Topology of the Hydrophobic Membrane-bound Components of the Histidine Periplasmic Permease

COMPARISON WITH OTHER MEMBERS OF THE FAMILY*

Raili E. Kerppola and Giovanna Ferro-Luzzi Ames‡

From the Department of Molecular and Cellular Biology, Division of Biochemistry and Molecular Biology, University of California, Berkeley, California 94720

(Received for publication, August 15, 1991)

The membrane-bound complex of periplasmic permeases comprises two hydrophobic proteins which have been hypothesized to be integral membrane-spanning proteins. We have investigated the topological organization of the hydrophobic components of the Salmonella typhimurium histidine permease, HisQ and HisM. Both proteins are digested by trypsin and proteinase K when either inside-out or right-side-out membrane vesicles are used. Therefore, these proteins are exposed to both surfaces of the membrane. Digestion with carboxypeptidase and binding studies with antibodies directed against the carboxyl terminus of HisQ and HisM have localized their carboxyl termini to the inside surface of the cytoplasmic membrane. Aminopeptidase digestion suggests periplasmic localization of their amino termini. Alkaline phosphatase fusions to HisQ and HisM indicate the existence of five spanners in both proteins. The periodicity and orientation of spanners and loops in HisQ and HisM match those of the five carboxyl-terminal spanners of MalF, the only other hydrophobic component of the periplasmic permeases for which topological information is available. An alignment of the sequences of all known hydrophobic components of periplasmic permeases is presented which indicates clear conservation of secondary structure and some conservation of primary sequence. The structural conservation of the components is discussed, and a role for a hydrophilic loop containing a conserved sequence (the EAA loop) is proposed.

Bacterial periplasmic transport systems (permeases) function on a large variety of substrates and require a receptor, the substrate-binding protein, that is located in the periplasm and is lost during osmotic shock resulting in loss of transport activity. These periplasmic permeases (also called “shock-sensitive”) generally consist of three to five protein components, one of which is the periplasmic substrate-binding protein, and the others are membrane-bound proteins (reviewed in Ames, 1986; Ames et al., 1990). In a typical permease with three membrane-bound components, two are hydrophobic and in some cases homologous to each other; they are usually depicted as spanning the membrane. It has been proposed that they form a pseudodimer (or a homodimer, in cases in which only one is present) (Ames, 1985). The third membrane-bound component has a hydrophilic sequence, despite being tightly membrane bound (Kerppola et al., 1991) and contains an ATP-binding site (Hobson et al., 1984; Higgins et al., 1985). The equivalent ATP-binding subunits from different periplasmic permeases share strong homology with each other (Ames et al., 1990; Mimura et al., 1991; Higgins et al., 1986) and are referred to as the “conserved components.” The membrane-bound proteins form a complex within the membrane with a subunit stoichiometry of two copies of the hydrophilic component to one each of the hydrophobic ones (Kerppola et al., 1991; Davidson and Nikaido, 1991). It has been proposed recently that the hydrophilic component spans the membrane complexed with the hydrophobic proteins (Kerppola et al., 1991). A model for the structure of conserved components has been predicted, and a structure-function analysis of the relationship between mutated residues and activity has been performed (Mimura et al., 1991; Shymala et al., 1991). In this model a structural feature accounting for the membrane-spanning property of conserved components is apparent. A somewhat different model which lacks this feature has been presented (Hyde et al., 1990). Periplasmic permeases are energized by ATP (Ames et al., 1989; Ames, 1996; Prossnitz et al., 1989; Joshi et al., 1989; Mimack et al., 1989; Dean et al., 1989), and the permeases for histidine (Bishop et al., 1989) and maltose (Davidson and Nikaido, 1990) have been reconstituted in proteoliposomes showing ATP hydrolysis concomitantly with transport (reviewed by Ames and Joshi, 1990).

The histidine permease of S. typhimurium is a well-characterized periplasmic permease composed of the periplasmic histidine-binding protein, HisJ, the homologous hydrophobic membrane proteins, HisQ and HisM, and the hydrophilic membrane protein, HisP. HisQ, HisM, and HisP form the membrane-bound complex (Kerppola et al., 1991). Genetic and biochemical studies of the histidine permease have shown that HisJ interacts with the membrane-bound complex (Ames and Spudich, 1976; Prossnitz et al., 1988). The deduced protein sequences (Higgins et al., 1982) show that HisQ and HisM are highly hydrophobic, with uncharged stretches that could function as membrane spanners. Hydrophaticity analysis identified three to five membrane spanners in each of HisQ and HisM (Ames, 1984, 1985). Here we define more precisely the topology of these proteins by identifying both the number and the orientation of the membrane spanners by using proteolysis by several enzymes, antibody binding, and alkaline phosphatase fusions as tools of analysis.

