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Catalytic Activities Associated With the Enzymes II of the Bacterial Phosphotransferase System

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The phosphotransferase system (PTS) in Escherichia coli is a multifunctional, multicomponent enzyme system. Its primary functions deal with carbon source acquisition, while its secondary functions are concerned with the regulation of bacterial physiology. The primary functions of the system include 1) extracellular detection, 2) unidirectional and exchange transmembrane transport, and 3) phosphoenolpyruvate-dependent and sugar phosphate-dependent phosphorylation of the sugar substrates of the system. The secondary functions include 1) regulation of the activities of adenylate cyclase and various non-PTS permeases and 2) regulation of the induced synthesis of several PTS enzymes. Both the primary and secondary functions appear to be elicited by the binding of a sugar substrate to an Enzyme II complex. One of these integral transmembrane enzymes, the mannitol Enzyme II (II^{mtl}), has been solubilized with detergent, purified to homogeneity, and reconstituted in an artificial membrane system. The molecular weight of this protein, II^{mtl}, is 60,000 daltons. It possesses an extracellular sugar binding site and distinct intracellular combining sites for sugar phosphate and phospho-HPr. An essential sulfhydryl group and an antibody combining site are localized to the cytoplasmic surface of the enzyme, while a dextran combining site is localized to the external surface. Preliminary experiments suggest that the different functions of the Enzyme II^{mtl} can be dissected by genetic and biochemical techniques. These studies emphasize the functional complexity of the PTS and its integral membrane protein constituents.

Key words: carbohydrates, transport, chemotaxis, regulation, phosphotransferase system, bacteria

Man, being the servant and interpreter of nature, can do and understand so much and so much only as he has observed in fact or in thought of the course of nature; beyond this he neither knows anything nor can do anything.

— Francis Bacon

During the past three decades considerable effort has been devoted to the study of transmembrane transport. Extensive kinetic studies of transport processes have been published, and much information has contributed to an understanding of the energy

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coupling mechanisms for different transport systems [1–6]. However, few of the bacterial permease constituents have been isolated, and in no case has the actual translocation mechanism been elucidated. Toward this end, we have devoted our attention to the bacterial phosphotransferase system (PTS), which uniquely couples the transmembrane translocation of its sugar substrates with sugar phosphorylation. This coupled process involves the participation of 2 general energy-coupling proteins, Enzyme I and HPr, as well as the sugar-specific Enzyme II complexes [7–9].

In this review, we shall discuss the functions of the PTS with emphasis on the recent work that has been performed in our laboratory. The mechanisms of transmembrane transport via the recently purified mannitol Enzyme II will be considered in some detail. Both unidirectional and bidirectional exchange transport will be discussed.

In extensive studies, the PTS has been shown to regulate the activities of a variety of non-PTS permease systems and the cyclic AMP synthetic enzyme, adenylate cyclase [1, 4, 8–11]. It also affects the transcriptional regulation of certain genes that code for protein constituents of the PTS. Preliminary studies suggest that an autoinduction-type mechanism involving exogenous (rather than intracellular) inducers is operative [12]. These studies, which emphasize the functional complexity of the bacterial phosphotransferase system, have been dealt with in a recently published review [1] and will not be reiterated here.

METHODS

The methods used in the studies reported here have either been published previously [13–16] or are presented in the figure and table legends.

RESULTS

Physiological Functions of the PTS in the E coli Cell

The early studies of Kundig, Ghosh, and Roseman [17] showed that the phosphotransferase system was capable of phosphorylating a variety of hexoses, including glucose, mannose, and fructose. Subsequent studies in the laboratory of E.C.C. Lin showed that hexitol utilization was also initiated by the PTS [18]. Figure 1 illustrates the enzymatic machinery involved in mannitol utilization in E coli. Extracellular mannitol is transported across the membrane and phosphorylated in a coupled process that requires the proteins of the PTS: Enzyme I, HPr, and Enzyme II^{mtl}. Subsequently, cytoplasmic mannitol-1-phosphate is oxidized to fructose-6-phosphate by NAD and a soluble mannitol phosphate dehydrogenase; fructose-6-phosphate is then metabolized via the glycoytic pathway.

The functions of the mannitol Enzyme II are listed in Table I. The enzyme is thought to function as the mannitol chemoreceptor, allowing the organism to recognize and respond to concentration gradients of this hexitol by swimming up the gradient [19, 20]. It also mediates 2 transport processes, termed unidirectional and bidirectional group translocation [9]. In these 2 processes, the sugar substrate is phosphorylated, on the one hand, at the expense of phosphoenolpyruvate (PEP); on the other hand, at the expense of mannitol-l-phosphate. Thus, the vectorial reactions of the PTS are inextricably linked to chemical reactions in the E coli cell.

