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MOLECULAR INTERACTIONS IN THE BACTERIAL PHOSPHOENOLPYRUVATE-PHOSPHOTRANSFERASE SYSTEM (PTS)*

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Molecular explanations are not generally available for the diverse physiological processes regulated by cell membranes. Despite intensive efforts from many laboratories, even the structures of cell membranes have not yet been established. Attempts to gain more concrete knowledge of the structure of membranes have often been channeled through studies of various biological functions associated with membranes. Perhaps the most useful and most intensively studied membrane function is the translocation of solutes across the membrane and the energy-coupling processes that take place during these reactions. Efforts have been made to elucidate the reaction sequences, molecular interactions, and possible conformational changes of proteins that take place during translocation processes in some biological and artificial membrane systems. Most of these studies have involved the application of various chemical or physical probes (ESR, NMR, fluorescence) which are either incorporated into the membranes or covalently linked to membrane components or transport substrates.

In only a few cases has it been possible to actually isolate and purify the membrane components involved in specific translocation processes and study some of their properties in vitro. One of these cases is the bacterial phosphoenolpyruvate-phosphotransferase system (PTS), which will be the subject of the following discussion. The bacterial PTS (1–3) has been shown to be responsible for the translocation and concomitant phosphorylation of a number of carbohydrates (4–7). While the PTS is found in many different bacteria (1, 8), its physiological role differs to some degree among the different genera of bacteria (9). For example, all carbohydrates are PTS transport-dependent in S. aureus (10), whereas only sugars of the D-gluco and D-manno configuration (fructose, hexitols, etc.) are transported via the PTS in the enteric bacteria E. coli and S. typhimurium (11). Other carbohydrates and amino acids in these organisms appear to be transported by other energy-dependent mechanisms – for example, direct involve-

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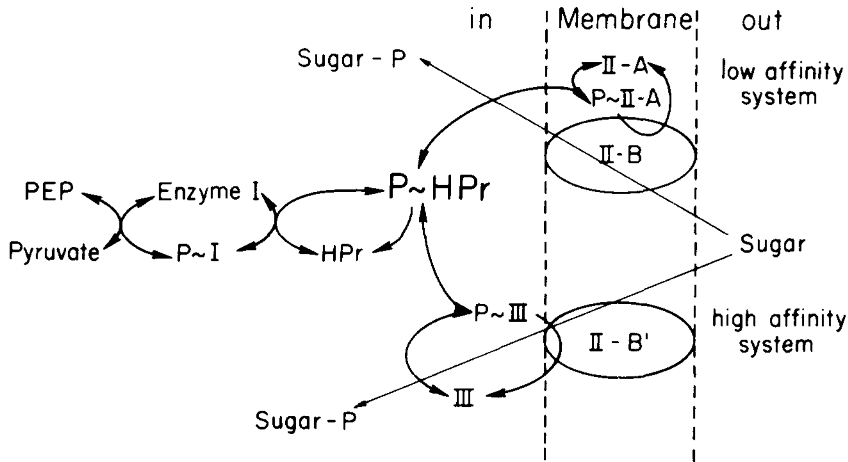


Fig. 1. Phosphate transfer and sugar transport via the PTS.

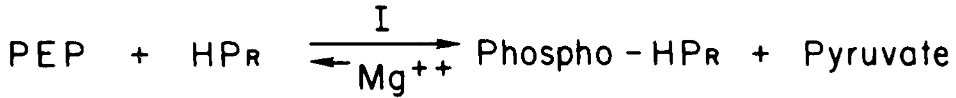
ment of the solute carrier proteins in the electron transport chain (12), proton or other cation-linked cotransport systems (13, 14), or connection via a coupling factor to the electron transport chain (15).

Besides its primary role as a translocating system for sugars, the PTS has been implicated in playing an important role in a number of other physiological processes (9) which will be discussed briefly later in this article.

A number of biochemical reactions that occur during phosphoryl transfer and carbohydrate translocation via the PTS have been studied (2, 3). The detailed reactions of the PTS as they have been elucidated in E. coli are shown in Fig. 1. The protein fractions that take part in this phosphoryl transfer are both cytoplasmic and membrane constituents and they can be classified into two groups: the general (not sugar-specific) proteins and the sugar-specific proteins.