*This work was supported by National Institutes of Health Grant DK12121 (to G. F.-L. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be sent.

1 V. Baichwal, D. Liv, and G. F.-L. Ames, unpublished data.
EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Media—The following Escherichia coli K-12 strains were used: TA3662, ΔΔIapAΔM72 Sm<sup>−</sup> lacZamK (bio inducible); ΔΔIapA ΔΔIamK (ΔΔI) carrying pFA17 (Amp<sup>+</sup>, hisQMP genes under the control of the Ap<sup>+</sup> promoter) (Hobson et al., 1984); TA3964, hisG611 hisQPM Pack pta700 carrying plasmid pFA53 (Amp<sup>+</sup>, hisQMP genes under the control of the Ap<sup>+</sup> promoter) (Prossnitz et al., 1988); CC118 (Manoil and Beckwith, 1985); GA176, CC118 carrying pFA136 (Amp<sup>+</sup>, hisQMP genes expressed from the phoA promoter); DB48, and DB139 ΔΔIapλ/ΔΔIamK ΔΔIM74 ΔΔIphoA PoiI phoR ΔΔIphoA ΔΔIphoB gsd gsk thi rpsL/ΔlacI857; ΔΔIamK ΔΔIphoA ΔΔIphoB gsd gsk thi rpsL/ΔlacI857 (ΔΔI) (Boyd et al., 1987). The transport genes are from the S. typhimurium histidine transmem transport operon in all cases. TA3662 and TA3964 were inoculated from frozen stock cultures into LB medium with ampicillin (50 μg/ml) and grown with aeration at 30 °C. Cells were then transferred to minimal medium (Rotth, 1970) supplemented with 0.4% glucose, 0.3 mM histidine, 0.05 mM thiamine, 50 μg ampicillin (TA3964) or with 0.4% glucose, 0.1 mM tryptophan, 0.05 mM thiamine, 0.1 mM biotin, 50 μg/mL ampicillin (TA3662).

Preparation of Membranes—TA3662 and TA3964 cultures (1 liter) were grown in minimal medium at 30 °C to A<sub>oo</sub> = 0.5 and then transferred to a 42 °C shaking water bath for 30 min to induce expression of HisQ, HisM, and HisP. Right-side-out vesicles (ROV)<sup>2</sup> were prepared essentially as described by Kacaba (1971) with modifications by Prossnitz et al., (1988). Inside-out membrane vesicles (IOV) were made by lysing cells in a French Press cell at 10,000 p.s.i. in 50 mM KPi, pH 7.0, 20 mM MgSO<sub>4</sub>. After lysis the suspension was centrifuged at 4,000 × g for 10 min to remove unbroken cells. The membranes were pelleted by centrifugation at 33,000 × g for 1 h and stored in aliquots in liquid nitrogen in 100 mM KPi, pH 6.6, 10 mM MgSO<sub>4</sub>, at −20 °C of protein/ml.

Proteolysis with Trypsin and Proteinase K—Membrane vesicles (100 μg of membrane protein total) were resuspended in 30 mM Hepes-HCl, pH 8.0, 1 mM CaCl<sub>2</sub> at 20 mg/ml final protein concentration. Equal volumes of proteinase K (Boehringer Mannheim) or N-tosyl-L-phenylalanine chloromethyl ketone-treated trypsin (Worthington) in the same buffer were added and the samples incubated at room temperature for 3.5 h. Digestion was stopped by adding equal volumes of either 100 mM phenylmethylsulfonyl fluoride (Sigma) in isopropyl alcohol (freshly dissolved) to proteinase K digestion samples or 10 mg/ml soybean trypsin inhibitor (Sigma) to trypsin digestion samples. After a 15-min incubation on ice, the membranes were pelleted in a microcentrifuge for 30 min at 4 °C. The membrane pellets were resuspended in 1 ml of ice-cold 20% trichloroacetic acid. After incubating on ice for 15 min the samples were centrifuged in a microcentrifuge at 0–4 °C for 15 min. The supernatant was carefully aspirated and the pellet washed with 1 ml of ice-cold acetone followed by a 15 min centrifugation and similar careful aspiration. The pellets were dried under vacuum and resuspended in Laemmli sample buffer with the addition of some Tris base if required to neutralize the remaining acid. Samples were boiled for 5 min and analyzed by SDS-PAGE and immunoblotting. For the analysis of HisM, samples were neither trichloroacetic acid precipitated nor boiled, since these treatments caused HisM to aggregate; instead, samples were frozen after addition of inhibitor and quickly thawed and loaded onto SDS gels immediately after the addition of the sample buffer.

