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Isolation and Characterization of Porins from *H. pylori*

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1. Introduction

The outer membranes of Gram-negative bacteria represent selective, permeability barriers to environmental molecules. This function is accomplished in two ways. First, outer membranes exclude many larger hydrophilic molecules, including enzymes and other proteins, and most hydrophobic ones, by virtue of their unique composition. It has been demonstrated in *Escherichia coli* that outer membranes are asymmetric bilayers containing a unique species of glycolipid, lipopolysaccharide (LPS), in its outer leaflet, and phospholipids in its inner leaflet. The highly negatively charged LPS is stabilized by divalent cations. The observations that *Helicobacter* outer membrane proteins are Triton X-100 insoluble in the absence of EDTA or NaCl *(1,2)*, that *Helicobacter* contains substantial amounts of LPS *(3)*, and that *Helicobacter* is resistant to highly hydrophobic antibiotics, such as trimethoprin and nalidixic acid *(4)*, lead one to believe that this organism is thematically similar.

Second, selective permeation of small hydrophilic molecules through the outer membrane occurs through the water-filled channels of proteins, termed porins. Such proteins have now been identified in a wide variety of bacteria, including *Helicobacter pylori*. The term, porin, has been somewhat misused to describe proteins with the biochemical features of known porins, including heat-modifiable behavior on sodium dodecyl sulfate-polyacrylamide gel electroporesis (SDS-PAGE). However, we use the term here to denote only those proteins with demonstrated ability to reconstitute channels in bilayer membranes.

Porins are perhaps the best-characterized outer membrane proteins. Their major function is in passage of small molecules through the outer membrane,

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although they fall into two main categories, the nonselective and so-called specific porins. These two classes of proteins differ by the demonstrated existence in the latter of a specific substrate binding site, which, at low concentrations, substantially accelerates passage of this substrate through the outer membrane. However, the distinction between these two classes has become blurred by some recent observations: (1) the *Rhodobacter* porin, previously termed nonspecific, was cocrystallized with an unidentified substrate in its channel *(5,6)*; (2) the sucrose-specific porin of *E. coli* could also function as a general porin *(7)*; and (3) the imipenem/basic amino acid porin OprD of *Pseudomonas aeruginosa* demonstrated homology to the nonspecific porin superfamily *(8)*.

Porins have been demonstrated to have a variety of roles in pathogenesis. These include a general *(9)* or specific *(10)* role in the passage of antibiotics into cells and a corresponding role in antibiotic resistance, as demonstrated when porins are deleted by mutation. They also possess immunomodulatory properties, including the ability to mitogenically activate B-cells, modulate host cell functions, and induce cytokine release *(11–13)*. In addition, porins have been demonstrated to have potential as vaccine components. Thus the study of porins in *Helicobacter* is a valid pursuit.

Our own studies were initiated in an attempt to clarify the known difficulties in treating *Helicobacter* infections in vivo, despite its apparent high degree of in vivo susceptibility. This discrepancy has been variously explained for subsets of antibiotic classes as being caused by reduction of antibiotic activity at low pH *(14)*, influence of slow growth in vivo on antibiotic susceptibility *(15)*, low bioavailability of certain antibiotics in the upper gastrointestinal tract *(16)*, development of antibiotic resistance *(17,18)*, recrudescence of infection *(19)*, or poor patient compliance because of the requirement for long periods of treatment *(20,21)*. However, few basic studies have been performed on the mechanisms of antibiotic uptake and action in *Helicobacter*; without such studies, the fundamental underlying reasons for reduction in antibiotic effectiveness in vivo cannot be properly investigated. The following methods describe procedures for porin isolation.

