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Localization to the inner surface of the cytoplasmic membrane by immunoelectron microscopy of enzyme I of the phospho*enol*-pyruvate:sugar phosphotransferase system of *Escherichia coli*

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ABSTRACT **The phospho*enol*pyruvate:sugar phosphotransferase system of *Escherichia coli* constitutes a major pathway for sugar translocation. It is composed of integral membrane proteins (enzyme II components) that recognize specific extracellular sugars as well as phosphocarrier proteins, one of which is called enzyme I. While enzyme I plays a role in energizing the enzyme II for sugar transfer, its precise cellular distribution had not previously been defined. This study was designed to elucidate the cellular location of this protein by immunoelectron microscopy. Enzyme I antibody bound to *E. coli* cryosections was visualized with protein A-gold. The gold particles in sections of wild-type *E. coli* were found primarily associated with the surface of the inner membrane. A strain of *E. coli* harboring a plasmid encoding the gene for enzyme I was also tested for its distribution of enzyme I. Consistent with the biochemically established overproduction of enzyme I, this strain showed an ≈80-fold higher density of gold particles per unit cell volume than the wild-type cells. The substantial overproduction of immunoreactive enzyme I was associated with a significant (≈20-fold) increase in the amount of that protein bound to the inner membrane. In addition, a substantial fraction of the total enzyme I accumulated within a 60-nm-wide zone in the vicinity of the inner membrane. A model to explain the zonal distribution of enzyme I under conditions of overexpression of the protein is presented.**

The phospho*enol*pyruvate:sugar phosphotransferase system (PTS) of *Escherichia coli* effects a group translocation reaction by which extracellular sugars are transported across the cell membrane concomitant with their phosphorylation (1). A number of sugar recognition proteins, termed enzyme IIs, some of which are inducible, are integral membrane proteins. The PTS components that are not sugar specific [enzyme I and heat-stable protein (HPr)] are constitutive and intracellular. In the case of enzyme I and HPr, purification studies have indicated that the proteins are soluble (2, 3). However, preparations of membrane vesicles, devoid of most cytoplasmic proteins, appear to contain some enzyme I and HPr (4, 5), suggesting that these proteins may be membrane associated. The studies reported here used immunoelectron microscopy (6–8) to visualize the precise *in vivo* distribution of enzyme I. The results support the idea that enzyme I is localized at the inner membrane by interaction with a limited number of binding sites.

MATERIALS AND METHODS

Growth of *E. coli* Strains. The strains of *E. coli* used in this study are listed in Table 1. The plasmid pDIA100 (10) encodes the gene for adenylate cyclase; the plasmids pEL06 (9) and pDS20 (11) encode genes for the PTS proteins HPr, enzyme I, and a fragment of enzyme III^{Glc}; and plasmid pDS48 (11) encodes the gene for enzyme III^{Glc}. The plasmid pEL06 resident in strain 600 results in an approximately 20- to 70-fold overexpression of enzyme I (9, 11). Strains were grown in LB medium (12) at 37°C with aeration. For studies involving the localization of β-galactosidase, cells were grown to midlogarithmic phase, at which point 5 mM isopropyl β-D-thiogalactopyranoside was added and the cultures were allowed to grow for another hour. Plasmids were maintained in the appropriate strains by including in the cultures ampicillin (30 μg/ml), kanamycin (50 μg/ml), or tetracycline (10 μg/ml). Overnight cultures of the strains were diluted 1:10 with fresh medium and grown for 4 hr. The cells were collected by centrifugation (10,000 × *g*) and then washed with phosphate-buffered saline (PBS) at pH 7.2. The washed cells were resuspended in 0.5% glutaraldehyde/0.6% tannic acid in PBS for 30 min at room temperature (7, 8). The fixed cells were washed once with PBS followed by several washes in 30% (vol/vol) glycerol in PBS or 2.3 M sucrose in PBS for cryoprotection (13). After the final wash, the wet pellet was thoroughly mixed with a Vortex mixer. Drops of the thick suspension were placed on cryomicrotome specimen pins and quickly frozen by plunging them into liquid nitrogen-cooled propane (14, 15). These specimens were stored in a liquid nitrogen reservoir for future sectioning.

Enzyme I and Antibody Reagents. Enzyme I was purified from *E. coli* strain 599 (9) as described (16). Samples of the purified protein were used for production of rabbit polyclonal antibody by standard procedures.

