Membrane Disposition of the *Escherichia coli* Mannitol Permease: Identification of Membrane-Bound and Cytoplasmic Domains†

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ABSTRACT: Two proteolytic fragments of the *Escherichia coli* mannitol permease (EII^{mtl}) have been identified on autoradiograms of sodium dodecyl sulfate–polyacrylamide gels and mapped with respect to the membrane. EII^{mtl} was selectively radiolabeled with either [^{35}S]methionine or a mixture of ^{14}C-labeled amino acids in *E. coli* minicells harboring a plasmid containing the mannitol operon. The intact permease (M_r 65 000) in everted vesicles derived from labeled minicells was cleaved by mild trypsinolysis into two smaller fragments (M_r 34 000 and 29 000). The 34 000-dalton fragment remained in the membrane and was insensitive to further proteolysis by trypsin. This fragment was identified as the N-terminal half of the protein by comparing the amount of the original [^{35}S]methionine label that it retained with the known differential distribution of methionine in the two halves of EII^{mtl}. The 29 000-dalton fragment, which was released into the soluble fraction and was sensitive to further trypsinolysis, therefore corresponds to the C-terminal half of the mannitol permease. Both fragments were shown to be antigenically related to EII^{mtl} by immunoblotting with anti-EII^{mtl} antibody. The 34 000-dalton fragment was further shown to form an oligomer under conditions which allow the intact enzyme to dimerize, suggesting that this domain plays an important role in EII^{mtl} subunit interactions. These results support a model in which EII^{mtl} consists of two domains of approximately equal size: a membrane-bound, N-terminal domain with a tendency to self-associate, and a cytoplasmic C-terminal domain. Finally, the techniques described in this paper should be of general use in studying the membrane disposition of *E. coli* proteins provided that the gene encoding the membrane protein of interest can be cloned and expressed in a minicell system.

The mannitol permease (enzyme II^{mtl} or EII^{mtl}) of the phosphotransferase sugar transport system (PTS) in *Escherichia coli* is required for the transport and concomitant phosphorylation of its sugar substrate mannitol by the following reactions:

$$\text{phosphoenolpyruvate (PEP)} + \text{HPr} \overset{\text{enzyme I}}{\leftrightarrow} \text{HPr–P} + \text{pyruvate} \quad (1)$$

$$\text{HPr–P} + \text{mannitol (out)} \xrightarrow{\text{enzyme II}^{mtl}} \text{HPr} + \text{mannitol-1-P (in)} \quad (2)$$

Enzyme I (EI) and HPr are general soluble phosphocarrier proteins which catalyze the transfer of phosphate from PEP to membrane-bound EII^{mtl} with subsequent phosphotransfer to the incoming sugar [for reviews, see Postma & Lengeler (1985) and Saier (1985)]. EII^{mtl} has been shown to be covalently phosphorylated during the course of these reactions (Roossien & Robillard, 1984; Waygood et al., 1984). However, the site of phosphorylation, as well as the binding sites for mannitol and HPr, are as yet unknown, as are most of the details of the structure of EII^{mtl}.

A model for the intramembrane topography of EII^{mtl} has been proposed on the basis of two indirect lines of evidence. Hydropathy analysis of the amino acid sequence deduced from the cloned and sequenced *mtlA* gene suggests the existence of two distinct domains: a very hydrophobic N-terminal domain, which probably spans the membrane several times, and a large hydrophilic C-terminal domain, which could extend into the cytoplasm (Lee & Saier, 1983). This model was supported by experiments using activity-modifying reagents and proteolytic enzymes to probe the permease from either side of the membrane. EII^{mtl} was easily modified on the inside surface of the membrane but was inaccessible to most reagents from the outside of the cell (Jacobson et al., 1983a). In this paper, we present direct evidence for this model employing a minicell system to selectively radiolabel EII^{mtl} in order to observe the effects of protease modification of the enzyme on sodium dodecyl sulfate (SDS)–polyacrylamide gels.