Our laboratory has had years of experience in porin isolation and characterization from numerous species. Thus, it is of some interest that, in our hands, methods that permitted the successful purification of crystallizable porin using starting materials that contained at least three other contaminating porins *(22)*, were unsuccessful for *H. pylori*. The described method is generally applicable for almost any porin and for most outer membrane proteins. It relies on the fact that porins form highly stable β-barrel structures, containing, in the known structures, 16 or 18 transmembrane β-strands *(23–25)*. Such structures will not easily renature once disassembled. However, they are highly resistant to attack by even the most powerful detergents (i.e., sodium dodecyl sulfate [SDS]),

Table 1
Porins of *Helicobacter pylori*

	Molecular mass of monomer, kDa[a]	Conductance, ns[b]
HopA	48	0.36
HopB	49	0.36
HopC	50	0.30
HopD	67	0.25
HopE	31	1.5

[a]Molecular mass refers to the apparent molecular mass observed when heat-denatured samples are run on SDS-PAGE.
[b]Average single channel conductance of the monomeric protein in 1.0*M* KCl.

since detergents cannot penetrate the ordered structure of porins. This gives rise to the property of heat-modifiability, by which porins migrate in a folded configuration on SDS-PAGE after solubilization at low-to-moderate temperatures in SDS, binding SDS only on the periphery of the porin molecule. If the nonheated porin runs as a native trimer (the usual oligomeric state of porins), it will appear at a higher apparent molecular mass or lower mobility than the monomeric, fully dissociated porin that has been preboiled in SDS. For some porins the primers can be disassociated into monomers after treatment in SDS at room temperature without affecting the β-barrel structure of the monomers. Alternatively, they might actually exist as monomers in the outer membrane. Such monomer porins run at a lower apparent molecular weight than when fully dissociated because of their more compact structure, and these proteins require more extensive heating to unfold them (i.e., 95°C for 20 min) *(2)*. The above properties permit one to utilize detergents and SDS-PAGE as purification procedures. The following procedures have permitted purification of milligram levels of five porins (HopA, HopB, HopC, HopD *[1]*, and HopE *[26]*) from *H. pylori* (Table 1), and nearly half a gram of OprP porin from *Pseudomonas aeruginosa*.

2. Materials

2.1. Porin Isolation Procedures

2.1.1. Isolation of Membrane Fractions

1. Brain-heart infusion (BHI) agar (Accumedia, Baltimore, MD).
2. 1% hemoglobin powder (Accumedia).
3. 20% (w/v) sucrose, 10 m*M* Tris-HCl, pH 8.0, containing 50 pg/mL of deoxyribonuclease 1 (Sigma, St. Louis, MO).
4. 70% (w/v) sucrose, 10 m*M* Tris-HCl, pH 8.0.

5. 18% (w/v) sucrose, 10 m*M* Tris-HCl, pH 8.0.
6. Reagent A: 2% Na_2CO_3, 0.02% NaK Tartrate, 0.1*M* NaOH, 1% SDS (added last).
7. Reagent B: 0.5% $CuSO_4$.
8. Reagent C: Mix 25 mL of reagent A with 1 mL of reagent B. Make fresh daily.
9. Folin Ciocalteu Reagent (BDH, Toronto, Ontario).

2.1.2. Identification of Heat Modifiable Proteins

1. 11% running gel (10 mL): 4.2 mL H_2O, 2.75 mL 40% (w/v) acrylamide (29:1 acrylamide/bis) (Bio-Rad, Hercules, California), 2.5 mL 1.5*M* Tris-HCl, pH 8.8, 0.18 mL 10% (w/v) SDS, 0.16 mL 5*M* NaCl, 0.07 mL 200 m*M* EDTA, 0.1 mL 10% ammonium persulfate, 0.04 mL TEMED.
2. 4% Stacking gel: (10 mL) 5.36 mL H_2O, 1.0 mL 40% acrylamide, 2.5 mL 0.5*M* Tris-HCl, pH 6.8, 1.0 mL 10% SDS, 0.1 mL 10% ammonium persulfate, 0.04 mL TEMED.
3. 10X SDS-PAGE running buffer: 30 g Tris-HCl, 10 g SDS, 144 g glycine. Make up to 1000 mL with distilled water.
4. 2X Solubilization mix: 2% SDS, 12 m*M* Tris-HCl, pH 8.0, 10% glycerol, 50 m*M* EDTA/100 mg bromophenol blue.
5. 0.375*M* Tris-HCl, pH 8.6.
6. Coomassie blue stain: 90 mL methanol, 20 mL acetic acid, 90 mL water, 200 mg Coomassie brilliant blue R250.
7. Destain solution: 290 mL distilled water, 80 mL methanol, 30 mL acetic acid.