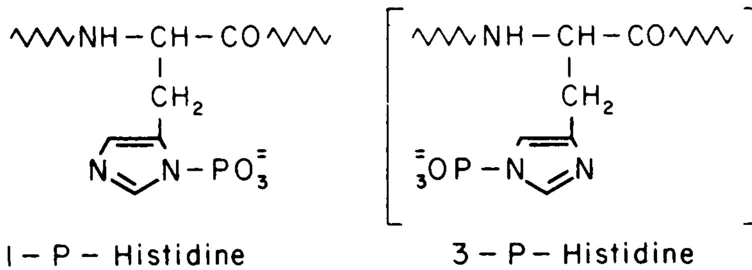
GENERAL PTS PROTEINS: ENZYME I AND HPr

The general PTS proteins, enzyme I and phosphocarrier HPr, are both cytoplasmic components. The main function of these proteins is the formation of phosphorylated HPr, which serves as the central phosphoryl donor for all membrane-associated PTS reactions (Fig. 2). Enzyme I has been purified close to homogeneity from several organisms; it has an approximate molecular weight of 80,000 daltons with an undetermined number of subunits. The phosphocarrier protein, HPr, has been isolated in homogeneous form from E. coli (16) and S. typhimurium. The protein consists of a single polypeptide chain with a molecular weight of 9,600 daltons. Both enzyme I and HPr seem to be constitutively synthesized, but under certain conditions elevated levels of both proteins have been detected (11). The structural genes for both proteins are located in an operon at 74 min on the S. typhimurium genetic map (17) (Fig. 3). The genes are linked together in the sequence ptsH (HPr)–ptsI (enzyme I). A promoter gene (ptsP)



I mole P incorporated /mole HPr

P linked to N-I of His imidazole ring



I - P - Histidine 3 - P - Histidine

Fig. 2. Phospho-HPr (E. coli).

for the PTS operon located between trzA and ptsH has recently been discovered (18).

Enzyme I catalyzes the transfer of the phosphoryl moiety of phosphoenolpyruvate to the N-1 position of a histidine imidazole ring of HPr, resulting in the formation of protein-bound phosphohistidine, a phosphoramidate. The N-1 position of the phosphate group in the histidine imidazole ring has been established by hydrolysis studies and by isolation and characterization of the phosphohistidine which is clearly distinguishable from its N-3 analogue (19). The product of this phosphoryl transfer, phospho-HPr, serves as general phosphoryl donor for all further phosphoryl transfer reactions involving the sugar-specific PTS proteins.

THE SUGAR-SPECIFIC PTS PROTEINS

As shown in Fig. 1, the transfer of the phosphoryl moiety from P~HPr to a given sugar requires a pair of proteins specific for that sugar or its analogues. The sugar-specific proteins of the PTS therefore comprise a family of pairs of sugar-specific proteins, each pair being necessary for the phosphorylation of one given sugar. As shown in Fig. 4, in a given pair at least one protein is a firmly bound membrane component. The operational (and rather unsatisfactory) nomenclature that we have used for the sugar-specific proteins of the PTS is as follows: the exhaustively washed membrane preparation containing the membrane-bound sugar-specific proteins is designated enzyme II; PTS proteins derived from the membrane are called II-A, II-B, II-B′, etc. When a sugar-specific protein is found in the soluble or cytoplasmic fraction it is called III or Factor III. Sugar-specific PTS proteins are either constitutively synthesized or inducible. Protein pairs of the II-A/II-B type (where both proteins are membrane-bound) so far have been found only in