Materials and Methods

Chemicals. [^{35}S]Methionine (1000 Ci/mmol), a mixture of 15 ^{14}C-labeled amino acids (55 mCi/milliatom of carbon), and [^{3}H]mannitol (19 Ci/mmol) were purchased from New England Nuclear (Boston, MA). ^{125}I-Labeled *Staphylococcus aureus* protein A (50 μCi/μg) was purchased from ICN Biomedicals, Inc. (Irvine, CA). L-1-(Tosylamido)-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin was purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of Membrane Vesicles. Minicells of *E. coli* strain MV1009 containing the plasmid pLC15-48, which encodes the mannitol operon, were isolated as described previously (Lee et al., 1981). EII^{mtl} was then selectively labeled during its synthesis with [^{35}S]methionine plus 2 μg/mL each of the other 19 unlabeled amino acids or with a mixture of 15 ^{14}C-labeled amino acids (N.E.N. NEC-445E) in the presence of 1 mM phenylmethanesulfonyl fluoride (PMSF) to inhibit proteolysis (Stephan & Jacobson, 1986). When ^{14}C-labeled amino acids were used, an amino acid supplement containing 2 μg/mL each of the remaining five amino acids was added to the incubation mixture. Unlabeled minicell membranes for use in immunoblotting (see below) were prepared by using a mixture of all 20 unlabeled amino acids (2

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μg/mL each). Everted membrane vesicles from all minicell preparations were prepared as described previously (Stephan & Jacobson, 1986), as were everted membrane vesicles from *E. coli* strain KL141 (Jacobson et al., 1983a). Vesicles prepared in this manner were found to be ≥90% everted as determined by the susceptibility of total EII^{mtl} activity in the intact vesicles to trypsin (Jacobson et al., 1983a). Protein concentration was estimated as described by Lowry et al. (1951).

Electrophoresis and Autoradiography. Labeled EII^{mtl} and its proteolytic products were detected on SDS–polyacrylamide (10%) gels run according to the method of Weber and Osborn (1969). Samples of control or trypsinized membrane vesicles were extracted in sample buffer containing 62.5 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 6.8, 2% SDS, 10% glycerol, 5% mercaptoethanol, 2.5 mM sodium thioglycolate, and 0.003% bromphenol blue at 100 °C for 5–10 min unless otherwise indicated. Gels were run for 18 h at 50 mA, treated for 15–30 min with 1 M sodium salicylate (pH 8.0) to enhance autoradiography (Chamberlain, 1979), and dried before exposure to Kodak XAR X-ray film for 1 day to 1 week at −70 °C. Radiolabeled bands were quantitated by using a Zeineh soft-laser scanning densitometer. Lanes containing molecular weight markers were cut off and stained separately with Coomassie brilliant blue. The gels showing subunit interactions (Figure 4) were run according to the method of Laemmli (1970).

Immunoblotting. Electrophoretic blotting of proteins from SDS–polyacrylamide gels onto nitrocellulose was carried out according to the method of Towbin et al. (1979) with the addition of 1% SDS to the transfer buffer. After blotting, unoccupied sites on the nitrocellulose membrane were blocked by incubation with 3% bovine serum albumin (BSA) in 0.9% NaCl/10 mM Tris-HCl (pH 7.4) (saline solution) at 40 °C for 45 min. The nitrocellulose was then washed in saline solution containing 0.005% Tween 20. EII^{mtl} and any antigenically related fragments were detected by incubation for 1 h at 20 °C with rabbit antiserum raised against the purified protein (Lee et al., 1981) diluted in 1% BSA, followed by washing in saline solution containing 0.005% Tween 20. The nitrocellulose membrane then was incubated with 5–10 μCi of ^{125}I-labeled *Staphylococcus aureus* protein A for 4 h at 20 °C (Burnette, 1981). The membrane was then washed as before, allowed to dry overnight, and autoradiographed on Kodak XAR film for 24 h at 20 °C. Labeled bands were quantitated as described above.

Measurement of EII^{mtl} Activity. EII^{mtl} activity was measured as the PEP-dependent phosphorylation of [^3H]mannitol as described previously (Jacobson et al., 1983a).