2.1.3. Selective Solubilization and Column Chromatography of Porins

1. 10 m*M* Tris-HCl, pH 8.0, 1.0% Triton X-100 (Sigma, St. Louis, MO), 2 m*M* $MgCl_2$.
2. 10 m*M* Tris-HCl, pH 8.0, 0.5% sodium lauryl sarcosinate (Sigma).
3. 10 m*M* Tris-HCl, pH 8.0, 3% octylpolyoxyethylene (OPOE) (Bachem Bioscience, Philadelphia, PA).
4. 10 m*M* Tris-HCl, pH 8.0, 0.08% *N,N*-dimethyldodecylamine-*N*-oxide (LDAO) (Fluka Chemika, Ronkonkoma, NY).
5. 1.0*M* NaCl.

2.1.4. Gel Purification

1. SDS-PAGE reagents (*see* Section 2.1.2.).
2. Coomassie blue stain.
3. 10 m*M* Tris-HCl, 0.08% *N,N*-dimethyldodecylamine-*N*-oxide, 1 m*M* EDTA, 150 m*M* NaCl.

3. Methods

3.1. Porin Identification and Isolation Procedures

3.1.1. Isolation of Membrane Fractions

1. Grow *H. pylori* for 3 d at 37°C in an atmosphere of 10% CO_2 on BHI agar that is supplemented with 1% hemoglobin after autoclaving. In our hands, this supported growth as well as, or better than, traditional growth procedures including microaerobic growth with a CampyPak system (Becton Dickinson, Cockeysville, MD) or growth on BHI agar with 5% sheep blood, or on chocolate agar (*see* Note 1). To gain large quantities of starting materials, two hundred 100 × 15 mm plates are each streaked out from plates grown for 2 d under the above conditions (*see* Note 1).
2. Harvest cells by scraping off the plates with a cotton swab, and resuspend in 20% (w/v) sucrose, 10 m*M* Tris-HCl, pH 8.0, containing 50 µg/mL of deoxyribonuclease I. The volume of sucrose should be approx 20 mL, and this should be adjusted so that the final cell resuspension is thick and viscous.
3. Disrupt the pooled cell suspension using a French pressure cell by two passages at 15,000 psi (*see* Note 1).
4. Remove unbroken cells by centrifugation at 1000*g* for 10 min.
5. Layer the supernatant in 10-mL quantities onto a sucrose gradient comprising (from bottom) 10 mL of 70% (w/v) sucrose and 20 mL of 18% (w/v) sucrose in a 40-mL centrifugation tube, and centrifuge at 65,000*g* for 4 h, or overnight.
6. Collect the membrane fraction, which appears as a whitish opaque band at the interface of the 18 and 70% gradients, by piercing the bottom of the tube with a needle and collecting the opaque fractions.
7. Add water to dilute out the sucrose, approx 4- to 5-fold.
8. Centrifuge the diluted fractions at 250,000*g* for 1 h at 4°C in a Beckman 60Ti rotor or equivalent.
9. Resuspend the pellet after centrifugation at about 20 mg protein/mL in 10 m*M* Tris-HCl, pH 8.0 (*see* Note 3).
10. Protein yields are determined by a modified Lowry assay that contains detergent *(27)*, and, in our hands, gives more accurate assays of membrane protein concentrations.
11. Starting with the cells harvested from 200 plates, isolate 850 mg of protein.