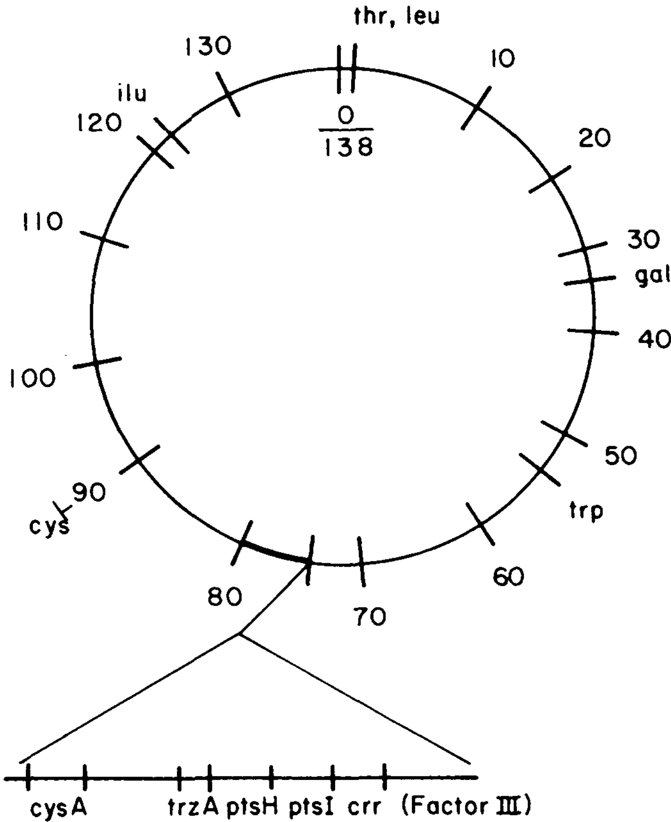


Fig. 3. Linkage map of Salmonella typhimurium showing the PTS gene region.

constitutive form, whereas the Factor III/II-B′-type system can be either inducible or constitutive. The inducible III/II-B′ protein pair was first discovered in S. aureus (20). In enteric bacteria (E. coli, S. typhimurium) the III/II-B′-type system has been shown to exist in constitutive as well as inducible forms. In any given bacterial cell many different sugar-specific pairs of PTS proteins can function simultaneously in the transfer of the phosphoryl moiety to a given sugar (or to groups of sugars), all utilizing the same central phosphoryl donor, P~HPr.

Where purified or homogeneous sugar-specific PTS proteins have been prepared, it has been shown that the sequence of phosphoryl transfer proceeds from P~HPr to one of the sugar-specific PTS proteins and then to the sugar. The last step, the formation of sugar phosphate, requires the second sugar-specific PTS protein, and this protein is invariably a membrane component.

In E. coli we can distinguish between two types of sugar-specific protein pairs which give rise to glucose-6-P – namely, a low-affinity system of the II-A/II-B type (K_m for glucose, 10^{-4} M) and a high-affinity system of the Factor III/II-B′ type (K_m for glucose, approximately 5×10^{-6} to 10^{-5} M). Both systems are constitutively synthesized, but the level of enzymatic activity depends to some degree on the physiological state of the

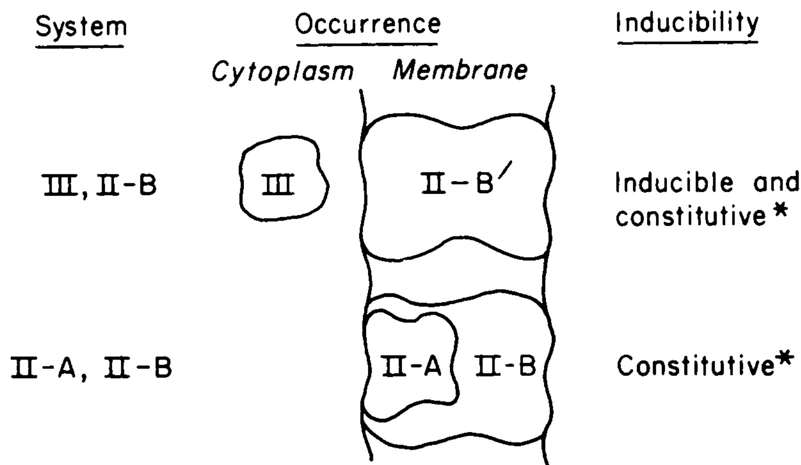


Fig. 4. Sugar-specific proteins of the PTS.

bacteria as well as on the carbon source of the growth medium.

The isolation, characterization, and phosphoryl transfer reactions catalyzed by the two glucose PTS of E. coli and S. typhimurium will be discussed in the following paragraphs as examples of the reactions catalyzed by the two types of pairs of sugar-specific PTS proteins.

ISOLATION AND CHARACTERIZATION OF THE LOW-AFFINITY GLUCOSE PTS FROM E. COLI (II-A/II-B SYSTEM)

The isolation and characterization of the low-affinity glucose-specific proteins from E. coli have been described (3) and the following represents a short summary of the status of the project to date. The components of the II-A/II-B system have been isolated from E. coli membranes by solubilization with a mixture of urea and 1-butanol and subsequent fractionation by ammonium sulfate, ion exchange chromatography, acrylamide gel electrophoresis, and isoelectric focusing (3). Restoration of active enzyme complexes capable of transferring the phosphoryl moiety from P~HPr to glucose required the II-A and II-B proteins, a divalent metal ion, and a specific phospholipid, phosphatidylglycerol (PG) (see Table I). No active complexes were formed in the presence of phosphatidylethanolamine, phosphatidylcholine, phosphatidic acid, or undecaprenolphosphate, while phosphatidylserine and cardiolipin were slightly active.