Results

Radiolabeling of proteins in minicells containing plasmid pLC15-48 results in the appearance of a major radiolabeled band on autoradiograms of SDS-polyacrylamide gels, corresponding to EII^{mtl} (Lee et al., 1981; Stephan & Jacobson, 1986). The enzyme has an apparent molecular weight of 60 000 when the Laemmli (1970) system is used (Lee et al., 1981) and of 65 000 when the Weber and Osborn (1969) gel system is used (Figure 1, time 0). Treatment of everted membrane vesicles derived from labeled minicells resulted in the appearance of two lower molecular weight bands at 34 000 and 29 000 in this gel system. As incubation with trypsin was continued, the 34-kilodalton (kDa) band was insensitive to further proteolysis, while the 29-kDa band, which appeared transiently in low amounts, was further degraded (Figure 1, times 2–60 min). A small amount of an endogenous fragment

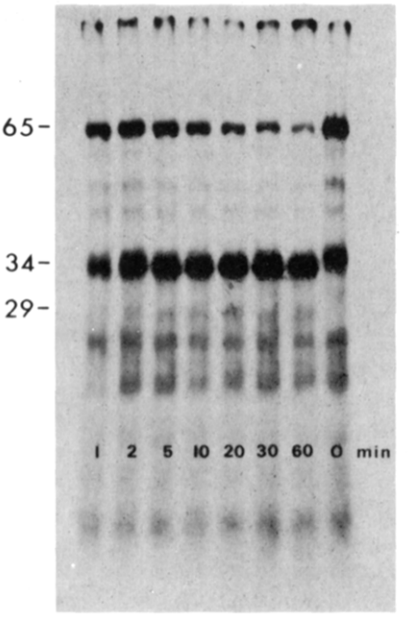

FIGURE 1: Trypsinolysis of the mannitol permease in everted vesicles. [^{35}S]Methionine-labeled everted membrane vesicles from minicells (0.75 mg/mL total protein) were treated with 0.5 μg/mL trypsin at 20 °C. Samples (12 μL) were added at the times shown to an equal volume of 0.5 mg/mL soybean trypsin inhibitor (SBTI). A portion of each sample was reserved for the measurement of EII^{mtl} activity (see Figure 2), and the remainder was extracted and electrophoresed as described under Materials and Methods. As a control, everted vesicles were incubated under identical conditions with trypsin plus SBTI. No proteolysis or inactivation of EII^{mtl} was observed compared to the zero time point sample over a 60-min incubation period (data not shown).

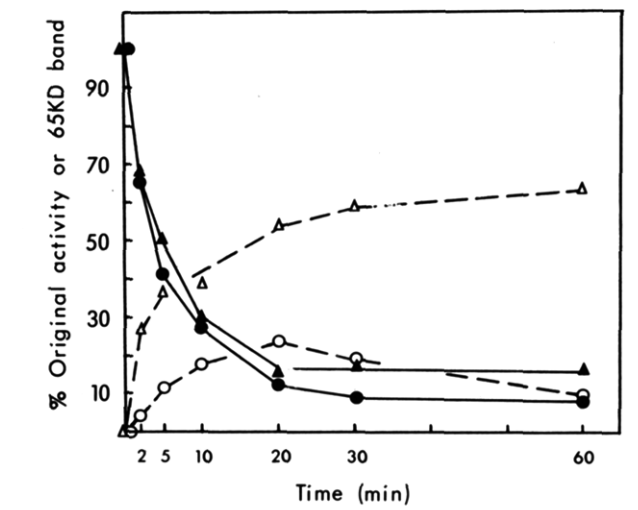

FIGURE 2: Quantification of trypsinolysis products and mannitol permease activity as a function of time. The autoradiogram shown in Figure 1 was scanned by densitometry to determine the relative amounts of the 65-kDa band (●) and 34-kDa band (△) as a function of time of proteolysis. The 29-kDa band (○) was quantitated relative to the original amount of permease by scanning densitometry of an immunoblot of unlabeled minicell membranes treated with trypsin as described in the legend to Figure 1. PEP-dependent EII^{mtl} activity (▲) was measured as described under Materials and Methods. The amount of the 34-kDa band is corrected for the small amount (19% of total radioactivity in the 65- + 34-kDa bands) of 34-kDa fragment appearing in the zero time point sample. Ordinate values represent the amount of intact permease, fragments, or activity relative to the original amount of 65-kDa protein or activity.

(M_r 34 000), which is antigenically related to EII^{mtl} (see below), was also apparent in the untreated sample (Figure 1, time 0) despite efforts to limit proteolysis in the preparation of minicell vesicles (see Materials and Methods).