Proteolysis with Carboxypeptidase P and Aminopeptidase M—Carboxypeptidase P and aminopeptidase M (amino acid arylamidase) (Boehringer Mannheim) digestion conditions were designed according to the directions given by the manufacturer. ROV and IOV membrane vesicles were thawed, sedimented in a refrigerated Sigma microcentrifuge (top speed, 30 min), and washed once with digestion buffer. For carboxypeptidase digestion vesicles were resuspended at a final protein concentration of 10 mg/ml in 50 mM sodium acetate, pH 4.4, 1 μM CaCl<sub>2</sub>, and 5 units of enzyme in a 20-μl final volume; after incubation at 37 °C for 3.5 h, an equal volume of Laemmli sample buffer (2-fold concentrated) was added, one-quarter of the sample was removed for HisM analysis, and the remainder boiled for 5 min. For aminopeptidase digestion the vesicles were resuspended at a final protein concentration of 10 mg/ml in 50 mM Tris-HCl, pH 7.5, 1 mM CoCl<sub>2</sub>, and 1 mg/ml aminopeptidase M in a 20-μl final volume. After incubation at 37 °C for 3.5 h, the reaction was stopped by the addition of an equal volume of 5 mM 1,10-phenanthroline and of 2-fold concentrated Laemmli sample buffer. One-quarter of the sample was removed for HisM analysis, and the remainder was boiled for 5 min.

Alkaline Phosphatase Fusions—In vivo fusions of TnphoA into plasmid pFA136 in GA176 were isolated using the A Tnpho method as described (Boyd et al., 1987). In vitro fusions Q1, M1, M2, M3, and M4 were generated from plasmid pFA160 with a fusion in hisP (obtained in vivo) using oligonucleotide-directed deletion mutagenesis as described (Boyd et al., 1987). In vitro fusions Q2 and Q3 were generated by a polymerase chain reaction gene-slicing method (Horton et al., 1989); fragments were synthesized with the desired fusion join points, using plasmid pFA136 as a template for hisQ fragments and pFA182 for the phoA fragment, and then cloned into a vector fragment of pFA182. Fusion locations were mapped by restriction enzyme digestion and by sequence analysis (Sequenase, U. S. Biochemical Corp.) of double-stranded plasmid templates purified with Qiagen plasmid purification columns (Qiagen, Los Angeles, CA). Alkaline phosphatase assays were performed in duplicate as described by Boyd and Beckwith (1989).

Miscellaneous Methods—Protein assays were performed with the method of Lowry et al. (1951). SDS-PAGE and immunoblotting were performed essentially as described (Kerpola et al., 1991). The antisera raised against carboxyl-terminal peptides from HisQ and HisM have been described (Kerpola et al., 1991). Immunoblots were quantitated using a Zeineh soft laser scanning densitometer (LKB Instruments). Binding of antibodies to membrane vesicles was measured as described by exposure of filter-retained vesicles to antisera and iodinated protein A (Prossnitz, 1989).

RESULTS

Proteolytic Digestion of HisQ and HisM—When oriented vesicles were exposed to proteinase K or trypsin, HisQ and HisM were digested in both ROV and IOV preparations indicating accessibility at both surfaces of the membrane. A typical experiment is shown in Fig. 1. About 50% of either HisQ or HisM in IOV is digested at 5 μg/ml trypsin or 10 μg/ml proteinase K. These proteins are more resistant to trypsin digestion when ROV are utilized, requiring about 1 mg/ml for 50% digestion. The similar pattern of susceptibility for HisQ and HisM is not surprising because of their sequence homol-

<sup>2</sup>The abbreviations used are: ROV, right-side-out vesicles; IOV, inside-out vesicles; Hepes, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

![FIG. 1. Quantitation of proteolytic digestion of oriented membrane vesicles.](image-url)
ogy. The susceptibility of the α and β subunits of F1 from the F-F, proton-translocating ATPase in ROV was shown to be very low in the same samples; since the F1 is located on the cytoplasmic surface of the membrane, these data indicate that the integrity of ROV is not disrupted by proteolysis. The higher resistance of HisQ and HisM to trypsin in ROV vesicles as compared with proteinase K may be caused by the relative scarcity of positively charged amino acid residues (i.e. sites of trypsin attack) in the external loops of integral membrane proteins than in the internal loops (Michel et al., 1986; von Heijne, 1986; see also “Discussion”). These results, which are in agreement with the solubility characteristics (Kerppola et al., 1991) and the predicted topology of HisQ and HisM (Ames, 1985), demonstrate that HisQ and HisM are integral membrane-spanning proteins.