Regulatory functions of the Enzyme II^{mtl} are also listed in Table I. The enzyme functions in the autoinduction of several proteins of the PTS. These include Enzyme I and HPr, the protein products of the PTS operon, as well as the mannose and glucose Enzymes II [12]. The Enzymes II have also been shown to play a role in the control

TABLE I. Physiological Functions of the Mannitol Enzyme II in E Coli

I. Catalytic functions
   A. Chemoreception
   B. Unidirectional and exchange group translocation
   C. Phosphoenolpryuvate- and mannitol-1-P-dependent sugar phosphorylation

II. Regulatory functions
   A. Autoinduction of PTS protein synthesis
   B. Control of non-PTS permease activity
   C. Control of adenylate cyclase activity

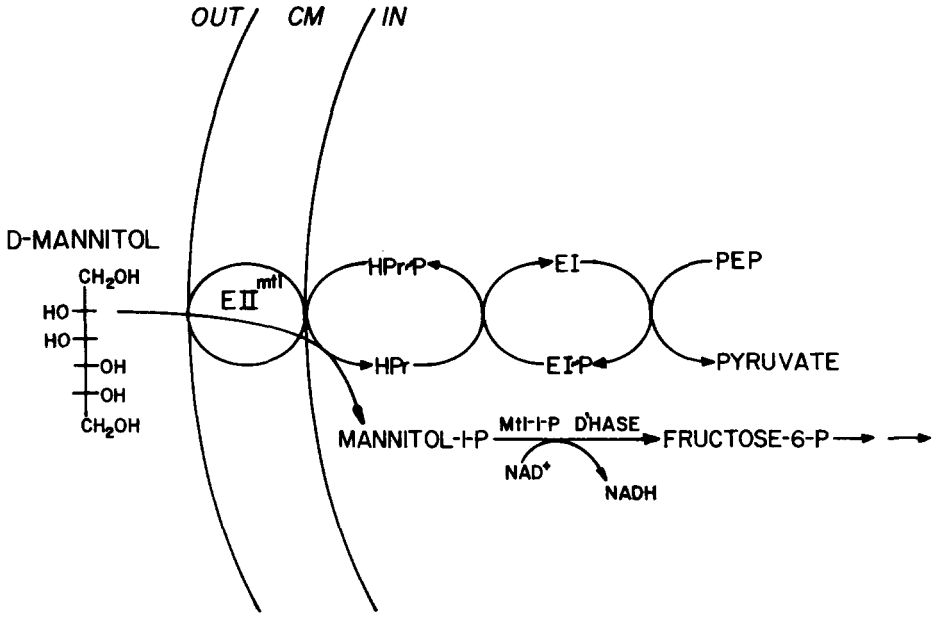

Fig. 1. Pathway of mannitol utilization in E coli.

of the activities of adenylate cyclase and certain non-PTS permeases. Up-to-date discussions of the regulatory functions of the PTS can be found in recent reviews [1, 4, 9].

Unidirectional and Exchange Group Translocation Catalyzed by the Enzyme II Complexes of the PTS

Extensive kinetic studies on the glucose transport system in the human red blood cell [21, 22] have shown that this system catalyzes 2 distinct transport processes (Fig. 2). In unidirectional transport, a glucose molecule, G_1, is transported from the external cell surface into the cytoplasm, where it is metabolized as a source of carbon and energy. The transport protein, after a period of time (Δt), can bind a second glucose molecule, G_2, on the external surface of the membrane and translocate it in the same direction. Because the sugar always moves in a single direction, we refer to the process as unidirectional transport.

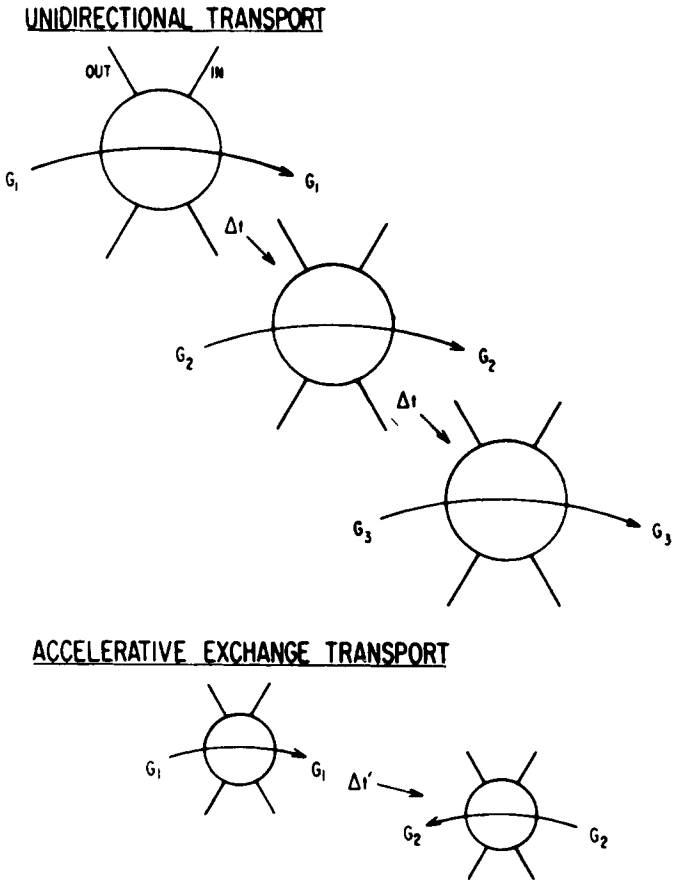

Fig. 2. Schematic illustration of unidirectional and accelerative exchange transport processes catalyzed by a permease system.