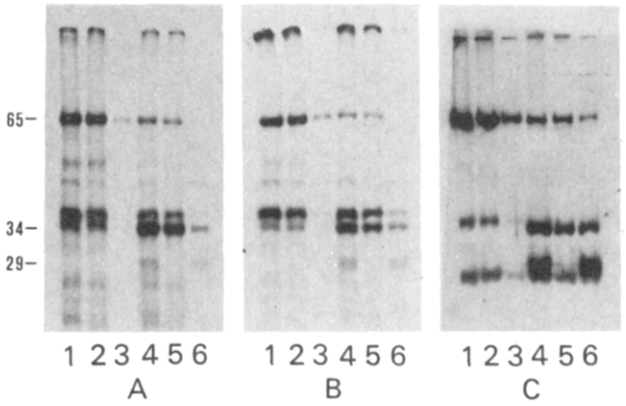

FIGURE 3: Localization of the 34- and 29-kDa fragments with respect to the membrane. Minicell membranes (0.86 mg/mL total protein) labeled with [^{35}S]methionine (6 μL, panel A) or ^{14}C-labeled amino acids (18 μL, panel B) or unlabeled (12 μL, panel C) were treated with H_2O (lanes 1–3) or 5 μg/mL trypsin (lanes 4–6) for 2 min at 20 °C, followed by the addition of an equal volume of 5 mg/mL SBTI. Each control and reaction mixture was then split into equal halves. Half of each mixture was kept on ice for 30 min and then extracted with sample buffer and electrophoresed (lanes 1 and 4). The other half was centrifuged at 12800*g* for 30 min at 4 °C. The pellets from these samples were resuspended, extracted with sample buffer, and electrophoresed (lanes 2 and 5). The supernatants were also extracted and electrophoresed (lanes 3 and 6). Panels A and B are direct autoradiographs, while panel C is an autoradiograph of an immunoblot. In all three panels: lane 1, control; lane 2, control pellet; lane 3, control supernatant; lane 4, trypsin treated; lane 5, trypsin treated, pellet; lane 6, trypsin treated, supernatant.

The results shown in Figure 1 are quantified in Figure 2. The rates of disappearance of the 65-kDa band and of the concomitant appearance of the 34-kDa band were determined by scanning densitometry. Figure 2 shows that loss of the 65-kDa band correlated with the loss of the PEP-dependent mannitol phosphorylation activity of EII^{mtl} and with the appearance of the 34-kDa band on the gel. The same pattern of trypsin degradation of EII^{mtl} was seen when everted vesicles from a non-minicell-producing strain, *E. coli* KL141, were treated with trypsin under similar conditions, and gels of the resulting digests were immunoblotted with anti-EII^{mtl} antibody (results not shown).

To localize these fragments with respect to the membrane, minicell membrane vesicles were treated with trypsin and centrifuged, and soluble and insoluble fractions were run in separate lanes. These results are shown in Figure 3 (panel A = [^{35}S]methionine labeling; panel B = ^{14}C-labeled amino acid labeling; panel C = immunoblotting). The 34-kDa fragment remained with the membrane fraction (Figure 3A–C, lane 5) while the 29-kDa fragment was released into the soluble fraction (Figure 3A–C, lane 6). Immunoblotting (Figure 3C) confirmed that the two fragments are products of EII^{mtl} degradation and further suggests that the 29-kDa fragment contains more epitopes to antibody, raised against the intact protein in detergent, than does the 34-kDa fragment (compare lane 6 in Figure 3A–C). These experiments also show that the 34-kDa fragment present even in untrypsinized samples (Figure 1) is derived from EII^{mtl} (lane 1, Figure 3A–C). The band appearing at approximately 40 kDa in all membrane samples shown in Figure 3, which was insensitive to proteolysis, corresponds to labeled mannitol 1-phosphate dehydrogenase (Lee et al., 1981) trapped within the everted vesicles.