Upon digestion with proteinase K an 11-kDa peptide was detected with antibody raised against HisQ only when ROV were utilized (Fig. 2). Since digestion of IOV does not yield detectable fragments and since the antibody used is directed against a carboxyl-terminal peptide of HisQ, these data indicate that the HisQ terminus is on the cytoplasmic surface. The 11-kDa HisQ peptide was the only proteolytic fragment detected by immunoblotting after proteolysis of any of the proteins from the membrane-bound complex.

Digestion with Carboxyl- and Aminopeptidases—To define more precisely the orientation of the transport proteins, we treated oriented membrane vesicles with exopeptidases specific to carboxyl or amino termini, carboxypeptidase P and aminopeptidase M, respectively. Resolution of the digestion products by SDS-PAGE, immunoblotting, and quantitation of the immunoreactive bands (Table I) showed that carboxypeptidase P digestion eliminated both HisQ and HisM immunoblot bands in IOV but not in ROV. Since the epitopes against which both HisQ and HisM antibodies are directed are at the proteins’ carboxyl termini (Prossnitz, 1989), the results support the notion that the carboxyl termini of both these proteins are located on the cytoplasmic surface of the membrane. Treatment with aminopeptidase M caused some increase in the mobility of HisM in ROV but not in IOV (Table I). This partial loss of HisM in ROV is likely caused by the products of aminopeptidase digestion being heterogeneous and resulting in a diffuse smear which could not be estimated accurately by densitometry. HisQ was not effectively digested by aminopeptidase, presumably because its highly hydrophobic amino terminus is not readily accessible to the enzyme; however, there was a slight increase in the mobility of the HisQ band in aminopeptidase-treated ROV (data not shown), indicating digestion of a small portion of the amino terminus of HisQ, in agreement with the amino terminus of HisQ being also localized on the outside.

Antibody Binding to Vesicles—The binding of the antisera to oriented membrane vesicles has provided another tool toward the characterization of the orientation of these proteins. ROV or IOV were exposed to the anti-HisQ or anti-HisM serum (Prossnitz, 1989) and the extent of antibody binding quantitated. Both antisera preferentially bound to IOV, with binding to ROV between 10 and 20% of the level of IOV binding; preimmune serum showed no preference and amounted to less than 5% (data not shown; Prossnitz, 1989). These results are in agreement with both HisQ and HisM carboxyl-terminal epitopes being present on the cytoplasmic side of the membrane.

Alkaline Phosphatase Fusions to HisQ and HisM—The proteolysis experiments described above yielded substantial information as to the topology of the permease proteins. To define further the orientation of HisQ and HisM in the membrane a genetic method developed specifically for the analysis of protein topology was employed (Manoil and Beckwith, 1986; Boyd et al., 1987; reviewed in Manoil et al., 1990). This method generates protein fusions of alkaline phosphatase to the protein of interest by the insertion into its structural gene of a transposon Tn5 derivative (Tn5-phoA) carrying the alkaline phosphatase gene lacking its own transcription, translation, and export signals. Alkaline phosphatase, a periplasmic protein, is expressed if the transposon inserts in frame into the gene of interest with the latter providing the transcription and translation signals. Since alkaline phosphatase is only active if located in the periplasm, only chimeric proteins that place the alkaline phosphatase on the periplasmic surface are active. The hydrophobic membrane spanners serve as translocation signals for the alkaline phosphatase moiety of the chimeric protein. Therefore, this method allows the detection of the number and orientation of the membrane spanners of an integral membrane protein by characterizing the sites of fusions with respect to their alkaline phosphatase activity.

Alkaline phosphatase fusions to HisQ and HisM were obtained by selecting for insertion of the Tn5-phoA transposon into a plasmid containing the hisQMP genes under control of their native dhuA promoter. Fig. 3 shows a schematic model of HisQ and HisM topology which was deduced from the results from both the proteolysis and antibodies experiments and is in agreement with the alkaline phosphatase fusion analysis. Each of HisQ and HisM contains five transmembrane spanners (QS1 to QS5, and MS1 to MS5), and four loops (QLP1 and QLP2, and MLP1 and MLP2 for those facing the periplasm; QLP3 and QLP4, and MLP3 and MLP4 for those facing the cytoplasm).