This process should be contrasted with that which is observed when the substrate sugar is present in appreciable concentrations on both sides of the membrane. A glucose molecule, G_1, moves first in one direction, and this movement is followed (or counteracted) by movement of a second glucose molecule, G_2, in the opposite direction (Fig. 2). Because the time for this exchange transport to occur is less than half the time necessary for unidirectional transport (ie, $\Delta t'$ is less than half of Δt in Fig. 2), we refer to the process as accelerative exchange transport.

While many transport systems that catalyze facilitated diffusion (such as the glucose transport system in the red cell) or substrate-proton symport (such as the lactose permease in E coli) have been shown to catalyze both of these processes [23, 24], other systems, such as those involving periplasmic solute binding proteins, catalyze only unidirectional transport [25, 26]. Still other systems, such as the anion transport system in the red blood cell [27] catalyze exchange transport processes exclusively. It seems reasonable that all transport systems that exhibit the phenomenon of exchange transport will display some common mechanistic features. Those for which this process cannot be demonstrated may translocate their substrates by a quite different mechanism.

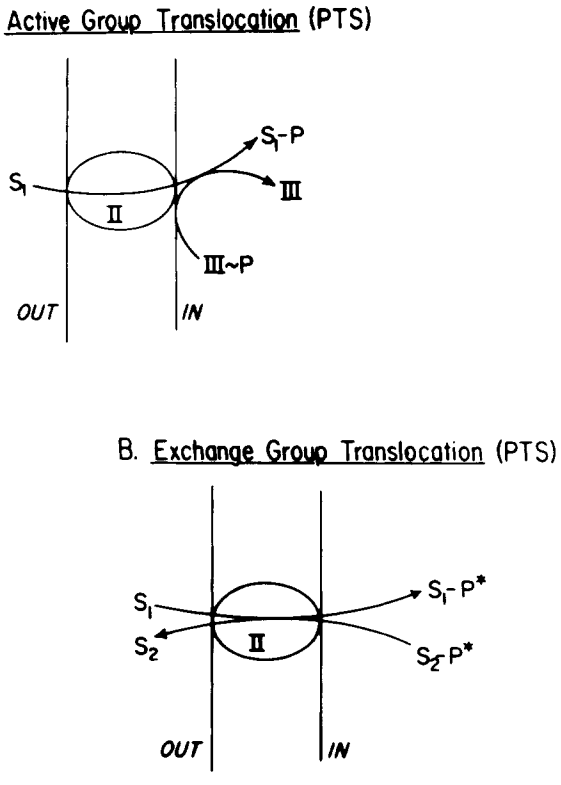

Fig. 3. Schematic illustration of unidirectional and bidirectional exchange group translocation catalyzed by an Enzyme II of the PTS.

An Enzyme II of the PTS can catalyze both unidirectional and exchange transport processes (Fig. 3). In the former process, sugar is translocated across the membrane and phosphorylated at the expense of phosphoenolpyruvate, the ultimate phosphate donor [7–9]. This process requires the energy-coupling proteins of the PTS, Enzyme I, and HPr. In some cases, a sugar-specific Enzyme III is also required [28, 29]. By contrast, the Enzyme II is both necessary and sufficient for catalysis of bidirectional-exchange group translocation (Fig. 3). In this process, a sugar on the external cell surface is brought into the cell and phosphorylated at the expense of an intracellular sugar phosphate. The sugar moiety of the latter molecule is expelled from the cell in a coupled process. Recently, the stoichiometry of this vectorial process has been demonstrated [M.R. Schmidt and M.H. Saier, Jr., unpublished results]. Employing an E coli mutant of J. Lengler that lacked mannitol phosphate dehydrogenase [32], it was shown that for every molecule of mannitol taken up and phosphorylated by exchange group translocation, a molecule of mannitol was expelled from the internal sugar phosphate pool. This demonstration of the stoichiometric relationship of the vectorial-exchange group-translocation process, together with the results of earlier studies [30, 31], provides a detailed description of these chemically driven vectorial reactions.