According to the amino acid sequence deduced from the sequenced *mtlA* gene, there are 19 methionines in the N-terminal half of the protein, but only 6 in the C-terminal half (Lee & Saier, 1983). Therefore, in the [^{35}S]methionine labeling system, 76% of the label should be in the N-terminal half of the protein and 24% in the C-terminal half. Thus, by measuring the relative amount of ^{35}S label retained in the 34-kDa fragment from [^{35}S]methionine-labeled minicells, one can determine which half of the protein is represented by this fragment. Table I shows that about 72% of the [^{35}S]-methionine label lost by the 65-kDa band during trypsin treatment is retained in the 34-kDa fragment. With ^{14}C-labeled amino acid labeling, on the other hand, about 50% of the label is retained in the 34-kDa fragment, as expected from taking into account the relative frequency of each labeled amino acid in the N-terminal half of the sequence and their relative specific activities in the labeling mixture (Table I). These results identify the 34-kDa fragment as the N-terminal half of EII^{mtl}, and, by deduction, the 29-kDa fragment as derived from the C-terminal half of the protein.

It has recently been shown that, when EII^{mtl} is extracted with SDS from the membrane under mild conditions, a dimeric form of the protein can be detected on SDS–polyacrylamide gels (Roossien & Robillard, 1984; Stephan & Jacobson, 1986). Figure 4 shows that the 34-kDa fragment produced by tryp-

Table I: Identification of the 34-kDa Fragment as the Amino Terminus[a]

label	amount of original label appearing in 34-kDa fragment (%)		
		predicted	
	found	if N-terminal	if C-terminal
^{14}C-labeled amino acids	50 ● 5	52[b]	48[b]
[^{35}S]methionine	72 ± 3	76[c]	24[c]

[a] Trypsinization of [^{35}S]methionine or ^{14}C-labeled amino acid labeled minicell membranes was carried out as described in the legend to Figure 1. Autoradiograms of three separate gels were scanned in each labeling system. [b] Calculated from the relative frequencies of the labeled amino acids in each half of EII^{mtl} and their specific radioactivities in the labeling mixture. [c] Predicted from the known amino acid sequence (see Results).

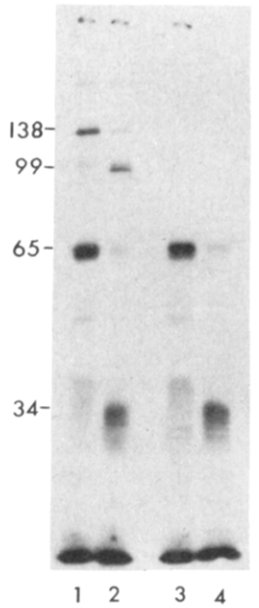

FIGURE 4: Oligomerization of the intact mannitol permease and the 34-kDa fragment. ^{35}S-Labeled minicell membranes (9 μL, 0.83 mg/mL total protein) were treated with either H_2O (control) or 1.5 μL of 35 μg/mL trypsin for 5 min at 20 °C. The reaction was stopped by adding an equal volume of 5 mg/mL SBTI. Extraction with sample buffer was performed either at 30 or at 100 °C as described (Stephan & Jacobson, 1986). Lane 1, control, 30 °C; lane 2, trypsinized, 30 °C; lane 3, control, 100 °C; lane 4, trypsinized, 100 °C.

sinolysis also appears as an oligomer on gels under these conditions. As shown previously, a portion of the intact EII^{mtl} is present as an apparent dimer after mild SDS extraction (Figure 4, lane 1). The 34-kDa fragment also partially associates into an oligomer (M_r 99000) under identical conditions (Figure 4, lane 2). The oligomer is sensitive to boiling, as is the dimer formed by the intact enzyme (Figure 4, lanes 3 and 4). That the 99-kDa band represents an oligomer of the 34-kDa fragment is shown by the fact that at 30 °C the 34-kDa band is reduced relative to the 100 °C sample by an amount corresponding to the amount of 99-kDa fragment appearing at the lower temperature (cf. lanes 2 and 4, Figure 4). An immunoblot of this gel also showed that the 99-kDa band reacted with anti-EII^{mtl} antibody (data not shown).

