27,5	5,41
SAg0628	*Group B Streptococcus*	recombinant His Tag	50,8	4,89
NMB1119	*Neisseria meningitidis*	recombinant His Tag	21,8	4,95

GST, glutathione-S-transferase.

5. Samples were automatically prepared for mass spectrometry analysis using the MAP II system (Bruker, Bremen, Germany). The instrument was programmed to perform sample desalting with ZIP-TIP (C18, Millipore, Bedford, MA).
6. Peptides were eluted from ZIP-TIPs with a saturated solution of α-cyano-4-hydroxy-cinnamic acid in 50% acetonitrile/0,1% trifluoro-acetic acid and directly loaded onto the SCOUT 381 multiprobe (Bruker, Bremen, Germany).
7. The samples were then allowed to air dry at room temperature.
8. Spectra were acquired on a Bruker Biflex II MALDI-TOF (Bremen, Germany) equipped with the SCOUT 381 multiprobe ion source in a positive-ion reflector mode. The acceleration voltage was set to 19 kV, and the reflector voltage was set to 20 kV. Typically, about 100 laser shots were averaged per spectrum from a 337-nm N2 laser.
9. Spectra were calibrated externally using a combination of standards [angiotensin II (1046.54 Daltons), substance P (1347.74 Daltons), bombesin (1619.82 Daltons), and clipped human ACTH18-39 (2465.20 Daltons) located in spots adjacent to the samples.
10. Peptides were selected in the mass range of 700–3000 Daltons.
11. Monoisotopic peak values were used for Mascot search on private databases. All searches were performed using a 200-ppm constraint window.

2.7. Image Acquisition and Analysis

1. Autoradiographs and Coomassie Blue-stained gels were scanned with a Personal Densitometer SI (Amersham Pharmacia) at 8 bits and 50 μm per pixel. Prior to image acqui-