To provide mechanistic information about the transphosphorylation reactions, Ada Rephaeli carried out in our laboratory, detailed kinetic analyses of the reactions catalyzed

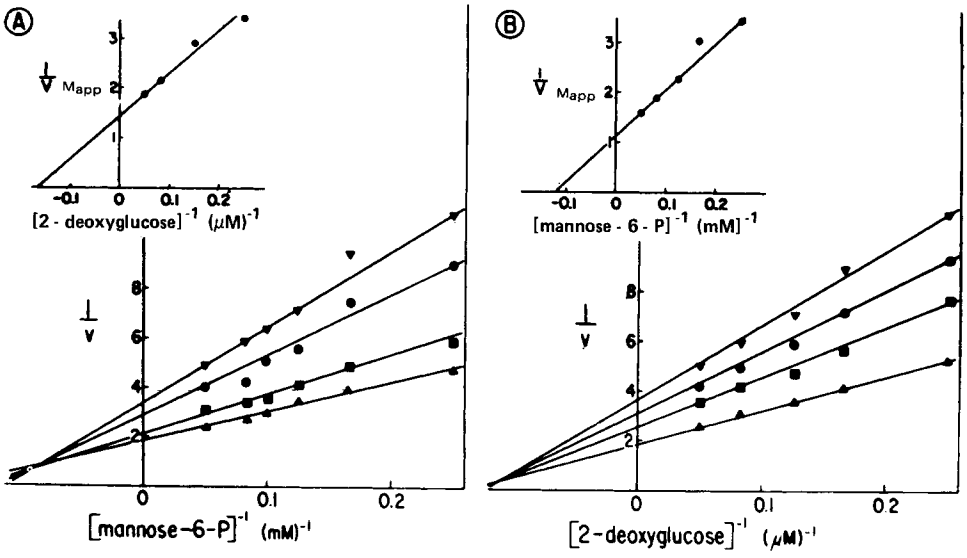

Fig. 4. Kinetic analysis of transphosphorylation catalyzed by the mannose Enzyme II of the PTS (A.W. Rephaeli and M.H. Saier, Jr., unpublished results).

by the glucose and the mannose Enzyme II complexes [33, 34]. Some of her kinetic studies are depicted in Figure 4. The results for the mannose Enzyme II can be summarized as follows: 1) The 2 substrates bind to the surface of the Enzyme II complex in a random fashion. That is, either sugar or sugar phosphate can bind to the enzyme first, and the alternate substrate binds second. There is no obligate order of substrate binding. This result is expected for a transport mechanism in which one substrate (the sugar) approaches the Enzyme II complex from the extracellular side of the membrane while the other substrate (the sugar phosphate) approaches the complex from the cytoplasmic side. 2) The reaction mechanism was shown to be of the "sequential" type. That is, while the 2 substrates can bind to the enzyme complex in random order, both must be present on the surface of the enzyme for the reaction to occur. This result (Fig. 4) is not consistent with a "ping-pong" type mechanism in which one substrate (ie, the sugar phosphate) binds to the enzyme first, modifies it, and then dissociates from the enzyme surface before the other substrate (ie, the sugar) binds. Direct transfer of the phosphoryl moiety from the sugar phosphate to the sugar is implied. 3) The 2 substrates were shown to bind in a positively cooperative fashion. Binding of one substrate (ie, the sugar) to the enzyme was found to enhance the affinity of the enzyme complex for the other substrate (ie, the sugar phosphate). Assuming that the 2 substrates bind to opposite surfaces of the membrane, this result leads to the suggestion that the binding of the sugar to the external side of the Enzyme II complex generates a signal, presumably a conformational change through the enzyme complex, to the cytoplasmic surface. It is possible that such a process is important not only to transport, but also to chemoreception. If 2 subunits are involved, this signal must be transmitted to the polypeptide chain adjacent to the one to which the sugar substrate is bound (see below). 4) Binding of a series of sugar substrates to the Enzyme II^{man} complex exhibited the same relative affinities as did the homologous series of sugar phosphate substrates. Thus, the relative order of binding affinities for

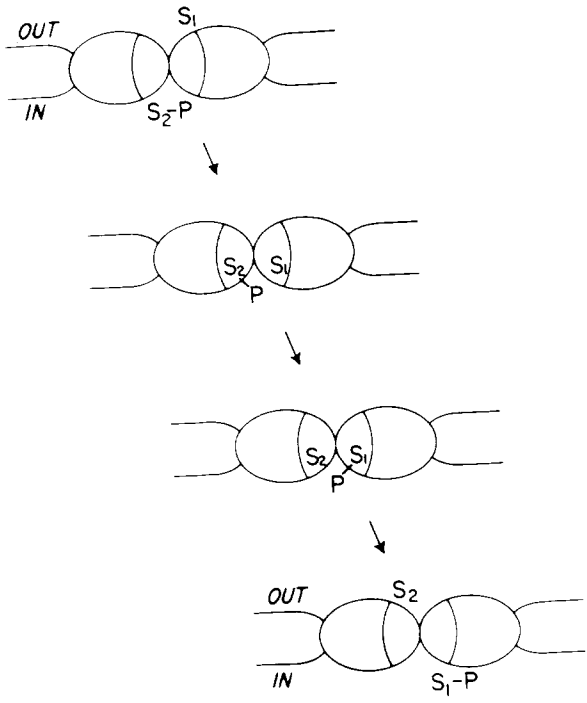

Fig. 5. Possible mechanism of exchange group translocation catalyzed by an Enzyme II complex of the PTS.