3.1.1.1. Protein Assay

1. Add 5 µL and 10 µL of a 1–10 dilution of each protein sample to a 13-mm borosilicate glass tube, and prepare a standard curve using additions of 0, 5, 10, 15, 20, and 25 µL of 0.1% BSA (in water).
2. Add 1 mL of reagent C, and let tubes stand at room temperature for 15 min.
3. Add 100 µL Folin Ciocaloteu reagent, vortex immediately, and let stand at room temperature for 30 min.
4. Read absorbance at 650 nm against a reagent blank.

3.1.2. Identification of Heat Modifiable Proteins

As discussed in the introduction, porins generally comprise heat modifiable proteins. To examine heat modifiable proteins in *H. pylori*, two-dimensional (unheated vs heated) SDS-PAGE is utilized. It was necessary to do this, since, generally speaking, there are no freely available assays to follow porin purification. Thus, SDS-PAGE mobility and heat modifiable behavior are general criteria that can be monitored during purification.

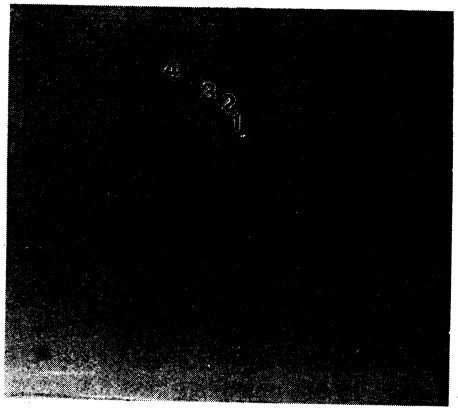

Fig. 1. Two dimensional SDS-polyacrylamide gel identifying heat modifiable proteins. Spots appearing left of the diagonal represent dissociated aggregates; spots appearing to the right of the diagonal are proteins that have become denatured only after heating. Spots 1, 2, 3, and 4 represent HopA, B, C, and D, respectively. Molecular masses (kDa) are indicated on the right. Reproduced with permission from ref. *1*.

1. Solubilize samples containing 30 µg of membrane proteins at room temperature (23°C) in SDS-containing solubilization-reduction mix and load into 6-mm wells on a 10 × 150 × 1.25 mm 4% SDS-polyacrylamide stacking gel with a 100 × 150 × 1.25 mm 11% running gel (*see* Note 2). The gel is run at 100 V for 4–5 h, using a conventional discontinuous buffer system *(28)*.
2. Excise a vertical gel strip comprising a single lane, equilibrate in 0.375*M* Tris-HCl, pH 8.6, for 1 min, and then wrap in cellophane and heat to 95°C for 20 min in the same buffer (*see* Note 3).
3. Remove the strips from the cellophane and overlay on a second SDS-PAGE gel that has been poured so that it contains only an 11% separating (running) gel and not a stacking gel (*see* Note 3); initiate electrophoresis by applying a constant voltage of 100 V.
4. Place gels in a clean tray and stain with Coomassie blue stain for 10 min. Following staining, place the gel in destain solution and agitate. Frequent changes of the destaining solution allows for more rapid visualization of the bands.
5. Proteins that do not demonstrate heat-modifiable behavior run identically in both dimensions and appear on a diagonal running from the top left-hand to bottom right-hand corner. Off-diagonal spots to the left of the diagonal are oligomer-forming proteins. Off-diagonal spots to the right of the diagonal are proteins that retain at least part of their folded structure in the presence of SDS at low temperature. A typical result for *H. pylori* is shown in Fig. 1.