β-Galactosidase. The β-galactosidase activity of strains KL16 and 600 were measured by the method of Miller (17). The activities were strain KL16 = 185 Miller units and strain 600 = 69 Miller units. Rabbit antibody against *E. coli* β-galactosidase was from Cappell Laboratories.

Immunoelectron Microscopy. Specimen pins were mounted on the holder of a Cryonova cryomicrotome (LKB) and 60- to 80-nm-thick sections were cut with a freshly broken glass knife at −98°C. Ribbons of dry sections were collected from the edge of the knife on freezing drops of sucrose (13); they were then transferred to Formvar-coated electron microscope grids, which were placed on liquefied gelatin. Immunolabeling of the sections for revealing antigenic sites and staining of the sections on the grids for generating contrast of the cellular structures were accomplished by floating the grids on drops of solutions placed in Petri dishes. The treatments were done in the following sequence (all solutions were made in PBS): 0.02 M glycine, preimmune serum (1:1000 dilution), immune serum (1:1000 dilution), protein A-gold (Jansenn Life Sciences, Piscataway, NJ) containing 10^9 particles per ml (15 nm), PBS followed by deionized water, 2% aqueous uranyl acetate oxalate, deionized water, and 4% aqueous polyvinyl alcohol (12). Finally, the grids were drained, leaving very thin films on the sections. The

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Abbreviations: PTS, phospho*enol*pyruvate:sugar phosphotransferase system; HPr, heat-stable protein; BSA, bovine serum albumin.

Table 1. *E. coli* K-12 strains used

Strain	Relevant genotype	Derivation	Source or ref.
KL16	Wild type		H. L. Kornberg
DG40	Δ(*ptsI-crr*)	KL16	CGSC 5915*
DS166	Δ(*ptsI-crr*), *recA*	DG40	9
599	Δ(*ptsI-crr*)/ pEL06	DS166	9
600	Δ(*ptsI-crr*)/ pEL06, pDIA100	DS166	9
626	Δ(*ptsI-crr*)/ pEL06, pDS48	DG40	9
372	Wild type/pBR322	1100*	
375	Wild type/pDS20	1100	

pEL06 carries a gene for kanamycin resistance; pDS48 carries a gene for ampicillin resistance; pDS20 carries a gene for ampicillin resistance; pDIA100 carries a gene for ampicillin resistance.
E. coli Genetic Stock Center, Department of Human Genetics, Yale University School of Medicine, New Haven, CT.

specimens were examined by a transmission electron microscope at 100 kV using a small beam spot and a 20-μm objective aperture. The gold label was visible at ×30,000 to ×50,000 instrumental magnification. Quantitative analyses from random micrographs were done in an IDEAS (FHC, Maine) image analysis computer using a stereology program (18). The results were expressed as the number of gold particles per μm^3 of cell volume at the 15% error level.

Since the gold particles corresponding to enzyme I immunoreactivity were nonrandomly distributed (see Figs. 1, 3, and 5) and appeared to be concentrated in the vicinity of the inner membrane, the distances of the particles from the membrane were determined. Electron micrographs containing gold particles were projected through an enlarger onto sheets of paper. The membrane profiles of the projected images were traced and the distances of the gold particles from the membrane were measured. The data were tabulated in an IBM AT computer spread sheet with a Lotus program, and a graphical presentation of the distribution was made using the Cricketgraph program on a Macintosh computer (see Fig. 2).

PTS Activity. PTS activities in toluene-treated cells (19) of strains 372 and 375 were determined. The activities at 30°C for methyl α-glucoside phosphorylation were 2.64 (strain 372) and 2.72 (strain 375) $\mu mol \cdot mg^{-1} \cdot hr^{-1}$. The corresponding activities for 2-deoxyglucose phosphorylation were 1.45 (strain 372) and 1.65 (strain 375) $\mu mol \cdot mg^{-1} \cdot hr^{-1}$. Methyl α-glucoside uptake into intact cells of strains KL16 and 626 was carried out as described by Solomon and Lin (20). Within experimental error, the uptake activities were indistinguishable. These data indicated that overproduction of enzyme I and HPr did not enhance PTS activity.