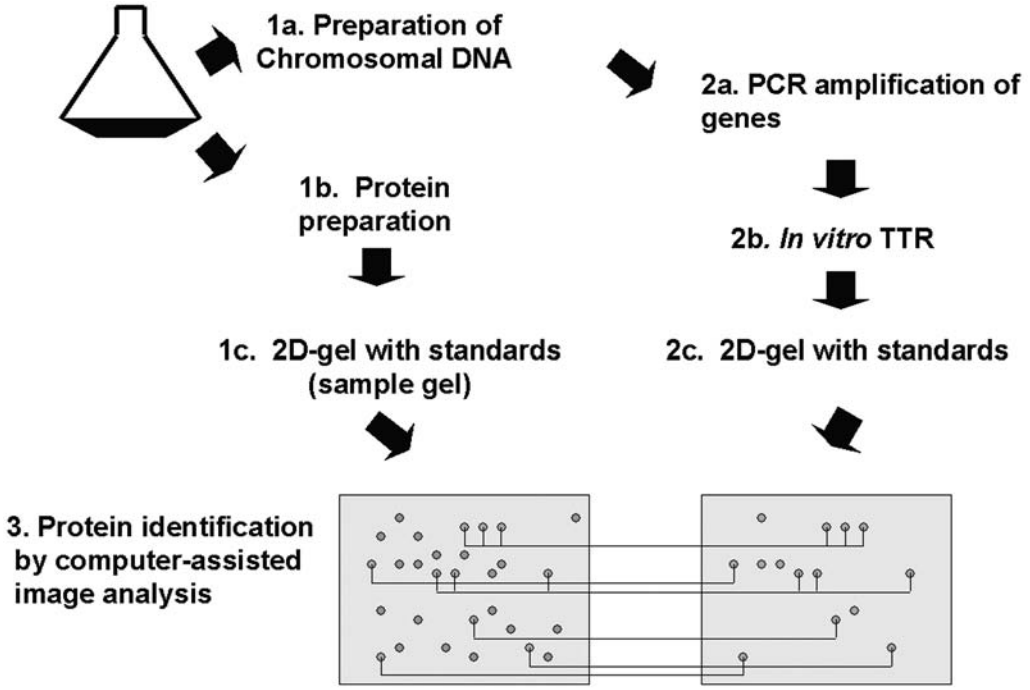

Fig. 1. Schematic representation of the procedure for protein spot identification by in vitro transcription/translation reactions. A bacterial culture is used for chromosomal DNA and total protein preparation (steps 1a. and 1b.). Proteins are mixed with the set of protein standards and then separated on a 2-D gel (sample gel, step 2c.) whereas chromosomal DNA is used for gene amplification (step 2a.). The amplified genes are transcribed and translated in vitro in the presence of ^{14}C-labeled amino acids (step 2b.). TTRs are pooled, mixed with the set of protein standards, and then separated on 2-D gels (step 2c.). Finally, the 2-D gels are autoradiographed and superimposed on the sample gel using computer-assisted image analysis (step 3.). Spot matching allows the immediate identification of protein spots on the sample gel.

sition of autoradiographs, the positions of the reference proteins were manually reported on the films.

2. Image analysis was performed using Image Master 2D Elite software, version 3.10 (Amersham Pharmacia Biotech).

3. Results

3.1. Description of Strategy

The technology is represented schematically in **Fig. 1**. Total proteins and chromosomal DNA are prepared from the organism under investigation. Total proteins are mixed with selected standard proteins and resolved by 2-DGE to generate the "sample gel," whereas chromosomal DNA is used as template for gene amplification. The amplified genes are cloned in appropriate expression vectors for in vitro protein synthesis. TTRs are carried out in the presence of ^{14}C-labeled amino acids and resolved by 2-DGE in the presence of the same standard proteins used for the sample gel. The 2-D gels are first stained for standards localization and then dried and autoradiographed for TTR visualization. Finally, autoradiographs are superimposed *in silico* on the sample gel

using the reference standards as common coordinates. Should the proteins encoded by the genes used in TTRs be present in the sample gel, they will match the TTR products, thus allowing their immediate identification.

3.2 Application of the Strategy

The potentiality of the procedure is illustrated here by analyzing two membrane proteins of *Neisseria meningitidis* group B (MenB), a bacterial pathogen under intense investigation in our laboratories for the development of an effective vaccine ***(19,20)***. In particular, we were interested in (1) determining whether the outer membrane protein OMP4 (a product of the NMB gene) and the chaperonin heat shock protein Hsp60 (a product of the NMB1972 gene) were present in the membrane protein preparation of the MC58 strain ***(19)***; and (2) the extent of conservation of these two proteins in different MenB clinical isolates.

To answer the first question, 125 μg of a membrane protein preparation of MenB cells were mixed with 20 standard proteins (*see* Materials and Methods and **Table 1**), resolved onto a 2-D gel, and subsequently stained with Coomassie Blue **(Fig. 2A)**. The addition of the standard proteins to both the sample proteins and TTRs (*see* below) is a fundamental step of the entire procedure in that the standard proteins are subsequently utilized to superimpose the sample gel properly on the TTR gels. Standard proteins should be selected to have an even distribution throughout the gel. This in fact allows the software algorithm to compensate for the local gel distortions with high accuracy.

In a parallel experiment, plasmid pET-0382 and pET-1972 carrying the NMB0382 and NMB1972 genes, respectively, were used for in vitro transcription and translation reactions. The TTR products were then mixed with the standard proteins and resolved on 2-D gel. Finally the gel was autoradiographed. As shown in **Fig. 2B**, the main com-