3.1.3. Selective Solubilization and Column Chromatography of Porins

1. Suspend samples containing 100 mg of membrane proteins from Section 3.1.1. to a final volume of 10 mL in 10 m*M* Tris-HCl, pH 8.0, containing 1% (v/v) Triton X-100 and 2 m*M* $MgCl_2$ at room temperature. Use an 18–22-gage needle and syringe to make resuspension easier.
2. To ensure complete suspension, sonicate the sample briefly (15 s) with a narrow probe sonicator (Fisher dismembranator [Pittsburgh, PA] or equivalent at a power giving use to cavitation-formation of small bubble in the suspension).
3. Centrifuge the suspension at 180,000*g* for 1 h on a Beckman 60Ti rotor (Fullerton, CA) or equivalent.
4. Remove the supernatant and resuspend the pellet, as above, in 10 mL of 10 m*M* HCl, pH 8.0, 0.5% (w/v) sodium lauryl sarcosinate.
5. Centrifuge samples as per step 3. At this stage, the pellet contains largely outer membrane proteins.
6. Resuspend the pellet as above in 5 mL of 10 m*M* Tris-HCl, pH 8.0, 3% octylpolyoxyethylene (OPOE), and centrifuge at 180,000*g* for 1 h.
7. Save the supernatant; repeat step 6 and compare the supernatant from this step with the previous OPOE-soluble protein preparation.
8. Separate the OPOE-soluble samples by fast protein liquid chromatography (FPLC) on a Mono Q HR 5/5 anion exchange column (Pharmacia, Toronto, Ontario), with elution using a salt gradient of 0–1*M* NaCl in a column buffer of 10 m*M* Tris-HCl, pH 8.0, 0.08% *N, N* dimethyldodecylamine-*N*-oxide (LDAO) (*see* Note 4). Run portions of all fractions collected on SDS-PAGE and collect fractions containing the heat-modifiable proteins of interest and pool as appropriate.

3.1.4. Gel Purification

1. Solubilize FPLC fractions in solubilization mix at room temperature for 10 min.
2. Load a solubilized sample containing up to 500 µg of protein into a single preparative well (*see* Note 4) and run on an 11% SDS-PAGE gel.
3. Excise thin, vertical gel slices from the left and right sides of the gel and stain with Coomassie blue. Use these stained strips as a guide to precisely excise, by use of a razor blade, bands corresponding to the proteins of interest from the unstained gel (*see* Note 5).
4. Crush gel slices are crushed and soak individually over night at 4°C in 0.5 mL of a buffer consisting of 10 m*M* Tris HCl, pH 8.0, 1 m*M* ethylene diamine tetraacetate (EDTA), 150 m*M* NaCl, 0.08% LDAO (*see* Note 4). This can be done using a standard test tube on a tube roller, but agitation is not absolutely necessary. Aspirate the liquid with a pipet and filter to remove any remaining acrylamide. The extraction process can be repeated if the initial extraction gives a low yield of protein. Store the eluted proteins are stored by freezing at –70°C.

3.2. Assay of Pore-Forming Activity

A full description of the assay procedures utilized to assay porins is not within the scope of this chapter, since such assays are highly technical and, for the system with the greatest utility (planar lipid bilayers), specialized apparatus is required. Instead, a general description is presented here.

3.2.1. Planar Lipid Bilayer Methods

This method has two major advantages and two potential disadvantages, compared to the liposome methods described in Section 3.2.2. One advantage is that virtually any porin can be assayed, regardless of its channel size. In addition, the method has single molecule sensitivity and requires extremely modest amounts of porin (ng) to yield a substantive amount of information about the channel formed by the porin. One potential disadvantage is that only about 1 in 10,000 molecules is actually assayed (i.e., enters the planar bilayer and forms channels). The porin must be extremely pure so that one has reasonable certainty that it is the purified protein species, rather than a contaminant, that is forming transbilayer channels. In practice, if a protein is 99% or more pure and forms channels at a concentration of 500 pg/mL, experience has taught us that it is this protein that almost certainly is forming the channels. A second concern is created by the fact that ion movement (i.e., current) is used to assess channel formation. It can be difficult to determine the exact size of biological molecules capable of passing through the channel (i.e., the exclusion limit of the porin), except by extrapolation to other channels, where both kinds of studies have been performed. The planar lipid bilayer method requires specialized apparatus (outlined below) and some training in its use. However, it is a very productive, rapid procedure for determining channel properties.

The apparatus is diagrammed in Fig. 2. The central part comprises a chamber that is machined from a 5 × 2.5 × 3 cm block to create two equal compartments separated by a 1-mm Teflon divider. One of these compartments contains a viewing window, and the Teflon divider is perforated by a 0.1–2 mm^2 hole.