Immunoblots. Immunoblots of the enzyme I and its antiserum were carried out by standard methods (21). *E. coli* cells were harvested after 4 hr of growth in LB medium at 37°C. After centrifugation, the cell pellets were washed in 10 mM Tris·HCl, pH 7.5/0.15 M NaCl (TBS) and then resuspended in TBS and briefly sonicated in an Ultrasonic cell disrupter. The disrupted cell preparation was centrifuged at 10,000 × *g* for 30 min to remove particulate material. Proteins in the supernatant solution were precipitated with 10% trichloroacetic acid. The precipitate was washed with deionized water and then dissolved in 10% SDS for PAGE. One portion of the gel was stained with Coomassie blue and the other portion was used for transfer of protein to cellulose acetate paper. After transfer, the cellulose acetate paper was first soaked in 5% bovine serum albumin (BSA) dissolved in TBS for 30 min at 37°C. The membrane was then washed three times for 5-min periods with TBS containing 0.1% BSA. The washed membrane was treated with a 1:1000 dilution of the enzyme I immune serum in TBS containing 1% normal rabbit serum for 2 hr at room temperature under constant agitation. The immune serum-treated paper was washed three times for 5-min periods with 0.1% BSA in TBS at room temperature. The paper was then incubated with radioiodinated protein A (Amersham) at 2.5 × 10^5 cpm/ml in TBS containing 0.1% BSA and a 1:20 (vol/vol) dilution of gelatin (45%; Sigma) for 2 hr at room temperature. The membrane was then washed twice in TBS containing 0.1% BSA followed by two washes in deionized water. Immunoreactive proteins were detected by autoradiography.

RESULTS

Association of Enzyme I with a Limited Number of Sites at the Surface of the Inner Membrane. Cryosections of a wild-type strain of *E. coli* (KL16; see Table 1) were prepared and treated with antiserum directed against enzyme I as described. The bound antibody was then immunolocalized with protein A-gold. Electron micrographs of some representative sections are shown in Fig. 1 *a* and *b*. Examination of many such micrographs provided convincing evidence that most of the cellular enzyme I is normally in close association with the inner membrane.

Since the sections prepared from the wild-type strain of *E. coli* showed a relatively sparse labeling by antibody to enzyme I, we were interested in examining sections prepared from a strain of *E. coli* (strain 600; see Table 1) that produces higher than normal levels of enzyme I as a result of hyperexpression of the enzyme I gene from a plasmid (9, 10). Fig. 1 *c* and *d* shows some micrographs from this study. The

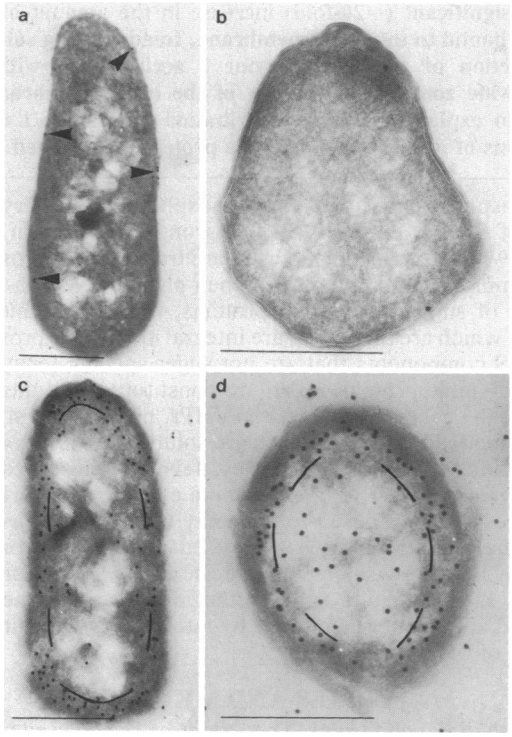

FIG. 1. Immunolabeling of enzyme I in cryosections of wild-type and enzyme I overproducer strains of *E. coli*. (*a* and *b*) Labeled cryosections of wild-type *E. coli*. (*c* and *d*) Immunolabeled enzyme I overproducer cells. Broken lines in *c* and *d* were drawn to demarcate a zone 60 nm wide in the vicinity of the inner membrane, in which enzyme I immunoreactivity is enriched. The arrowheads in *A* indicate the regions of the inner membrane where there are gold particles. (Bars = 0.5 μm.)

immunolabeling in these sections is denser than in sections from wild-type cells. While much of the enzyme I in these sections is associated with the inner membrane, the bulk of the gold labeling occurs in the close vicinity of the inner membrane.