Discussion

EII^{mtl}, the mannitol permease, is a membrane-bound transport protein which performs multiple functions in *E. coli*. This enzyme is responsible for the transport and phosphorylation of mannitol, as well as for chemoreception of mannitol during chemotaxis (Lengeler et al., 1981). These functions require the interaction of the permease with other proteins such as HPr (and possibly enzyme I) and also presumably with proteins involved in chemotactic signaling (Segall et al., 1985). The disposition of EII^{mtl} with respect to the membrane will thus probably reflect this multiplicity of functions and the several binding sites which are required.

Previously, the determination of the membrane disposition of the EII^{mtl} polypeptide relied on two indirect lines of evidence. First, the amino acid sequence of the protein was deduced from the cloned and sequenced *mtlA* gene. Hydropathy analysis of this sequence revealed seven major regions of hydrophobic amino acids, possibly the membrane-spanning portions of the protein (Lee & Saier, 1983). These hydrophobic regions are confined to the first N-terminal 336 amino acids, which have an overall relative hydrophobicity of +0.90, similar to such membrane-bound proteins as bacteriorhodopsin and the lactose permease (Kyte & Doolittle, 1982; Foster et al., 1983). The remaining, C-terminal half of the protein has an overall hydrophobicity similar to soluble proteins. These observations have led to a model in which the enzyme consists of a membrane-bound domain plus a cytoplasmic domain of about equal size (Lee & Saier, 1983). The other indirect line of evidence, which supported this model, relied on observations of the accessibility of the protein to activity-modifying reagents when they were presented from either side of the membrane. The activity of the enzyme was impervious to proteases and other reagents when right-side-out membranes (e.g., spheroplasts) were treated. However, EII^{mtl} was sensitive to such reagents when everted (e.g., French press) vesicles were treated (Jacobson et al., 1983a). These results also suggested that a large portion of the protein extends into the cytoplasm.

This paper presents the first direct evidence that such a model of the membrane disposition of the permease is correct. When radiolabeled EII^{mtl} in everted minicell membrane vesicles was treated with trypsin, two fragments were produced (Figure 1). One fragment, a 34-kDa polypeptide, remained in the membrane and was insensitive to further proteolysis. The other fragment, a 29-kDa polypeptide, was released into the soluble fraction and further degraded (Figures 1 and 2). The same two fragments were observed when membranes of normal cells (*E. coli*, strain KL141) were trypsinized, making it likely that the protein is correctly inserted in the minicell membrane.

The membrane-bound 34-kDa fragment should be the hydrophobic, N-terminal half of the EII^{mtl}, according to predictions from the sequence data and hydropathy analysis. This prediction was confirmed by the results of differential labeling with either [^{35}S]methionine or a mixture of ^{14}C-labeled amino acids as shown in Figure 3 and quantitated in Table I. The 29-kDa fragment, which was released into the soluble fraction and was sensitive to further proteolysis (Figures 1–3), therefore represents the C-terminal half of the protein. Apparently, this half of the protein has very little noncovalent interaction with the membrane-bound domain, since it was readily released from the membrane under the mild conditions of proteolysis and centrifugation. This result confirms the prediction that a large portion of the protein extends into the cytoplasm as inferred from its ready accessibility to modifying reagents from the inside (Jacobson et al., 1983a). Since the PEP-dependent activity of the permease was lost concomitantly with cleavage of the two fragments, however, it is clear that the covalent association of both domains is necessary for mannitol phosphorylation to occur. Similar results were obtained when purified EII^{mtl} was treated with trypsin (Jacobson et al., 1983b), suggesting that the region sensitive to proteolysis is also exposed in detergent solution. Interestingly, although the 29-kDa fragment appeared only transiently in low amounts when radiolabeled minicell membranes were used (Figure 1), it was much more reactive with anti-EII^{mtl} antibody than the 34-kDa fragment (Figure 3C). This result is consistent with the 29-kDa fragment being water soluble and presumably therefore much more antigenic than the 34-kDa fragment.

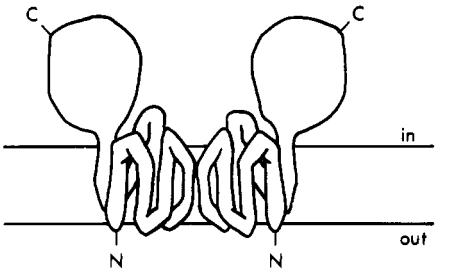

FIGURE 5: Model for the membrane disposition of the mannitol permease. See text for details (N = amino terminus, C = carboxy terminus).