The hole is anointed at its edges with a lipid solution (typically 1.5% oxidized cholesterol or 1.5% diphytanoyl phosphatidyl choline in n-decane), and dried under a jet of hot air to provide a surface to which a membrane can adhere. Each compartment is then filled with 6 mL of a salt solution (e.g., 1*M* KCl). Subsequently, the hole is wiped with a Teflon rod onto which 5 µL of one of the above lipids has been pipeted. The lipid will cover the hole (assessed by measuring a high resistance when a voltage is passed across two electrodes dipping into the two compartments). Within a short time the lipid thins out until it forms a bilayer. This can be observed using a short focal length telescope in incident light coming from a suitable focused light source, such as a microscope light, since the observed lipid changes from multicolored to black, because of the optical properties of lipid globules and lipid bilayers, respectively. This gives the methodology its alternate name, black lipid bilayers.

To the electrode dipping into the solution in one compartment, a direct current voltage source is attached. To the electrode in the other, a current amplifier (Keithly 427, Cleveland, OH), oscilloscope to monitor the amplified signal (Tektronix 511 A, Beaverton, OR), and rapid response chart recorder (Houston

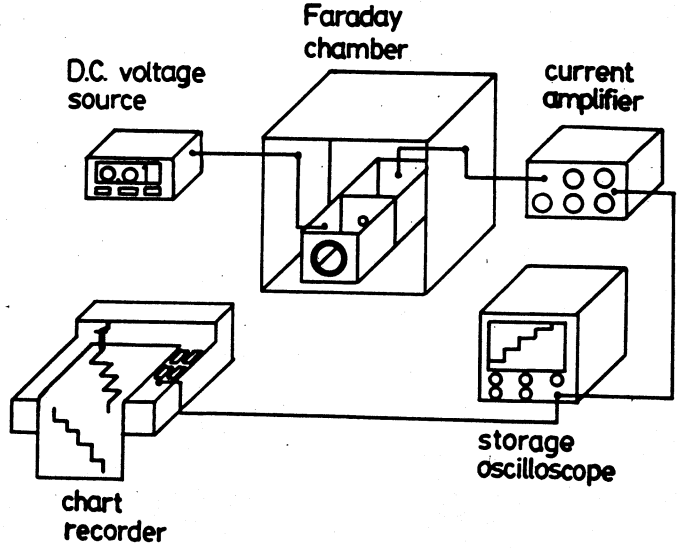

Fig. 2. Schematic representation of planar lipid bilayer model membrane system. Electrodes connected to the voltage source and current amplifier are placed in an aqueous salt solution within the Teflon chamber, and a lipid bilayer is painted across the hole separating the chamber compartments. Conductance increases caused by the insertion of porins into the bilayer are monitored on the oscilloscope and recorded on the chart recorder. Reproduced with permission from ref. *37*.

Instruments 4512, Austin, TX) are attached. With the naked membrane, application of a voltage (typically 10–50 mV) results in a very small current (approx 2 pA), since bilayers have little permeability to ions. If a porin in detergent solution is added to the compartment on one side of the membrane, it will become diluted to a very low detergent concentration, and because of its avidity for membranous (lipid) environments, it will spontaneously incorporate into the membrane, forming a conduit for the movement of ions through the channel. This can be observed, after signal amplification, as a stepwise increase in conductance in both real time on the chart recorder and at a faster time resolution on the oscilloscope.

Some of the channel properties that can be easily checked by varying experimental parameters are whether the channel is water-filled, whether there is a strong selectivity for cations over anions (or vice versa), the influence of lipid composition (usually none), whether the channel aggregates in the membrane, the effect of voltage on channel properties, whether the channel permits only unidirectional flux of ions, and the variability in the sizes of individual channels. Refer to the literature for exactly how these properties are studied

(29–31). The major readout, however, is average single channel conductance of the channel in given salts, which is itself proportional to the volume of the channel and its geometry. The single channel conductance can range from 10,000–11,000 pS for individual porin channels (*see* Table 1 for *Helicobacter* porin properties).