Several fusion events were obtained in vivo in both hisQ and hisM, and the precise sites of insertion were determined by sequencing the fusion join points and are indicated in Fig. 3. The HisQ (1–24, 2–19, 1–2, and 2–2) and HisM (1–19 and 1–11) fusions with high activity obtained in vivo were found to be in hydrophilic regions which were assigned to loops QLP1, QLP2, and MLP1 and MLP2 (Fig. 2 and Table II). The location of the remaining hydrophilic segments, QLP3, QLP4, MLP3, MLP4, and MLP5, and the carboxyl termini of both proteins, were determined by generating fusions to these segments by in vitro procedures. Alkaline phosphatase fusions to the amino

![Image](2331)
Fig. 3. Topological models of HisQ and HisM. The locations of the alkaline phosphatase join points in HisQ and HisM are shown with arrows; for alkaline phosphatase activities, refer to Table II. The overall charge of loops shown in parentheses was calculated from the predicted sequence (Higgins et al., 1982). Spanners in HisQ and HisM are labeled as S1 to S5, with the prefixes Q and M, respectively. Hydrophilic loops are labeled L with the respective prefixes Q and M and with the suffixes p (1 and 2) or c (1 and 2) for periplasmic and cytoplasmic loops, respectively. The amino and carboxyl termini are labeled Q-NH2 and M-NH2, and Q-COOH and M-COOH, respectively. The EAA sequence is indicated by a box. The drawing is not to scale.

Table II

| Fusion site | Activity | Fusion activity |
|-------------|----------|----------------|
| histQ-phoA  |          |                |
| Q1          | 14       | 1.140          |
| 1-24        | 47       | 1.224          |
| 2-19        | 48       | 1.239          |
| Q2          | 0.7      | 1.410          |
| 1-2         | 24       | 1.489          |
| 2-2         | 51       | 1.533          |
| Q3          | 0.3      | 1.541          |
| histM-phoA  |          |                |
| M1          | 6        | 1.839          |
| 1-19        | 33       | 1.892          |
| 1-11        | 34       | 1.964          |
| M2          | 9        | 2.075          |
| 2-29        | 15       | 2.171          |
| M3          | 26       | 2.231          |
| M4          | 1.5      | 2.336          |

* Units have been defined previously (Boyd and Beckwith, 1989).
* The numbers indicate the histQ and histM nucleotide, respectively, at which the sequence is fused to nucleotide 1 from TnphoA. Numbering is from Higgins et al. (1982).

Discussion

A combined biochemical and genetic approach has made possible a thorough characterization of the transmembrane orientation of the hydrophobic membrane-bound components of the histidine permease. The accessibility of HisQ and HisM to proteases at both surfaces of the membrane together with alkaline phosphatase fusion and hydropathy analyses indicates that HisQ and HisM are integral membrane proteins with five membrane-spanning segments each. The use of carboxy- and aminopeptidases, together with antibody binding experiments, has established that the carboxyl termini are on the inside and the amino termini on the outside surface of the membrane. The topological model based on this information (Fig. 3) includes five spanners and disagrees with the tentative three-spanner model postulated previously (Ames, 1985); a fusion protein would not attain a conformation compatible with correct subunit assembly.
Comparison with Other Hydrophobic Transport Components—In contrast to the ATP-binding subunits of periplasmic permeases which are highly homologous to one another, the hydrophobic components are not, with the exception of a short sequence located in a hydrophilic region, which contains the well conserved triplet glutamate, alanine, alanine, and after 2 residues a hydrophobic residue followed by a well conserved glycine (the EAA sequence). Even though the homology across the entire family of these components is not high, except for the EAA sequence, several of them bear considerable similarity to each other in various combinations (e.g. HisQ and HisM, which are homologous to each other (Ames, 1985) and to GlnP). As proposed previously, this speaks in favor of a hypothesis which pictures an ancestral permease already composed of hydrophobic and hydrophilic components, which would have undergone evolution to accommodate different substrates (Ames, 1986) rather than a hypothesis in which for each permease of differing specificity the ATP-binding subunit would have been recruited independently to energize the transport process by unrelated hydrophobic membrane-spanning proteins (Hilles et al., 1987). Having established the topology of the hydrophobic subunits of the histidine permease, we conclude that its EAA sequence is located in a loop on the inside surface of the cytoplasmic membrane. Upon reexamination of the sequences of all the known hydrophobic transport components searching for common sequence and structural patterns, we found that in each of the known hydrophobic subunits there is a sequence either identical (12 sequences) or closely related (6 sequences) to the EAA sequence. In cases in which an EAA sequence is not obvious (LivH, OppB, SfbB-N, SfbB-C, Flub-B, N, FecC, FecD, and BtuC) an EAA loop is recognizable by numerous identities in the immediate neighborhood. The region bearing this homology is located in all cases at the same approximate distance from the carboxyl terminus as in HisQ and HisM; in the case of SfbB and FlubB there are two such homologous sites because both proteins appear to be internal duplications. In the iron hydroxamate and iron citrate transport systems the glutamate has been replaced by aspartate. By analogy to the histidine permease, this region, which we call the EAA loop, is presumably located in each protein on the inside surface of the cytoplasmic membrane. An alignment of all known EAA loop sequences with a consensus for this sequence using the Genalign program was obtained and is presented in Fig. 4. The sequences, 45 residues long and including the EAA homology, are listed in order of relatedness. When number of matches between sequences is compared, we find that the sequences cluster and form subgroups that share stronger than average homology within the group. It was pointed out previously that the EAA sequence is located 80–100 residues from the carboxyl terminus of these proteins (Deen and Hofnung, 1985). In this analysis, the EAA sequence is found between 95 and 130 residues from the carboxyl terminus.