the sugar series was glucose > mannose > 2-deoxyglucose > N-acetylglucosamine > fructose, while that for the sugar phosphate series was glucose-6-P > mannose-6-P > 2-deoxyglucose-6-P > N-acetylglucosamine-6-P > fructose-6-P. The similarity in relative substrate binding within these 2 homologous series suggested that the kinetically distinguishable sugar and sugar phosphate binding sites might actually represent 2 different conformations of a single active site of the protomeric species. Assuming this to be the case, one must invoke the simultaneous participation of two adjacent subunits to account for a sequential transphosphorylation mechanism. On the basis of these results and considerations, we proposed the model for an Enzyme II complex depicted in Figure 5. As illustrated in the figure, the complex is thought of as an array of 2 (or more) protein subunits with the single substrate binding site localized to the center of the complex. The complex consequently exhibits approximate bilateral symmetry, and the active sites of the adjacent protomers form a transmembrane channel [35]. For the complex to catalyze transphosphorylation, a sugar (S_1) must approach from the external surface of the membrane while the sugar phosphate (S_2-P) approaches the complex from the cytoplasmic side (Fig. 5). The 2 substrates then bind to the adjacent active sites in an almost symmetrical array, such that the phosphate group of S_2-P is midway between S_1 and S_2. Then, as a result of nucleophilic attack by the 6-hydroxyl group of S_1 on the phosphorus atom of S_2-P, displacement of phosphate to S_1 occurs (Fig. 5). This phosphoryl transfer reaction is presumably accompanied by minor conformational changes in the subunits so that S_1-P must be released into the cytoplasm while S_2 is expelled from the cell into the external medium. The net result is vectorial-exchange group translocation.

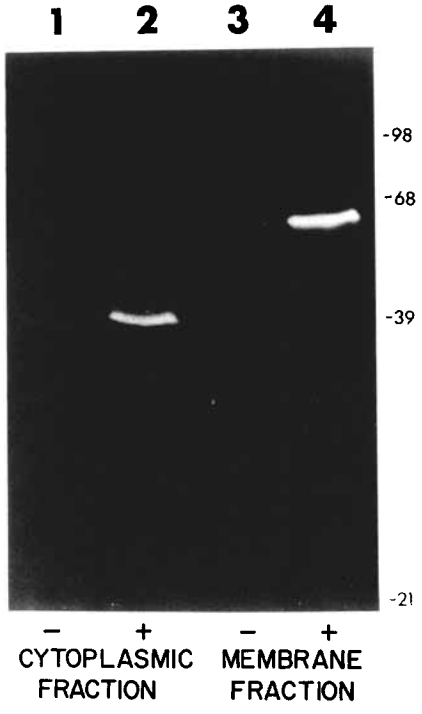

Fig. 6. Proteins coded for by plasmid pLC15-48 and synthesized in an E coli minicell strain (C.A. Lee, G.R. Jacobson, and M.H. Saier, Jr., unpublished results). The (–) and (+) indicate the absence and presence of cyclic AMP and inducer (mannitol) prior to analysis. Molecular weights of standard proteins are indicated on the right.

Properties of Purified Mannitol Enzyme II From E coli

To further our understanding of the mechanism by which the Enzymes II catalyze sugar transport, we felt that it would be necessary to obtain an Enzyme II complex in pure form. Therefore, Gary Jacobson solubilized the mannitol Enzyme II from the membrane, employing doxycholate in the presence of a high salt concentration and purified the protein to apparent homogeneity, employing hydrophobic chromatographic and hydrophobic ion-exchange procedures [16]. The purified Enzyme II complex consisted of a single polypeptide chain of 60,000 molecular weight that catalyzed both the phosphoenolpyruvate-dependent and the mannitol-1-phosphate-dependent phosphorylation of mannitol.

Considerable published evidence [19, 20] supports the conclusion that the Enzyme II functions as the mannitol chemoreceptor as well as the protein that transports and phosphorylates this sugar. The synthesis of all 3 activities has been shown to be induced by growth of the cells in the presence of mannitol [36, 37]. To establish that the Enzyme II alone catalyzes these functions, Cathy Lee and Gary Jacobson cloned the mannitol operon and studied its expression in minicells of E coli [38]. Some of the results of their study are shown in Figure 6.

In response to inducer (mannitol) and cyclic AMP, only 2 proteins were synthesized (Fig. 6). One of these was a cytoplasmic protein having a molecular weight of

40,000 and the properties of mannitol-1-phosphate dehydrogenase. The other was an integral membrane protein with a molecular weight of 60,000. It had the immunological and physicochemical properties of the Enzyme II^{mtl}. Since a single membrane protein was synthesized in response to inducer and cyclic AMP, it must be concluded that the mannitol Enzyme II not only catalyzes the 2 sugar phosphorylation reactions, it also serves as the mannitol chemoreceptor, permease, and kinase in vivo. The second conclusion has recently been partially confirmed. The purified Enzyme II^{mtl} has been reconstituted in an artificial phospholipid vesicular membrane (Huangosomes) [39]. The Enzyme II catalyzed the transport of mannitol across the membrane both by exchange group translocation and by facilitated diffusion (J.E. Leonard, unpublished results). That the mannitol Enzyme II could catalyze facilitated diffusion had been concluded from earlier studies with whole cells [40, 41].