With a slightly different setup in which only a voltage source and a multimeter (Keithly 610, Cleveland, OH) are connected to the electrodes, one can measure macroscopic conductance, and determine such properties as voltage dependence, selectivity for one ion over another, and whether the channel contains a specific binding site for a given substrate (*see* ref. *30* for procedures).

3.2.2. Liposome Procedures

One can also utilize liposomal procedures to measure channel properties. These are useful for large channels like the OmpF porin of *E. coli*, but are technically challenging with many possible experimental pitfalls, as evidenced by disparities between two groups studying *Pseudomonas* OprF porin by one of these procedures, liposome swelling *(32,33)*. In addition, they are exceptionally difficult to perform with charged substrates, require high concentrations of soluble substrates, and cannot be utilized for small pores or specific porins, unless the substrate is known. Nevertheless, in the hands of skilled practitioners, such methods do yield pore exclusion limits equivalent to those measured in vivo. It must be emphasized that both these and the above procedures are models and must eventually be confirmed by in vivo assessments. For details of how these procedures are performed, *see* refs. *31*, *34*, and *35*.

4. Notes

1. Other growth media work quite adequately, and it appears that the expression of porin proteins is not dramatically changed by using different media *(2)*. In addition, our preliminary data suggest that the nongrowing coccoid forms yield porin preparations that are virtually indistinguishable from those obtained from the growing spiral forms. One important factor is that the plates must not be dry or the cells will not grow. It is useful to store agar plates at 4°C shortly after pouring them so that condensation will form, keeping the plates moist. Media supplemented with hemoglobin can be stored for 3 mo or longer at 4°C without showing decreased cell growth, although other media, such as that supplemented with β-cyclodextrin, do not appear to support cell growth if the media are a month or more old.

   When growing large amounts of cells, it may be preferable to use larger culture plates (150 × 15 mm or larger). To achieve sufficient growth on a plate, it is necessary to streak out a large number of cells; streaking out single colonies will not produce significant growth. The cells from a single plate are usually only sufficient to streak out 10–15 additional plates.

2. When disrupting cells for membrane preparations, a sonicator, or glass bead agitator, can be utilized instead of a French pressure cell although the efficiency of cell breakage is decreased. The efficiency may be increased by freezing the cells at –20°C prior to disruption. Separate batches of cells can be frozen and then pooled together prior to disruption.
3. After cell membranes have been collected, they may be directly resuspended in a detergent solution if a solubilization procedure using the given detergent is to follow. Larger gels (100 × 150 × 1.25 mm) tend to give better results, but minigels (80 × 60 × 0.75 mm) may also be used. Larger gels facilitate the handling of gel strips, and it is easier to place the larger gel strip on the second separating gel without damaging it. When heating a gel strip, it is wrapped in cellophane, but precautions to make it completely water tight are not necessary, because small leaks will not cause a significant loss of protein from the gel. Heating must be carried out at 95°C for 20 min; trimeric porins may dissociate after heating to 65°C for 10 min, but heat modifiable monomers require further heating to ensure that they fully unfold. It should be noted that at least one heat modifiable porin, OprF of *Pseudomonas aeruginosa*, is quite stable to boiling in the presence of SDS, and extensive heating is required to unfold this protein *(36)*. Placement of the gel strip on a secondary separating gel can be achieved by sliding the gel strip between the glass plates, or by separating the glass plates, inserting the gel strip, and then repositioning the glass plate. The latter method has proven to be quite successful, since it minimizes damage to the gel strip, and it enables a tighter contact between the gel strip and the separating gel. A stacking gel may be used for the second dimensional gel run, but experience has shown that this produces smears and streaks, instead of compact spots.
4. In our hands, OPOE is the detergent of choice for porin purification, since it yields porin of crystallizable quality (Egli and Hancock, unpublished results). It is quite expensive, however, and we replace it with *N,N*-dimethyldodecylamine-*N*-oxide (LDAO) for column chromatography, since this latter detergent has similar properties but a lower critical micellar concentration, such that a fourfold lower concentration can be utilized in column buffers to maintain proteins in solution.