A quantitative approach to estimating the distribution of enzyme I was taken. The number of ImmunoGold particles associated with the cryosections of both the wild-type (KL16) and the enzyme I overproducer (600) cells were determined from random samples of electron micrographs and analyzed stereologically. The numerical densities of the gold particles were found to be $14/\mu m^3$ cell volume for wild-type cells and $1114/\mu m^3$ cell volume for the overproducer strain. This measurement indicates that the ImmunoGold binding is ≈80 times higher in the enzyme I overproducer strain than in the wild-type strain. The enzyme I overproducer strain contains 20–70 times more enzyme I than the wild-type cells (ref. 11; unpublished studies). The reasonable correspondence between the biochemically estimated enzyme content of cells and the quantitative values for ImmunoGold binding is consistent with the interpretation that the measurement of the number of ImmunoGold particles is related to the amount of cellular enzyme I.

The value of 1114 gold particles of 15 nm diameter per μm^3 of cell volume was calculated to occupy 0.8% of the cell space. Since the concentration of enzyme I is ≈1% of the total cytoplasmic protein in the hyperexpressed cells (≈1 mg/ml), it was calculated that if each of the enzyme I molecules was tagged by a gold particle, 1.8% of the cell volume would be occupied by gold particles. Therefore, under the conditions of these experiments, at least 44% of the cellular enzyme I molecules have interacted with enzyme I antibody and are associated with a gold particle derived from protein A-gold.

The distances of the gold particles relative to the inner membrane were measured from a large number of cryosections. These data have been expressed as the percentage of the gold particles present at the inner membrane and in 15-nm zones, within the cytoplasm, measured from the inner membrane. In Fig. 2, the results are presented as histograms. In the wild-type cell, ≈28% of the gold particles were at the inner membrane and this dropped to 6% in the next zone and remained at that low level in all the other zones (Fig. 2*A*). In the enzyme I overproducer strain, 8% of the gold particles were at the inner membrane. The fraction of the total gold particles then increased in successive zones followed by a gradual decline to a low level. The density of gold particle distribution was high within a 60-nm zone (Fig. 2*B*), in which ≈50% of the total gold particles were present. This is in contrast to a random distribution of the gold particles not associated with the inner membrane in the cytoplasm of the wild-type cell (Fig. 2*A*). From the data on the total number of gold particles per μm^3 of cell volume in the wild-type and enzyme I overproducer strains and the fraction of the total gold particles that are membrane associated (Fig. 2, zones 1), it was possible to calculate the relative number of membrane sites occupied by enzyme I in the two strains. The calculations indicate that the wild-type strain has 3.9 binding sites occupied per μm^3 of cell volume compared to 89 binding sites occupied per μm^3 of cell volume in the enzyme I overproducer strain. It therefore appears that there is insufficient enzyme I in wild-type cells to fully saturate the available enzyme I binding sites. When the cellular content of enzyme I is increased by hyperexpression from a plasmid, the membrane becomes saturated with enzyme I and the excess protein accumulates in the vicinity of the inner membrane.

Specificity of the Distribution of Enzyme I in the Region of the Inner Membrane. To prove that the demonstrated distribution of enzyme I was not an artifact of the methodology, an examination was made of the distribution in cryosections of

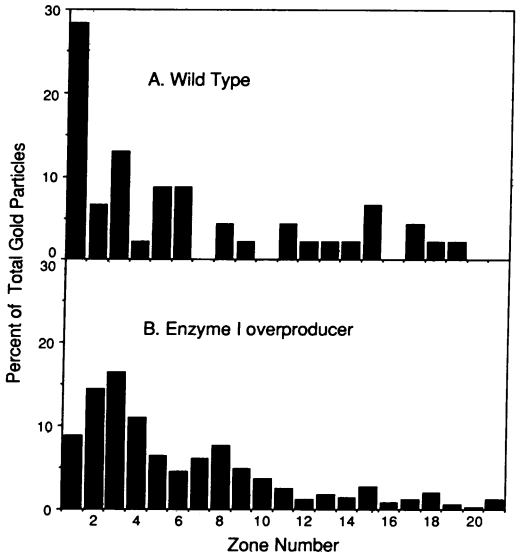

FIG. 2. Distribution of the ImmunoGold labeling by enzyme I in the wild type and enzyme I overproducer strains of *E. coli*. The percentages of the total ImmunoGold particles at the membrane and in successive 15-nm-wide zones from the cytoplasmic membrane up to the approximate midpoint of average sections of the wild-type (*A*) and enzyme I overproducer (*B*) strains are shown. Zone 1, 0 nm (i.e., at the membrane); zone 2, all particles 0.1 nm from the edge of the membrane up to 15.0 nm from the membrane; zones 3–21, successive 15-nm zones up to 300 nm, which is the approximate midpoint of the cells.