Sidedness Properties of The Enzyme II^{mtl}

Recent work with chemical reagents has confirmed the suggestion that the Enzyme II^{mtl} spans the membrane. Preliminary evidence for this suggestion was obtained by kinetic studies with dead-end inhibitors [34]. The nonphosphorylatable glucose analogue, 6-deoxyglucose, inhibited transphosphorylation catalyzed by the mannose Enzyme II by a mechanism that was shown to be competitive with respect to the sugar substrate but noncompetitive with the sugar phosphate phosphoryl donor. This glucose analogue was also a potent inhibitor of sugar uptake in whole cells. By contrast, glucose-6-sulfate and glucosamine-6-phosphate, 2 inactive sugar phosphate analogues, inhibited the reaction by a mechanism that was competitive with respect to the sugar phosphate substrate but noncompetitive with respect to the sugar. In these cases, the inhibitors were not inhibitory when sugar uptake was examined in whole cells. This result leads to the tentative conclusion that the sugar binding site expresses itself only on the external surface of the membrane while the sugar phosphate binds exclusively to the cytoplasmic surface of the Enzyme II complex.

That the Enzyme II does, in fact, span the membrane was shown using membrane impermeable reagents. Under appropriate conditions p-chloro-mercuriphenylsulfonate (pCMPS) exhibits specificity for sulfhydryl groups in the protein and does not permeate the membrane [42]. This reagent was found to inhibit completely Enzyme II^{mtl} activities when present on the cytoplasmic surface of the enzyme complex but had no effect when added externally [M.H. Saier, Jr., and M.R. Schmidt, unpublished observation]. The same had been shown previously by Hagenauer-Tsapis and Kepes [43] for the glucose Enzyme II. Additionally, antibody prepared against homogeneous Enzyme II^{mtl} was found to inhibit the activity of the enzyme only when present on the cytoplasmic side of the membrane [G.R. Jacobson, C.A. Lee, and M.H. Saier, Jr., unpublished observation].

A recent report from Robillard's laboratory has shown that low molecular weight α-1, 6-glucan found in crude extracts of E coli (2,500 daltons) interacts with and activates the PEP-dependent phosphotransferase activity of the PTS [44]. Although the site of action of the glucan was not determined, the authors suggested that the polysaccharide activates at the level of the phospho-Enzyme I-HPr complex or the HPr-Enzyme II complex of the PTS. In pursuing this problem, we found that commercially available α-1, 6-glucan (dextran) of an average molecular weight of 40,000 inhibited the transphosphorylation reaction catalyzed by the mannitol Enzyme II. Moreover, it appeared to do so by binding to a site on the external surface of the Enzyme II complex (M.R. Schmidt and M.H. Saier, Jr., unpublished observations). Thus, the Enzyme II^{mtl} appears to span the

membrane with binding sites for sugar and dextran on the external side of the bilayer and sites for phospho-HPr, sugar phosphate, pCMPS, and anti-Enzyme II^{mtl}-specific antibody on the cytoplasmic surface of the membrane.

Evidence That Transphosphorylation Depends Upon Transient Subunit Interactions

It was suggested (Fig. 5) that vectorial transphosphorylation (exchange group translocation) depends on subunit interactions. We have observed that the properties of this reaction show 3 distinct differences from those of the phosphoenolpyruvate-dependent reaction, differences that would not be expected based on the fact that a single protein catalyzes both reactions. The properties that show these differences include the pH-activity profiles, the temperature dependencies of the 2 reactions, and the enzyme concentration dependencies. The anomalous results to be described appear to be explainable by the postulate that while a single Enzyme II^{mtl} subunit catalyzes the phosphoenolpyruvate-dependent reaction, 2 subunits must come into direct contact to catalyze the sugar phosphate:sugar transphosphorylation reaction.

In early studies [30, 45], we showed that for 6 different Enzyme II complexes, the pH optima for the phosphoenolpyruvate:sugar-phosphotransferase activities were between pH 7 and pH 10, but the pH optima for the transphosphorylation reactions were on the acidic side, between pH 4 and pH 7. This observation has been confirmed with the purified mannitol Enzyme II. The pH optimum for mannitol phosphorylation with phosphoenolpyruvate as the phosphoryl donor was 9.5, but that for the mannitol-1-phosphate: mannitol transphosphorylation reaction was 6.0 [C.A. Lee, J.E. Leonard and M.H. Saier, Jr., unpublished results).

The temperature dependencies of the 2 reactions were studied over a temperature range of 0°C to 40°C, with the pure enzyme dissolved in detergent (lubrol) micelles. Arrhenius plots of the data (log v vs $1/T°K$) gave a straight line for the PEP-dependent reaction, but a break in the curve at about 23°C was obtained for the transphosphorylation reaction [J.E. Leonard, unpublished results]. Similar data were generated for the native enzyme in E coli membrane fragments, but a transition at about 13°C was observed for the transphosphorylation reaction. Therefore it appeared that the transphosphorylation reaction was more sensitive to the fluidity of the hydrophobic environment in which the Enzyme II^{mtl} was imbedded than was the PEP-dependent reaction.