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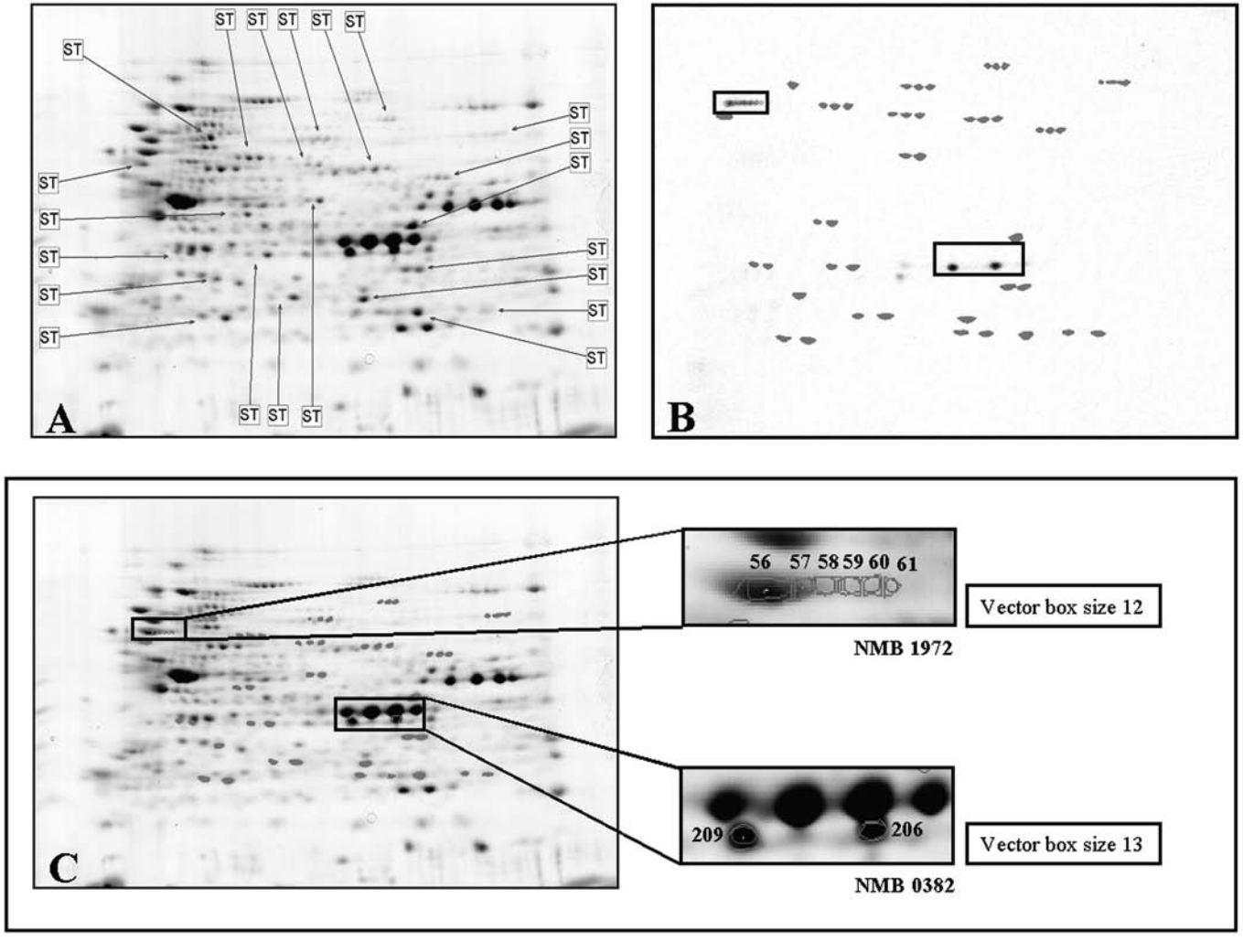
Fig. 2. *(see facing page)* Matching analysis of TTR 2-D gel spots with sample 2-D gel spots. **(A)** Sample gel. A membrane protein preparation of MenB (125 μg) was loaded onto a 2-D gel in the presence of the 22 standard proteins (1 μg each, spots assigned with green arrows labeled ST) and stained with Coomassie Blue. **(B)** Autoradiograph of a TTR 2-D gel. Radiolabeled NMB 1972 and NMB 0382 were synthesized in vitro. Aliquots (20 μg of total proteins) of each reaction were mixed together, combined with the 22 standard proteins (1 μg each), and loaded onto a 2-D gel. The gel was first stained with Coomassie Blue to visualize the standard proteins and then dried and autoradiographed. The TTR products are circled in red and boxed on the autoradiograph. The standard position (solid blue spots) was determined by superimposing the autoradiograph on the corresponding Coomassie Blue-stained gel. **(C)** Superimposition of the TTR autoradiograph (B) on the sample gel (A) using Image Master 2D Elite, 3.1 software. The software creates the combined gel shown in C in which the TTR gel is superimposed on the sample gel using the reference proteins (solid blue spots). The combined gel is then scanned for TTR spots located within a fixed area (vector box) from any spot of the sample gel. If a TTR spot is within the selected vector box range from one protein of the sample gel, the two spots are automatically matched (boxed spots). **(D)** Details of the matching areas are highlighted in C. The panel shows the extent of matching accuracy between TTR spots (circled in red) and the corresponding spots on the sample gels (circled in blue). The "comet-like" spots of the NMB 1972 gene product synthesized in vitro match spot numbers 56–61 of the sample gel, identified as NMB 1972 protein by mass spectrometry, within a vector box size of 12. Similarly, spots 206 and 209, identified as two different NMB 0382 isoforms by mass spectrometry, match the two autoradiographic spots obtained by TTR of the NMB 0382 gene, within a vector box size of 13. ST, standard.