another protein (β-galactosidase) generally assumed to be cytoplasmic. Fig. 3 shows the results of such a study. In cryosections of both the enzyme I overproducer strain (Fig. 3*a*) and the wild-type strain (Fig. 3*b*), β-galactosidase immunoreactivity is randomly distributed throughout the cytoplasm. In contrast, the enzyme I overproducer strain shows substantial localization of enzyme I at and in the vicinity of the inner membrane (Fig. 3*c*). We conclude from this experiment that the immunoelectron microscopic procedure used here can distinguish between a membrane-localized and a cytoplasmic protein.

The distribution of enzyme I and β-galactosidase immunoreactivity in a 60-nm zone of the cytoplasm close to the inner membrane compared to the remainder of the cytoplasm of cryosections of strain 600 was calculated (Table 2). The data indicate that enzyme I immunoreactivity is 4–18 times more

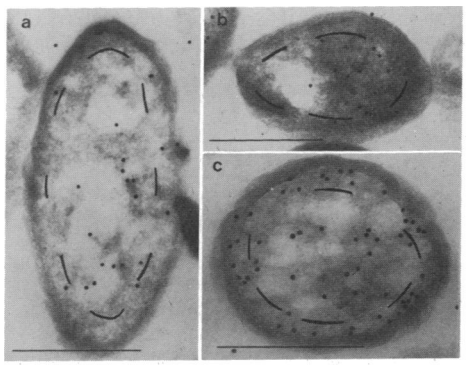

FIG. 3. A comparison of the immunolabeling of enzyme I and β-galactosidase in cryosections of wild-type and enzyme I overproducer strains of *E. coli*. (*a*) Immunolabeling for β-galactosidase in enzyme I overproducer strain. (*b*) Immunolabeling for β-galactosidase in wild-type *E. coli*. (*c*) Immunolabeling for enzyme I in enzyme I overproducer strain. See legend to Fig. 1 for a description of the broken line. (Bars = 0.5 μm.)

dense in the region of the cytoplasm near the inner membrane than in the remainder of the cytoplasm. In contrast, the β-galactosidase immunoreactivity is randomly distributed in the cytoplasm. These quantitative measurements support the idea that enzyme I is specifically concentrated in the vicinity of the inner membrane.

Specificity of the Immunolabeling of Enzyme I. The labeled cryosections shown in Fig. 1 were prepared by using a polyclonal antibody that was made from a purified preparation of enzyme I. To ensure that the demonstrated immunolabeling was, in fact, due to a specific immune reaction against enzyme I, the monospecificity of the antiserum preparation was examined by an immunoblot (Fig. 4). It can be seen that the antiserum produces only one band of immune labeling in extracts of the overexpression strain 600 (Fig. 4*B*, lane 1) and that this band is coincident with that corresponding to purified enzyme I (lane 2). Further evidence that the immune reaction is specific comes from the observation that an extract of a strain of *E. coli* in which the genes for HPr and enzyme I are deleted (strain DS166; see Table 1) shows no bands of immunoreactivity (lane 3).

A variety of control experiments were done on cryosections to show that the type of labeling observed in Fig. 1 was specific for enzyme I. Fig. 5*d* shows a representative cryosection of the enzyme I overproducer strain labeled with the enzyme I antiserum. Evidence that the labeling is specific comes from the finding that omission of the immune serum abolishes the ImmunoGold labeling pattern in cryosections of the enzyme I overproducer strain (Fig. 5*a*). Another line of evidence documenting the specificity of the immunolabeling was provided by the experiment shown in Fig. 5*b*. When a cryosection of the enzyme I overproducer strain was labeled with enzyme I antiserum under the same conditions as used for Fig. 5*d* and then treated with purified enzyme I, all of the immunolabeling was displaced. A final test of the specificity of the immunolabeling of the cryosections is shown in Fig. 5*c*. In this case, the sections were prepared from a strain of *E. coli* (DS166; see Table 1) that does not produce enzyme I because the relevant genes have been deleted. It can be seen that, while the enzyme I overproducer strain shows substantial immunolabeling with the enzyme I antiserum (Fig. 5*d*), there is no labeling in the strain that does not produce enzyme I (Fig. 5*c*).