The third parameter distinguishing the PEP- and mannitol-1-P-dependent reactions was the Enzyme II concentration dependencies of these reactions (Fig. 7). While a plot of the activity of the former reaction was linear with enzyme concentration, that of the mannitol-1-P-dependent reaction showed marked upward curvature (Fig. 7). When this latter reaction rate was plotted versus the *square* of the enzyme concentration, a straight line resulted (insert to Fig. 7). This behavior would be explained readily if the former reaction, corresponding to unidirectional transport, was catalyzed by a single monomer while the bidirectional transphosphorylation reaction required the simultaneous participation of two subunits.

To explain these results, we propose the minimal subunit interactions depicted in Figure 8. The mannitol Enzyme II is asymmetric with an essential sulfhydryl group on the cytoplasmic surface of the enzyme complex. As proposed, the majority of the subunits exist as dissociated species within the phospholipid bilayer. Lateral diffusion of 2 such subunits must occur to bring them together so the transphosphorylation reaction can occur.

This postulate clearly can explain the enzyme concentration dependency depicted

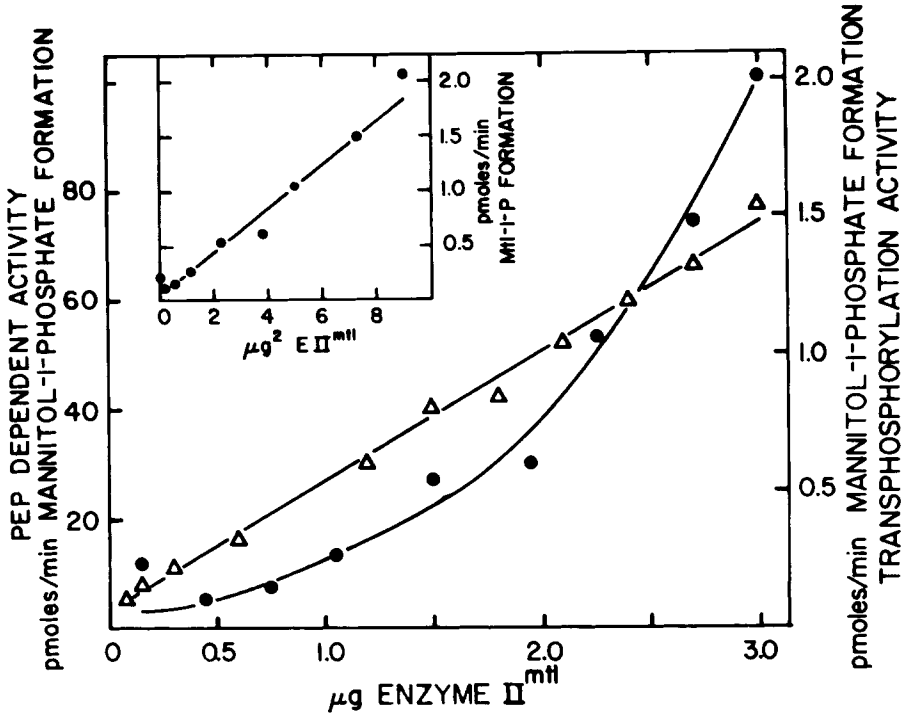

Fig. 7. PEP-dependent and mannitol-1-P-dependent phosphorylation of mannitol as a function of Enzyme II^{mtl} concentration. (Insert: activity of the transphosphorylation reaction plotted versus the square of the Enzyme II concentration. The lubrol concentration was maintained constant.) (J.E. Leonard and M.H. Saier, Jr., unpublished results.)

in Figure 7, but it also provides explanations for the pH and temperature dependencies. To understand the pH dependency of the mannitol Enzyme II-catalyzed transphosphorylation reaction, 3 facts must be recognized. First, the rate of the transphosphorylation reaction is much less than that of the PEP-dependent reaction. Second, the pH optimum for this reaction is pH 6, although the PEP-dependent reaction shows maximal activity at a basic pH. Finally, the isoelectric point of the purified Enzyme II (the pH at which the enzyme bears no net charge) was found to be pH 6. A brief consideration of these facts reveals that 2 subunits of an integral membrane protein might be expected to come together most frequently at its isoelectric point, because at any other pH the net charge of the protein would be appreciable, and electrostatic repulsion would tend to keep the subunits apart. Thus, the pH curve for transphosphorylation may not be a measure of the inherent activity of the dimer but of the tendency of the individual subunits to come together with the formation of the active dimeric complex.

The temperature dependency of the transphosphorylation reaction may be similarly explained. Since the subunits are predominantly dispersed, this association to give an active dimer will be dependent on the fluidity of the hydrophobic medium in which the proteins are suspended. If this environment passes through the liquid-solid transition so that the medium becomes relatively viscous, lateral diffusion will be inhibited, thus preventing the association of monomers. This fact would cause the transphosphorylation reaction to be more temperature dependent below the transition temperature than above it.