The most striking result of these studies was the fact that the components of the II-A/II-B system could not be mixed in any random fashion, but for an enzymatically

TABLE I. Restoration of Enzymatic Activity with the Isolated Components of the Glucose-Specific II-A/II-B System of E. coli

Components Present in Incubation Mixture *	Methyl α-Glc-6-P (μmole/30 min)
II-A, II-B, Mg^{2+}, phosphatidylglycerol	0.145
II-A, II-B, Mg^{2+}	0.010
II-B, Mg^{2+}, phosphatidylglycerol	0.000
II-A, Mg^{2+}, phosphatidylglycerol	0.005

*The incubation contained a P~HPr generating system.

TABLE II. Effect of Order of Mixing on the Formation of Enzymatically Active II-A/II-B Complexes

Sequence*	Methyl α-Glc-6-P (μmole/30 min)
II-B + Mg^{2+} + PG + II-A	0.121
II-B + PG + Mg^{2+} + II-A	0.009
Mg^{2+} + PG + II-B + II-A	0.003

*PG = phosphatidylglycerol. The incubations contained a P~HPr generating system.

active complex to be formed they had to be reacted with each other in a specific order (Table II). Formation of active complexes between II-B and PG always resulted in the formation of aggregates which could be separated from unreacted components by centrifugation at 200,000 X g. All of the enzymatic activity was found in the sediments, which made it possible to study the correlation between the appearance of enzymatic activity and the amount of PG entering the II-B/PG compexes as well as the stoichiometry involved in the association of II-B and PG. As shown in Fig. 5, optimal enzyme activity in the presence of II-A was obtained when the II-B/PG complex reached the saturation point with respect to PG. At this stage the complexes contained one protein molecule for every 50 PG molecules.

PHOSPHORYL TRANSFER FROM P~HPr TO GLUCOSE VIA THE II-A/II-B SYSTEM

As indicated in Fig. 1, the phosphoryl transfer from P~HPr to sugar proceeds via a phosphorylated II-A protein. A summary indicating the ^{32}P transfer from ^{32}P~HPr to II-A is presented in Fig. 6. Catalytic amounts of enzyme I and HPr were used with ^{32}P-labeled PEP (2) as a ^{32}P~HPr generating system. Increasing amounts of II-A resulted in increasing amounts of ^{32}P transferred to protein; with no II-A present only a very small amount of ^{32}P-protein was formed and it represented the level of ^{32}P~HPr formed by the

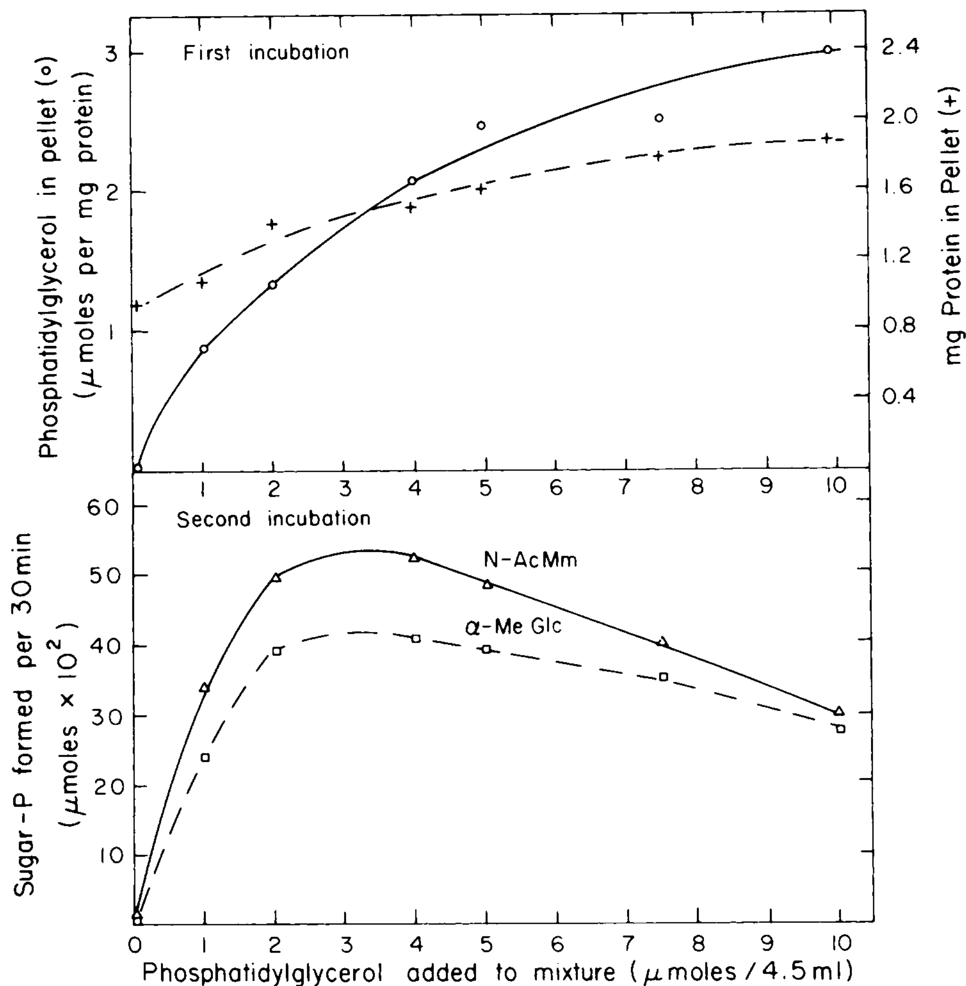
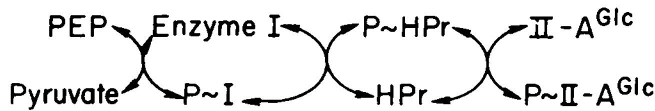