DISCUSSION

The PTS of *E. coli* plays a crucial role in the transport of a number of carbohydrates. The characteristics of this system have been thoroughly reviewed (1, 2). From the standpoint of the focus of this study, it is important to point out that the system is generally described as being composed of sugar-specific integral membrane proteins as well as general soluble proteins, one of which is enzyme I. The pathway of sugar transport coupled to phosphorylation involves phosphotransfer from enzyme I to HPr and then frequently to an integral sugar-specific membrane protein. Therefore, HPr must exhibit some degree of association with membrane proteins, although possibly transient. It might be the case that HPr

Table 2. Regional distribution of enzyme I and β-galactosidase activity

Strain	Antigen	Particles per μm^2		
		Inner membrane zone (A)	Central zone (B)	A/B
600	Enzyme I	292	73	4.0*
		888	50	17.8†
		470	76	6.2‡
600	β-Galactosidase	33	38	0.9§
KL16	β-Galactosidase	35	72	0.5¶

The areas (expressed in μm^2) were determined by following Simpson's rule (22); the gold particles were quantitated as described. Strain 600 = enzyme I overproducer; strain KL16 = wild-type; zone A = 60 nm zone near inner membrane; zone B = remainder of cytoplasm. Data in the table were from the following cryosections: *, Fig. 3*c*; †, Fig. 1*c*; ‡, Fig. 1*d*; §, Fig. 3*a*; ¶, Fig. 3*b*.

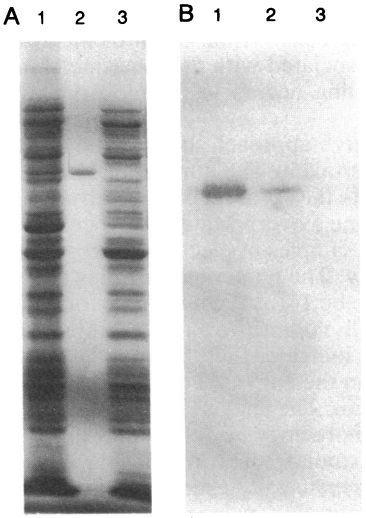

FIG. 4. Immunoblot analysis of antiserum to enzyme I. (*A*) Coomassie blue-stained SDS/PAGE as follows: lane 1, broken cell extract of strain 600 (enzyme I overproducer), 12.4 μg of cell protein; lane 2, purified enzyme I, 6.0 μg of protein; lane 3, broken cell extract of strain DS166 (PTS deletion mutant), 3.9 μg of cell protein. (*B*) Immunoblot as follows: lane 1, purified enzyme I, 0.2 μg of protein; lane 2, extract of strain 600, 1.0 μg of cell protein; lane 3, extract of strain DS166, 1.0 μg of cell protein.

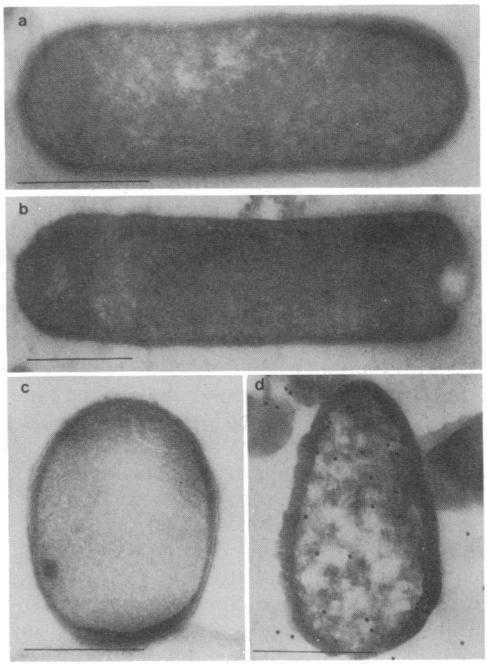

FIG. 5. Specificity of immunolabeling of enzyme I in the enzyme I overproducer strain. Preparations of the cells and labeling of the cryosections were performed as described in *Materials and Methods* with the exceptions described in the legends to the figures. (*a*) Immune serum omitted. (*b*) Section was treated with immune serum followed by purified enzyme I. (*c*) Section was prepared from the PTS deletion mutant strain DS166. (*d*) Control labeling with immune serum. (Bars = 0.5 μm.)