ST
A
B
C
56 57 58 59 60 61
Vector box size 12
NMB 1972
209
206
Vector box size 13
NMB 0382

ponents of the NMB 1972 and NMB 0382 gene products were resolved on the autoradiograph in "comet-like" spots and two spots, respectively, corresponding to spots having different pI but identical molecular weight. Only minor intermediates, most likely represented by prematurely interrupted TTR products, were visible on the autoradiograph.

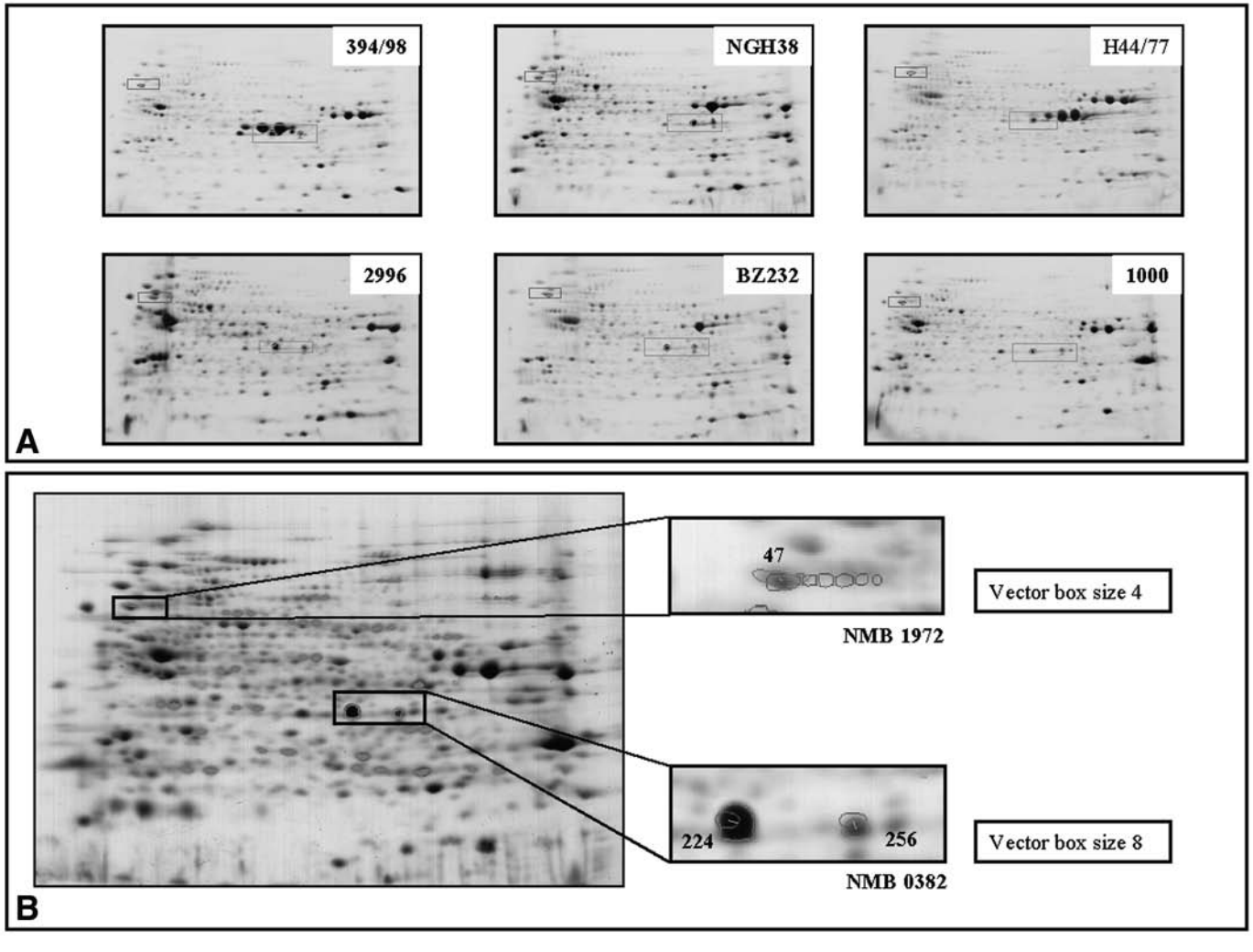
To determine whether the proteins were present in the MenB MC58 membrane preparation, the autoradiograph was superimposed *in silico* on the sample gel using the Image Master 2D Elite 3.1 program. Using the reference proteins, the software first creates a combined gel in which the TTR gel is superimposed on the sample gel and then scans the combined gel for TTR spots located within a fixed area (vector box) from any spot of the sample gel. If a TTR spot is within the selected vector box range from one protein of the sample gel, the two spots are automatically matched. As shown in **Fig. 2C**, the two NMB 0382 TTR spots matched two spots on the sample gel when a vector box size of 13 was selected. Similarly, the NMB 1972 "comet-like" TTR spots matched the corresponding protein spots 56–61 using a vector box size of 12. The details of the matching areas are given in **Fig. 2C**, showing the extent of accuracy of the matches between the TTR spots (circled in red) and the spots of the sample gel (circled in blue).