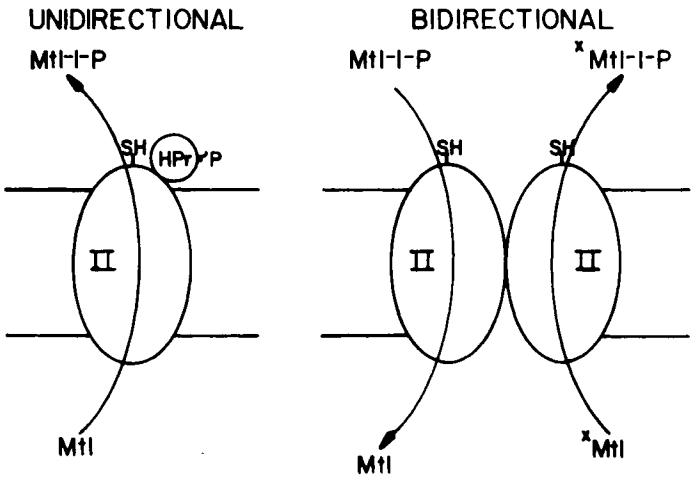

Fig. 8. Model suggesting subunit dependencies for unidirectional and bidirectional groups translocation catalyzed by the mannitol Enzyme II of the PTS.

The three experiments illustrating qualitatively different behavior for the pH, temperature, and enzyme-concentration dependencies of the 2 phosphorylation reactions are thus explained, assuming that unidirectional PEP-driven transport requires one subunit while bidirectional, sugar phosphate-dependent vectorial transphosphorylation depends on the transient formation of a functional dimer. The speculative nature of this suggestion should be emphasized, however, particularly in view of the observation that the purified Enzyme II^{mtl} used in these studies had been partially proteolytically cleaved into 2 unequal polypeptide chains [J.E. Leonard, unpublished result]. Further biochemical and biophysical studies on the intact Enzyme II will be required to substantiate or refute the proposed explanation.

Genetic Dissection of the Enzyme II^{mtl}

Since the mannitol Enzyme II is a multifunctional enzyme, it might be expected that the individual activities associated with the protein may depend on the integrity of different amino acid residues, moieties, and/or conformations of the protein molecule. Interactions with phospholipids or other proteins may also be important for some but not other activities attributable to the Enzyme II. With this possibility in mind, attempts were made to dissect the catalytic functions of the enzyme employing biochemical and genetic techniques. Only limited success has resulted from the application of protein-specific reagents, but greater success resulted when genetic techniques were employed. Using a positive selection procedure for mutants defective in the enzyme II^{mtl} [46, 47], John Leonard isolated several distinct classes of mutant enzymes. In these studies, chemotactic, transport, and both PEP- and mannitol-1-P-dependent phosphorylation activities were estimated. Some of the mutants showed parallel increases or decreases in all activities assayed. However, one class of mutants exhibited reduced transport and phosphorylation activities while the chemoreception activity was enhanced. Other mutants were defective for transport and chemoreception but retained phosphorylation acitivites. These studies revealed that the chemoreception, transport, and phosphorylation activities

are overlapping but distinct and can be altered in a nonparallel fashion. The application of physicochemical and kinetic techniques to the wild type and mutant forms of the Enzyme II^{mtl} may reveal the mechanistic basis for the distinct catalytic functions of the protein.

CONCLUDING REMARKS

The Enzyme II complexes of the bacterial phosphotransferase system are multifunctional integral membrane proteins. They sense concentration gradients of sugars, thereby serving as chemoreceptors; they transport their sugar substrates across the membrane by at least 2 (and probably 3) distinct mechanisms, and they phosphorylate the incoming sugar in coupled processes. These proteins also serve as components of a regulatory system that controls the activities of other permeases and of adenylate cyclase, and they apparently regulate the rates at which some of the proteins of the PTS are synthesized [1]. Kinetic studies of the transphosphorylation reactions catalyzed by the mannose Enzyme II complex showed that the reactions occurred by a random bi-bi sequential mechanism in which substrate binding is cooperative. Further studies conducted with the mannitol Enzyme II showed that the enzyme, a single polypeptide chain of 60,000 molecular weight, spans the phospholipid bilayer in an asymmetric fashion. Indirect experiments comparing the PEP-dependent and mannitol-1-phosphate-dependent: mannitol phosphorylation reactions have led to the tentative conclusion that while subunit interactions are required for the latter reaction, the former is catalyzed by the monomeric species. While our studies have allowed us to propose specific mechanisms by which the Enzymes II mediate sugar transport and phosphorylation, further investigations dealing with the physicochemical and catalytic properties of the proteins will be required to confirm or refute these postulates. The availability of mutant Enzyme II^{mtl} proteins, which are specifically altered in one or more of the assayable activities of the enzyme, may facilitate these studies.

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