Recently, EII^{mtl} has been shown to form a dimer which can be detected on SDS–polyacrylamide gels when membranes are extracted under mild conditions (Roossien & Robillard, 1984; Stephan & Jacobson, 1986). It seems likely that this dimer is held together primarily by noncovalent, hydrophobic interactions, since it can be dissociated by boiling and by the nonionic detergent Lubrol PX (Roossien & Robillard, 1984; Stephan & Jacobson, 1986; Figure 4, lanes 1 and 3). Since the 34-kDa N-terminal fragment is the most hydrophobic portion of the protein, we presumed that it could be responsible for these subunit interactions. The experiments shown in Figure 4 demonstrate that the 34-kDa fragment can itself oligomerize but appears as a 99-kDa band on gels which could represent a trimer. This association of more than two polypeptide fragments may result from the loss of steric hindrance present in the intact protein due to the large C-terminal domain.

We have incorporated the present data and previous results into a model of EII^{mtl} disposition with respect to the membrane as shown in Figure 5. The seven membrane-spanning portions of the protein predicted from the sequence would suggest that the extreme N-terminus is on the outside of the cell (Lee & Saier, 1983), while the C-terminus extends into the cytoplasm as also shown by the present study. The large C-terminal domain is shown to make very little contact with the membrane-bound portion of the protein, since it spontaneously

dissociates from the membrane after proteolysis. Furthermore, it seems likely that there is a narrow exposed region between the two domains and close to the surface of the membrane, which is a preferred site of proteolysis by trypsin. This site probably is found between amino acid residues 335 and 351 since this stretch contains three lysine residues (335, 338, and 340) and two arginine residues (350 and 351) (Lee & Saier, 1983), and cleavage in this region would produce fragments approximately equal in size to those shown in this report. The model also depicts the mannitol permease as a dimer in the membrane, held together primarily by interactions between the intramembrane domains, although direct evidence for an intramembrane dimer remains a subject for further work (Stephan & Jacobson, 1986). This model, with a large cytoplasmic domain, fulfills the requirement for multiple protein binding sites on the inside of the cell needed to perform the multiple functions of the mannitol permease.

Finally, we believe that the techniques described in this report could be of general use for studying the overall disposition of *E. coli* membrane proteins provided that (1) the gene for the protein of interest can be cloned and expressed in a minicell system and (2) the protein is sensitive to proteolysis at either or both faces of the cytoplasmic membrane.

Registry No. Mannitol permease, 91386-44-6.

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Complete Sequence and Structure of the Gene for Human Adenosine Deaminase†

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ABSTRACT: The nucleotide sequence of the human adenosine deaminase gene was determined. The gene was isolated in a series of overlapping λ phage clones containing human germ line DNA. A total of 36 741 base pairs were sequenced, including 32 040 base pairs from the transcription initiation site to the polyadenylation site, 3935 base pairs of 5′-flanking DNA, and 766 base pairs of 3′-flanking DNA. The gene contains 12 exons separated by 11 introns. The exons range in size from 62 to 325 base pairs while the introns are 76–15 166 base pairs in size. The area sequenced contains 23 copies of Alu repetitive DNA and a single copy of an "O" family repeat. All but one of these repeat sequences are located in the first three introns or the 5′-flanking region. The apparent promoter region of the gene lacks the "TATA" and "CAAT" sequences often found in eucaryotic promoters and is extremely G/C rich. Contained within this region are areas homologous to other G/C-rich promoters, including six decanucleotide sequences that are highly homologous to sequences identified as functional binding sites for transcription factor Sp1.

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4), a part of the purine catabolic pathway, catalyzes the irreversible deamination of adenosine and deoxyadenosine. Deficiency of adenosine deaminase activity in humans is associated with an autosomal recessive form of severe combined immunodeficiency disease (Giblett et al., 1972; Martin & Gelfand, 1981; Thompson & Seegmiller, 1980).

Adenosine deaminase purified from erythrocytes (Schrader et al., 1976; Daddona & Kelley, 1977) and granulocytes (Wiginton et al., 1981) is a soluble monomeric protein with a molecular weight of approximately 41 000. Adenosine de-

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