Having demonstrated that both OMP4 and Hsp60 are present in the membrane protein preparation of the MC58 strain, we then asked whether the two proteins were also conserved in six different MenB isolates. To this aim, their membrane proteins were resolved on 2-D gels **(Fig. 3A)**, and the gels were matched with the TTR autoradiograph. The matching analysis revealed that the two proteins were highly conserved in all six strains. **Figure 3B** gives a detailed analysis of the matching experiment with the MenB 1000 strain 2-D map. In this strain, the matching spots were subjected to mass spectrometry analysis ***(8)***, which confirmed the identity of the spots (data not shown).

4. Discussion

The growing importance of proteomics analysis in the understanding of biological processes is pushing research toward the identification of new, more efficient methods for protein identification. Although it is well recognized that new concepts in protein separation must be applied for complete proteome characterization ***(21)***, the methods based on 2-DGE will remain the first choice until alternative, innovative approaches have reached the mature state of general applicability. Once a pool of proteins have been separated on a 2-D gel, each protein spot must be processed and eventually identified. Although spot processing can be carried out either *in situ*, after spot picking, or on a membrane, after electroblotting ***(22)***, the method for spot identification is mass spectrometry. However, as already pointed out, the approach is hampered by problems such as high costs, complexity of the analysis and relatively low sensitivity. Here, we propose an alternative approach, which may offer some advantages over the current methods, at least for some specific applications. The method is based on 2-D gel separation

Fig. 3. *(see facing page)* Matching analysis of TTR 2-D gel spots with 2-D maps of six different MenB isolates. **(A)** Membrane preparations of six MenB strains ***(20)*** were separated on 2-D gels, and the gels were matched with the TTR autoradiograph. Red boxes indicate the spots matching the OMP4 TTR product, whereas blue boxes highlight the Hsp60 matches. **(B)** Detailed matching analysis of the membrane preparation of MenB 1000 strains. For details, *see* **Fig. 2**.


394/98
NGH38
H44/77
2996
BZ232
1000
A
47
NMB 1972
Vector box size 4
224
256
NMB 0382
Vector box size 8
B

of radiolabeled synthetic proteins derived from TTRs of PCR-amplified genes. The gel is autoradiographed and the autoradiograph is superimposed on the sample gel whose protein spots have to be identified. Matching between spots from the autoradiograph and the sample gel immediately allows protein identification. The method is sufficiently accurate and sensitive and, when compared with other procedures, relatively simple, rapid, and inexpensive.