shuttles between an association with membrane-bound phosphoacceptors and cytoplasmic enzyme I. However, there are some lines of evidence suggesting that the "soluble" proteins of the PTS might be associated with the integral membrane proteins to form a functional sugar transport complex (23, 24). Gachelin (25) showed that *E. coli* permeabilized with toluene had a higher PTS activity than broken cell extracts, suggesting that intact cells contained an organized unit of the soluble proteins of the PTS with the specific integral membrane proteins. In Kaback's studies (4) of PTS-dependent transport of methyl α-glucoside in membrane vesicles prepared from *E. coli*, he found phospho*enol*pyruvate-dependent sugar uptake in wild-type bacteria but not in bacteria that did not make enzyme I; these findings were interpreted in the framework of an association of some fraction of the cellular enzyme I and HPr with the inner membrane. A similar set of experiments was carried out by Saier *et al.* (5), who also suggested that a fraction of the soluble PTS proteins was associated with the inner membrane. On the basis of the observed hydrophobicity of enzyme I, as well as the nature of the interactions of enzyme I and HPr, Misset and Robillard (26) suggested that the PTS may exist *in vivo* as a multiprotein complex.

The immunoelectron microscopic studies presented in this paper support and extend these ideas. We show here that at least one-third of the cellular enzyme I of wild-type *E. coli* (Fig. 2) exists associated with the inner membrane. Furthermore, by taking advantage of our capability to vary the cellular content of enzyme I by hyperexpression of the protein, evidence is presented that there are a limited number of inner membrane binding sites for the enzyme I protein.

The use of immunochemistry coupled with electron microscopy of cryosections has evolved as a powerful tool for determining the physiological location of proteins in microbes (6). Lazdunski has effectively used this approach for the localization in *E. coli* of elongation factor Tu (27) and colicins (28, 29). Since it is essential in a study of this nature to demonstrate the specificity of the immune reaction, a number of controls were performed. First, a Western blot (Fig. 4) demonstrated that the antiserum used reacted only with a protein(s) that migrated on acrylamide gels to the same position as authentic enzyme I. Second, we showed (Fig. 5) that preimmune serum from the same rabbit that produced our immune serum gave no immunolabeling of cryosections of the *E. coli* enzyme I overproducer strain. Third, authentic purified enzyme I was able to displace antibody bound to membrane-associated enzyme I in the sections. Last, there was no immune serum-dependent labeling by protein A-gold when the sections were prepared from a strain of *E. coli* deficient in enzyme I.

An unusual observation in this study concerns the distribution of enzyme I in the enzyme I overproducer strain (Figs. 1, 3, and 5). The protein that is not associated with the inner membrane binding sites accumulates in a zone ≈60 nm wide that is close to the inner membrane. Since β-galactosidase does not show this zonal distribution (Fig. 3), the observed pattern is not an artifact of the methodology used here. Since there is no well-identified structure in this 60-nm zone that could account for the banding of enzyme I, we considered the possibility that this accumulation is related to the specific enzyme I binding sites. We favor the hypothesis that the milieu of the cytoplasm inhibits a free and rapid diffusion of cytoplasmic enzyme I. The accumulation of the protein close to the membrane might reflect the rapid exchange of bound enzyme I with that in the cytoplasm resulting in a net hydrophobic drift of the protein to the region of the inner membrane. It should be noted that an identical process might be occurring in wild-type cells; however, the lower density of immunoreactive enzyme I makes it difficult to evaluate. It would be of interest to examine the distribution of other proteins of *E. coli* that interact with the inner membrane to determine whether, under conditions of hyperexpression, a similar banding phenomenon occurs.