   An important factor in obtaining reproducible solubilization results is the concentration of protein in the sample. The starting concentration in detergent should be 10 mg/mL. Changing this concentration will vary the amount of protein extracted in each solubilization step. A concentration of 10 mg/mL of detergent will enable the extraction of HopE in the Triton X-100-soluble fraction, while HopA, B, C, and D will be extracted in the OPOE-soluble fraction. When fractions are resuspended during solubilization, a large amount of granular material will not resuspend, as the fractions contain insoluble material (possibly peptidoglycan fragments). However, this granular material must be meticulously agitated to release any soluble proteins bound to them. If this is not done, a considerable portion of the porin proteins may be found in the final pellet, but an extraction using 3% OPOE with 0.4*M* NaCl will usually solubilize the remaining porin.

After detergent solubilization, the samples can be further purified using FPLC, or they can be directly cut out of a preparative gel. Samples for FPLC are normally dialyzed in the starting buffer, which contains 0.08% LDAO. However, occasionally, this results in the entire sample fluting in the void volume. This can be solved by leaving the sample in 3% OPOE, as this ensures that the porins will remain soluble and will enhance binding to the column. Typically, the porins cannot be purified after one column run, but if partially purified fractions are pooled and lyophilized, separation can be achieved. This, however, may result in proteins that are not active in pore-forming assays.

5. As with the 2D gels, better results are usually achieved using larger gels, which permit better protein separation. When cutting unstained bands from a gel, it is often difficult to obtain a pure species, even when stained sections are present as a guide. It should be noted that after staining the guide slices in Coomassie blue, they may have to be placed in water to swell them to the original size, since staining may dehydrate and shrink the gel. To improve the chances of obtaining a pure band, a number of very thin slices can be excised from the region where the band of interest is, as this decreases the chances of including other proteins. Similarly, changing the acrylamide concentration can aid in separating proteins with similar molecular masses.

When eluting a protein from a gel slice, a greater yield is achieved if the gel is crushed into small fragments. The volume of liquid used is dependent on the size of the gel slice, but 0.5–1 mL is usually sufficient. Following elution, the proteins may be concentrated by ultrafiltration or by precipitation, and then they should be frozen at –70°C. Any pore-forming assays should be done as soon as possible after gel elution, as some gel purified proteins rapidly lose their pore-forming ability. Freezing at –70°C usually does not affect porin activity, and can ensure longer retention of activity.

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Methods for the Identification of *H. pylori* Host Receptors

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1. Introduction

Bacterial attachment to host receptors is a prerequisite for colonizatio epithelial cell surfaces, in particular, continuously renewing mucosal surf such as the gastrointestinal tract. Microbes express adhesion molecule interactions with eukaryotic cell surface proteins or glycoconjugates, suc glycoproteins and glycolipids *(1)*. The combination of high receptor spec ity *(2)* and restricted receptor distribution will target bacteria to specific sues, i.e., cell populations. This is referred to as tissue tropism and p determines the niche a bacterium is able to occupy. In addition, compet between bacterial species for space and nutrients selects for bacteria ab colonize specific niches. Bacteria unable to adhere to the epithelial cells mucus lining will be exposed to the local nonspecific host defense mechan (such as peristalsis and turnover of the epithelial cell populations and the m layer) and eventually removed. The biological relevance of adherence a initial step in the infectious process has focused interest to the struc involved in these processes. Bacterial adhesins and host receptors are potential targets for novel antimicrobial drug design *(3)*. Antimicrobial a could be chemically coupled to soluble high-affinity receptor analogs an pathogens, such as *H. pylori*, once they are targeted by the complex. So receptor analogs would competitively interfere with bacterial attachment lizing the same mechanism as naturally occurring scavenger molecul human secretions, such as milk and saliva. Receptor analogs could be d oped for high-affinity interactions and would thereby be efficient inhibit

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