As far as accuracy is concerned, we have previously shown that the method is highly accurate in terms of its capacity to establish with a high degree of confidence whether a given protein is present in the protein mixture under investigation. In fact, mass spectrometry analysis of the sample gel spots matching the TTR spots revealed that all matching spots corresponded to the expected proteins ***(8)***. The most serious problem, which weakens the degree of accuracy of this procedure, is the resolution power of 2-D electrophoresis. Proteins having similar molecular weight and isoelectric point are often hardly resolved on 2-D gels, and protein comigration may lead to misinterpretations during the comparative analysis of TTR gels with sample gels. Although in our experience comigration events are relatively rare (in the course of a MALDI-TOF analysis of bacterial protein 2-D maps obtained on mini-gels, we found that only 3 of 254 spots were constituted by more than one protein species) and should not affect the interpretation of proteins resolved in more than one isoform (the probability that two isoforms of the same protein comigrate with two protein species is expected to be very low), the possibility that TTR products superimpose on unrelated proteins cannot be ruled out.

With regard to the sensitivity of the proposed procedure, in general, the lowest sensitivity limit of all proteomic approaches based on 2-D gel separation is the sensitivity limit of the staining system. In other words, for a protein to be characterized, it must be visible on the gel. However, although mass spectrometry-based methods often fail to assign a name unambiguously to a poorly visible spot, in the case of our procedure the sensitivity of the staining system is truly the sensitivity limit of the procedure. The more sensitive the staining system used for protein detection, the more sensitive the procedure proposed here is.

Finally, one of the advantages of the procedure proposed here is that it can be utilized by any molecular biologist with relatively simple instrumentation. Gene amplification and TTRs can be completed in 1 wk, and, if a simple robotic station is available, up to 100 reactions can be carried out simultaneously. If appropriate TTR pools are run on the same gel, 50–100 TTRs can be analyzed in 2 wk by a single scientist. Finally, gel autoradiography usually takes few hours, and the available software packages for image acquisition and analysis allow for processing of several gels per day ***(7)***.

The most critical aspect of the procedure is to guarantee a consistently accurate superimposition of the sample gel on the TTR gels. This can only be achieved if good sets of standard proteins and advanced software for image analysis are available. Standard proteins are indispensable, representing the hallmark of the software used for gel normalization. Because gel distortions and aberrant migrations of proteins have a local and unpredictable behavior, it is fundamental for the subsequent analysis to use several standard proteins, which are evenly distributed in the gel.

In addition to standard proteins, the availability of proper software packages for gel analysis is also critical. Although software distributors are working on more and more sophisticated packages, we found some of the products already available on the market

to be more than adequate. In this study, we have utilized a software from Pharmacia Biotech, but a preliminary analysis with Melanine ***(22)*** has provided comparable satisfactory results.

We consider this procedure particularly amenable for bacterial proteomics analysis in consideration of the fact that post-translational modifications, which may alter protein pI, are relatively limited. Obviously, substantial protein modifications such as endoprotease digestion cannot be detected with this procedure, whose main deliverable property is to establish whether a protein with the molecular weight and pI of the TTR gene product is present in the protein pool under investigation.

Although this procedure can be utilized for the elucidation of whole bacterial proteomes, we believe that the method is particularly useful for the analysis of subsets of specific proteins. In fact, whereas with 2-D gel/mass spectrometry the presence or absence of specific proteins can be established only after scanning a large portion of the gel, with this procedure their identification is much simpler since only their coding genes need to be amplified and used for the analysis. In the particular example presented here, we were interested in knowing whether two specific proteins were present and conserved in the MenB membrane protein subproteome of different clinical isolates. The superimposition of the autoradiograph of TTR products on the 2-D maps of the membrane fractions of the different isolates allowed us to demonstrate rapidly the conservation of the two antigens in all the strains analyzed. This kind of analysis will be invaluable to the identification of new vaccine candidates against MenB.