These data suggest that *E. coli* enzyme I is associated with a membrane binding site *in vivo*. It is tempting to speculate that this binding site is an integral PTS protein. We imagined that, if this were the case, the increased membrane association of enzyme I resulting from hyperexpression of enzyme I might affect sugar transport activity. However, assay of sugar uptake in intact cells or sugar phosphorylation in cells permeabilized with toluene showed no evidence for a significant difference in these activities between wild-type and enzyme I overproducer strains. These observations suggest that, if the enzyme I binding sites detected here are related to the transport of methyl α-glucoside or 2-deoxyglucose, the membrane association of enzyme I is not the limiting factor in sugar transport. Clearly, additional studies will be necessary to elucidate the nature of the site or sites that account for the inner membrane association of enzyme I as well as the possible physiological ramifications of the interaction of enzyme I with the inner membrane.

1. Postma, P. W. & Lengeler, J. W. (1985) *Microbiol. Rev.* **49**, 232–269.
2. Meadow, N. D., Kukuruzinska, M. A. & Roseman, S. (1984) in *The Enzymes of Biological Membranes*, ed. Martonosi, A. (Plenum, New York), Vol. 3, pp. 523–559.
3. Danchin, A. (1987) *Microbiol. Sci.* **4**, 267–269.
4. Kaback, H. R. (1968) *J. Biol. Chem.* **243**, 3711–3724.
5. Saier, M. H., Jr., Cox, D. F., Feucht, B. U. & Novotny, M. J. (1982) *J. Cell. Biochem.* **18**, 231–238.
6. Smit, J. & Todd, W. J. (1986) *Ultrastructure Techniques for Microorganisms* (Plenum, New York).
7. Guan, T., Ghosh, A. & Ghosh, B. K. (1985) *J. Bacteriol.* **164**, 107–113.
8. Tinglu, G., Ghosh, A. & Ghosh, B. K. (1984) *J. Bacteriol.* **159**, 668–677.
9. Liberman, E., Saffen, D., Roseman, S. & Peterkofsky, A. (1986) *Biochem. Biophys. Res. Commun.* **141**, 1138–1144.
10. Roy, A. & Danchin, A. (1982) *Mol. Gen. Genet.* **188**, 465–471.
11. Saffen, D. W., Presper, K. A., Doering, T. L. & Roseman, S. (1987) *J. Biol. Chem.* **262**, 16241–16253.
12. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
13. Tokuyasu, K. T. (1984) in *Immunolabelling for Electron Microscopy*, eds. Polak, J. M. & Varndell, I. M. (Elsevier, Amsterdam), pp. 71–82.
14. Dubchet, J., Adrian, M., Lepault, J. & McDowell, A. W. (1985) *Trends Biochem. Sci.* **10**, 143–146.
15. Griffith, G., McDowell, A., Back, R. & Dubchet, J. (1984) *J. Ultrastruct. Res.* **89**, 65–78.
16. Weigel, N., Waygood, E. B., Kukuruzinska, M. A., Nakazawa, A. & Roseman, S. (1982) *J. Biol. Chem.* **257**, 14461–14469.
17. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 353–355.
18. Weibel, E. R. (1979) *Stereol. Methods* **1**, 41–49.
19. Harwood, J. P., Gazdar, C., Prasad, C., Peterkofsky, A., Curtis, S. J. & Epstein, W. (1976) *J. Biol. Chem.* **251**, 2462–2468.
20. Solomon, E. & Lin, E. C. C. (1972) *J. Bacteriol.* **111**, 566–574.
21. Tsang, V., Peralta, J. & Simons, A. R. (1983) *Methods Enzymol.* **92**, 377–391.
22. Chemical Rubber Co. (1943) *Handbook of Chemistry and Physics* (Chemical Rubber Co., Cleveland), p. 260.
23. Reddy, P., Meadow, N., Roseman, S. & Peterkofsky, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8300–8304.
24. Liberman, E., Reddy, P., Gazdar, C. & Peterkofsky, A. (1985) *J. Biol. Chem.* **260**, 4075–4081.
25. Gachelin, G. (1969) *Biochem. Biophys. Res. Commun.* **34**, 382–386.
26. Misset, O. & Robillard, G. T. (1982) *Biochemistry* **21**, 3136–3142.
27. Bernadac, A. & Lazdunski, C. (1981) *Biol. Cell* **41**, 211–216.
28. Geli, V., Baty, D. & Lazdunski, C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 689–693.
29. Baty, D., Knibiehler, M., Verheij, H., Pattus, F., Shire, D., Bernadac, A. & Lazdunski, C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1152–1156.