Fig. 5. Interaction of II-B with phosphatidylglycerol. Two mg of II-B were reacted with 50 μmoles of $CaCl_2$ and the indicated amounts of phosphatidylglycerol for 20 min at 4°C, followed by centrifugation at 250,000 X g. The resulting pellets were suspended and assayed for protein (+) and phosphatidylglycerol (o) content (upper frame) and for enzymatic activity with II-A and a P ~ HPr generating system (lower frame).

generating system. One of the surprising facts was that phosphoryl transfer from P~HPr to II-A did not require the presence of II-B or the phospholipids. The phosphoryl linkage in II-A-P has not yet been clearly established but preliminary experiments indicate the possibility that it is an acylphosphate.

Phosphoryl transfer from P~HPr to II-A was also demonstrated with isolated small membrane vesicles as shown in Fig. 7. Small membrane vesicles were obtained by passage of the isolated E. coli membranes through a French pressure cell and isolation of a fairly uniform population of small membrane vesicles by glycerol density centrifugation. Incubation of these membrane vesicles with the ^{32}P~HPr generating system gave rise to ^{32}P-



Incubation Mixture *	^{32}P-Protein cpm
Complete 50 μg II-A^{Glc}	13223
Complete 100 μg II-A^{Glc}	28497
Minus II-A^{Glc}	2021
Minus Enzyme I	116
Minus HPr	235
Minus Mg^{++}	346

*Complete incubation mixture (0.17 m): ^{32}PEP, 0.2 μm, sp.A. 10^7 cpm/μm; Enzyme I, 5 μg; HPr, 4 μg; II-A^{Glc}, as indicated; $MgCl_2$, 1 μm; Bicine buffer, pH 7.5, 10 μm; DTT, 0.2 μm; 15 min., 37°

Fig. 6. Transfer of ^{32}P from PEP to II-A^{Glc}.

labeled membranes which could be isolated by glycerol density centrifugation as indicated in Fig. 7. Addition of glucose to the isolated ^{32}P-labeled membrane resulted in the rapid formation of glucose 6-phosphate, indicating that the ^{32}P label in the membranes was indeed associated with the II-A PTS protein.

THE HIGH-AFFINITY GLUCOSE PTS OF E. COLI (III/II-B′ SYSTEM)

The sugar-specific protein pair of the high-affinity glucose system of E. coli (see Fig. 1) consists of the soluble cytoplasmic protein, designated Factor III^{Glc}, and one membrane-bound protein, II-B′. The designation II-B′ for the membrane protein of the high-affinity glucose PTS is used to indicate that this membrane protein is different from the II-B protein, which is part of the low-affinity PTS (II-A/II-B system) discussed earlier.