A preliminary comparison of the topological models for HisQ and HisM with that for MalF, the only other hydrophobic component of periplasmic permeases for which such information is available (Boyd et al., 1987; Froshauer et al., 1988), indicates that even though sequence homology is limited to the EAA loop, the structural homology between HisQ and HisM and MalF is extensive: the periodic pattern and the size of the loops and spanners are very similar throughout the length of the three proteins, excluding the long aminoterminal extension present in MalF. A comparison of the predicted topology of all other known hydrophobic subunits, with all the EAA loops aligned, indicates a similar structural conservation of a minimum of five transmembrane spanners and corresponding loops, with several of the proteins having additional predicted spanners and loops at their amino-terminal ends (Fig. 5). It is clear that this family of proteins has maintained a basic set of structural constraints, as indicated by their topological organization, even though they may have lost considerable sequence similarity. This is not surprising since proteins that perform similar functions are expected to maintain similar structural characteristics. In this respect, maintenance of a fixed distance between the EAA loop and the carboxyl terminus presumably indicates a structural requirement that is not yet understood.

As mentioned before, the hydrophobic components of periplasmic permeases do not have a high degree of sequence similarity. Exceptions are the arabinose, ribose, and galactose (Scripture et al., 1987),3 as well as the maltose and glycerol-phosphate permeases (Overduin et al., 1988) and iron hydroxamate, iron citrate, and vitamin B12 permeases, in which homology extends through the whole length of the operons. In our sequence analysis we found that the histidine transport operon bears strong homology to the glutamine transport

3 R. Hogg, personal communication.
The Topology of Periplasmic Permease Complex

---

operon (alignments not shown). Therefore, within this family, there clearly exist closely related subfamilies of transport systems.

We have shown previously that the hydrophobic components of the histidine transport system form a complex within the membrane (Kerppola et al., 1991). As HisQ, HisM, and GlnP are the smallest proteins of the set of hydrophobic components, it may be assumed that they represent the minimum structure necessary for the formation of a functional complex for this class of permeases. In addition, since the glutamine permease contains a single hydrophobic protein, GlnP, it may also be assumed that a homodimer of a single hydrophobic component organized as determined for HisQ and HisM, is sufficient to form an active complex. The presence of additional spanners and loops in other hydrophobic components may reflect the need for specialized functions. What is then the role for the loop that has been marked? The BtuC sequence published contains an erroneous C deletion which leads to premature termination; the sequence shown here is obtained by translation after the C has been reinserted (Kerppola, 1990).
bic components? Since our results indicate that HisQ and HisM provide the scaffolding upon which the HisP protein is folded (Kerppola et al., 1991; Kerppola, 1990) and since the EAA loop is located on the intracellular surface we propose that its function in all periplasmic permeases is in the interaction with conserved portions of the hydrophilic subunits (conserved components) in the membrane protein complex. The regions of highest sequence conservation in the latter ones are in portions of the nucleotide binding site whereas secondary structure conservation is more extensive, presumably including the entire nucleotide binding site (Mimura et al., 1991). It is possible that the EAA loop interacts directly with portions of the nucleotide binding site. Alternatively, it may interact with a well conserved sequence stretch which is modeled to be outside of the nucleotide binding pocket proper and has been postulated to be involved in signal transmission between the conserved and the hydrophobic components, the so-called glutamine-, glycine-rich linker (Mimura et al., 1991).