Acknowledgments

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References

1. Lockhart, D. J. and Winzeler, E. A. (2000) Genomics, gene expression and DNA arrays. *Nature* **405,** 827–836.
2. Futcher, B., Latter, G. I., Monardo, P., McLaughlin, C. S., and Garrels, J. I. (1999) A sampling of the yeast proteome. *Mol. Cell. Biol.* **19,** 7357–7368.
3. Gygi, S. P., Rochon, Y., Franza, B. R., and Aebersold, R. (1999) Correlation between protein and mRNA abundance in yeast. *Mol. Cell. Biol.* **19,** 1720–1730.
4. Varshavsky, A. (1996) The N-end rule: functions, mysteries, uses. *Proc. Natl. Acad. Sci. USA* **93,** 12142–12149.
5. Washburn, M. P. and Yates, J. R. III. (2000) Analysis of the microbial proteome. *Curr. Opin. Microbiol.* **3,** 292–297.
6. Pandey, A. and Mann, M. (2000) Proteomics to study genes and genomes. *Nature* **405,** 837–846.
7. Lopez, M. F. (2000) Better approaches to finding the needle in a haystack: optimizing proteome analysis through automation. *Electrophoresis* **21,** 1082–1093.
8. Norais, N., Nogarotto, R., Lacobini, E. T., Garaguso, I., Grifantini, R., Galli, G., and Grandi, G. (2001) Combined automated PCR cloning, in vitro transcription/translation and two-dimensional electrophoresis for bacterial proteome analysis. *Proteomics* **1,** 1378–1389.
9. Chevallet, M., Santoni, V., Poinas, A., et al. (1998) New zwitterionic detergents improve the analysis of membrane proteins by two-dimensional electrophoresis. *Electrophoresis* **19,** 1901–1909.

10. Montigiani, S., Falugi, F., Scarselli, M., et al. (2002) Genomic approach for analysis of surface proteins in *Chlamydia pneumoniae*. *Infect. Immun.* **70,** 368–379.
11. Gygi, S. P., Corthals, G. L., Zhang, Y., Rochon, Y., and Aebersold, R. (2000) Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. *Proc. Natl. Acad. Sci. USA* **97,** 9390–9395.
12. Grandi, G. (2001) Antibacterial vaccine design using genomics and proteomics. *Trends Biotechnol.* **19,** 181–188.
13. Tabor, S. and Richardson, C. C. (1985) A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc. Natl. Acad. Sci. USA* **82,** 1074–1078.
14. Pratt, J. M. (1984) Coupled transcription-translation in prokaryotic cell-free systems, in *Transcription and Translation: A Practical Approach* (Hames, B. D. and Higgins, S. J., eds.), IRL Press, New York, pp. 179–209.
15. Molloy M. P., Herbert B. R., Slade M. B., Rabilloud T., Nouwens A. S., Williams K. L., and Gooley A. A. (2000) Proteomic analysis of the *Escherichia coli* outer membrane. *Eur. J. Biochem.* **267,** 2871–288.
16. Herbert, B. R., Molloy, M. P., Gooley, A. A., Walsh, B. J., Bryson, W. G., and Williams, K. L. (1998) Improved protein solubility in two-dimensional electrophoresis using tributyl phosphine as reducing agent. *Electrophoresis* **19,** 845–851.
17. Doherty, N. S., Littman, B. H., Reilly, K., Swindell, A. C., Buss, J. M., and Anderson, N. L. (1998) Analysis of changes in acute-phase plasma proteins in an acute inflammatory response and in rheumatoid arthritis using two-dimensional gel electrophoresis. *Electrophoresis* **19,** 355–363.
18. Wilm, M., Shevchenko, A., Houthaeve, T., Breit, S., Schweigerer, L., Fotsis, T., and Mann, M. (1996) Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. *Nature* **379,** 466–469.
19. Tettelin, H., Saunders, N. J., Heidelberg, J., et al. (2000) Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. *Science* **287,** 1809–1815.
20. Pizza, M., Scarlato, V., Masignani, V., et al. (2000) Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science* **287,** 1816–1820.
21. Binz, P. A., Muller, M., Walther, D., et al. (1999) A molecular scanner to automate proteomic research and to display proteome images. *Anal. Chem.* **71,** 4981–4988.
22. Hoogland, C., Sanchez, J. C., Walther, D., et al. (1999) Two-dimensional electrophoresis resources available from ExPASy. *Electrophoresis* **20,** 3568–3571.