Since our analysis strongly suggests that all periplasmic permeases originated from a common ancestor, the fact that primary sequence conservation is extensive only in the conserved components, except for closely related permeases, supports the notion that structural conservation can replace sequence conservation in which folding pattern but not individual residues need to be conserved. Although the conserved components display extensive sequence conservation because of their major role in ATP hydrolysis, the binding proteins and the hydrophobic subunits have a specialized role in binding and possibly providing a passageway through the membrane for the substrate, therefore imposing a need for evolutionary sequence divergence.

Periplasmic permeases are members of a superfamily of transporters which we refer to as traffic ATPases (Ames et al., 1990) and which include several eukaryotic systems such as the gene product responsible for cystic fibrosis (cystic fibrosis transmembrane conductance regulator, CFTR), and the P-glycoprotein of the multidrug resistance system, MDR. In the eukaryotic membrane-bound components there is a specialization of the nucleotide binding site (conserved components) in the membrane protein complex. The overall organization of the eukaryotic traffic ATPases is analogous in all ways to that of the prokaryotic family members. It is likely that progress in understanding the more easily manipulated prokaryotic traffic ATPases will be very useful, providing working models for the eukaryotic counterparts.

Acknowledgments—We thank Robert Simon for the anti-F1 serum; Colin Manoil and Dana Boyd for strains and advice on the alkaline phosphatase fusion; and Robert Hogg for supplying the sequence of MglC before publication.

REFERENCES

Adams, M. D., Wagner, L. M., Graddis, T. J., Landick, R., Antonucci, T. K., Gibson, A. L., and Oxender, D. L. (1990) J. Biol. Chem. 265, 11436–11443

Amemura, M., Makino, K., Shinagawa, H., Kobayashi, A., and Nak-ata, A. (1985) J. Mol. Biol. 184, 241–250

Ames, G. F.-L. (1984) in Microbiology 1984 (Schlessinger, D., ed) pp. 13–16, American Society for Microbiology, Wash., D.C.

Ames, G. F.-L. (1985) Curr. Top. Membr. Transp. 23, 103–119

Ames, G. F.-L. (1986) Annu. Rev. Biochem. 55, 397–425

Ames, G. F.-L. (1990) Rev. Biochem. 141, 341–348

Ames, G. F.-L., and Joshi, A. (1990) J. Bacteriol. 172, 4133–4137

Ames, G. F.-L., and Spudich, E. N. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1877–1881

Ames, G. F.-L., Nikaido, K., Groarke, J., and Petitchory, J. (1989) J. Biol. Chem. 264, 3998–4002

Ames, G. F.-L., Mimura, C., and Siblayama, V. (1990) FEMS Microbiol. Rev. 75, 422–446

Angerer, A., Gaisset, S., and Braun, V. (1990) J. Bacteriol. 172, 572–578

Bell, A. W., Buckel, S. D., Grosbach, J. M., Hope, J. N., Kingsley, D. H., and Herndon, M. A. (1986) J. Biol. Chem. 261, 7855–7858

Bishop, L., Agbayani, R. J., Ambudkar, S. V., Maloney, P. C., and Ames, G. F.-L. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6963–6967

Boyd, D., and Beckwith, J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9446–9450

Boyd, D., Manoil, C., and Beckwith, J. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8525–8529

Boyd, D., Green, G., Boyd, D., Mc Govern, K., and Beckwith, J. (1988) J. Biol. Chem. 263, 4245–4260

Davidson, A. L., and Nikaido, H. (1991) J. Bacteriol. 173, 8946–8950

Dean, D. A., Davidson, A. L., and Nikaido, H. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9134–9138

Friedrich, M. J., de Veaux, L. C., and Kadner, R. J. (1986) J. Bacteriol. 167, 928–934

Froshauer, S., and Beckwith, J. (1983) J. Biol. Chem. 258, 10969–10963

Froshauer, S., Green, G., Boyd, D., McGovern, K., and Beckwith, J. (1988) J. Mol. Biol. 200, 501–511

Gowrishankar, J. (1989) J. Bacteriol. 171, 1923–1931

Higgins, C. F., Haag, P. D., Nikaido, K., Ardeshtir, F., Garcia, G., and Ames, G. F.-L. (1989) Nature 339, 725–727

Higgins, C. F., Hiles, I. D., Whalley, K., and Jamieson, D. K. (1985) EMBO J. 4, 1033–1040

Higgins, C. F., Hiles, I. D., Salmon, G. P., Gill, D. R., Downie, J. A., Evans, I. J., Holland, I. B., Gray, L., Buckel, S. D., Bell, A. W., and Herndon, M. A. (1986) Nature 323, 449–450

Hiles, I. D., Gallagher, M. P., Jamieson, D. J., and Higgins, C. F. (1987) J. Mol. Biol. 195, 125–142

Hobson, A., Weatherwax, R., and Ames, G. F.-L. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 733–737

Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 61–68

Hyde, S. C., Emsley, P., Hartshorn, M. J., Mimmack, M. M., Gileadi, U., Pearl, S. R., Gallagher, M. P., Gill, D. R., Hubbard, R. E., and Higgins, C. (1990) Nature 346, 362–365

Joshi, A., Ahmed, S., and Ames, G. F.-L. (1989) J. Biol. Chem. 264, 2126–2133

Kaback, H. R. (1971) Methods Enzymol. 22, 99–120

Kang, C. H., Shin, W.-C., Yamagata, Y., Goken, S., Ames, G. F.-L., and Kim, S.-H. (1991) J. Biol. Chem. 266, 23893–23899

Kerppola, R. E. (1990) Architecture of the Membrane-bound Component of the Histidine Permease of Salmonella typhimurium, Ph.D. thesis, University of California, Berkeley

Kerppola, R. E., Shyamala, V., Klebba, P., and Ames, G. F.-L. (1991) J. Biol. Chem. 266, 9857–9860

Koster, W., and Braun, V. (1986) Mol. Gen. Genet. 204, 435–442

Kustu, U., and Ames, G. F.-L. (1974) J. Biol. Chem. 249, 6074–6077

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275

Manoil, C., and Beckwith, J. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8219–8233

Manoil, C., and Beckwith, J. (1986) Science 233, 1403–1408

Manoil, C., Mekalanos, J. J., and Beckwith, J. (1990) J. Bacteriol. 172, 515–518

Michel, H., Weyer, K. A., Gruenberg, H., Dunger, I., Oesterhelt, D., and Lottspeich, F. (1986) EMBO J. 5, 1149–1158

Minamikawa, M. L., Gallagher, M. P., Pearl, S. R., Hyde, S. C., Booth, I. R., and Higgins, C. F. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8257–8260
Topology of Periplasmic Permease Complex

Mimura, C. S., Holbrook, S. R., and Ames, G. F.-L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 84–88
Noel, D., Nikaido, K., and Ames, G. F.-L. (1979) Biochemistry 18, 4159–4165
Nohno, T., Saito, T., and Hong, J.-S. (1986) Mol. & Gen. Genet. 205, 261–263
Overduin, P., Boos, W., and Tommassen, J. (1988) Mol. Microbiol. 2, 767–775
Petronilli, V., and Ames, G. F.-L. (1991) J. Biol. Chem. 266, 16293–16296
Prossnitz, E. (1989) In vitro Reconstitution of the Histidine Transport System of Salmonella typhimurium: Binding Protein Interactions, Ph.D. thesis, University of California, Berkeley
Prossnitz, E., Nikaido, K., Ulbrich, S. J., and Ames, G. F.-L. (1988) J. Biol. Chem. 263, 17917–17920
Prossnitz, E., Gee, A., and Ames, G. F.-L. (1989) J. Biol. Chem. 264, 5006–5014
Roth, J. R. (1970) Methods Enzymol. 17A, 3–35
Scripture, J. B., Voelker, C., Miller, S., O’Donnell, R. T., Polgar, L., Rade, J., Horazdovsky, B. F., and Hogg, R. W. (1987) J. Mol. Biol. 197, 37–46
Shyamala, V., Baichwal, V., Beall, E., and Ames, G. F.-L. (1991) J. Biol. Chem. 266, 18714–18719
Sirko, A., Hryniewicz, M., Hulanicks, D., and Bock, A. (1990) J. Bacteriol. 172, 3351–3357
Staudenmaier, H., Van Hove, B., Yaraghi, Z., and Braun, V. (1989) J. Bacteriol. 171, 2626–2633
Surin, B. P., Rosenberg, H., and Cox, G. B. (1985) J. Bacteriol. 161, 189–198
von Heijne, G. (1986) EMBO J. 5, 3021–3027