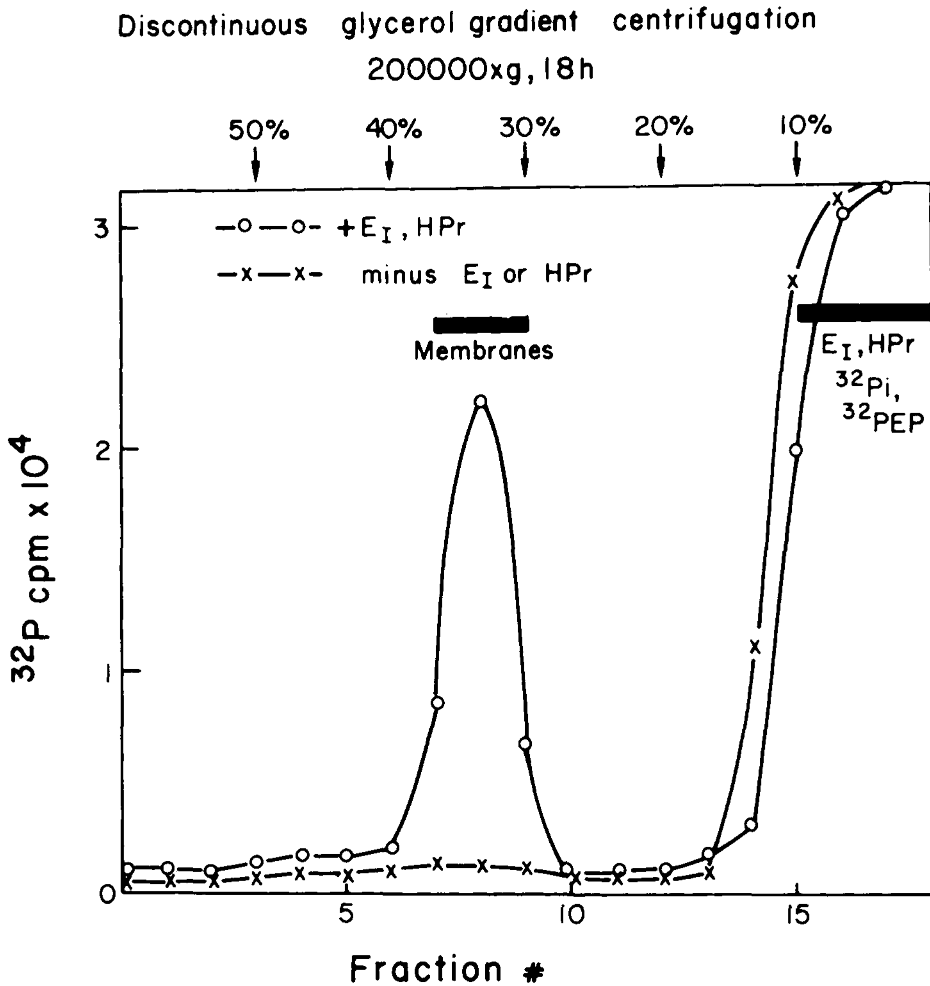


Fig. 7. Transfer of phosphate from ^{32}PEP to II-A in membranes (E. coli, K 235).

THE CYTOPLASMIC COMPONENT OF THE HIGH-AFFINITY GLUCOSE PTS IN E. COLI: FACTOR III^{Glc}

Factor III is a constitutively synthesized cytoplasmic PTS protein; it has a molecular weight of approximately 20,000 daltons, and it appears to be made up of 3 to 4 subunits. Factor III is a highly acidic protein with an isoelectric point of 4.03 as judged by the pH of the fraction containing III obtained through isoelectric focusing with a shallow pH gradient.

The gene locus (crr gene) coding for or regulating the synthesis of Factor III in S. typhimurium is linked closely to the PTS operon (Fig. 3) as indicated by deletion mapping (17). The crr gene is also cotransducible with ptsI and ptsH. However, recent studies have indicated that the crr gene is controlled independently from the PTS genes as indicated

TABLE III. Purification of III^{Glc} from E. coli

Fraction	Specific Activity*
Crude extract	n.d.†
200,000 × g supernatant	n.d.
30–80% amm. sulf.	0.05
Sephadex G75 column	0.2
DEAE column	2.1
Sephadex G75SF column	10.0
Isoelectric focusing	20.0

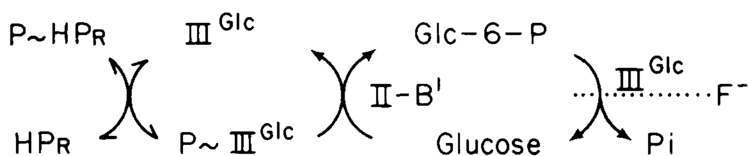
*Specific Activity = μmoles Methyl α-Glc-6-P formed per 30 min at 37°C. †n.d. = not determined.

by the discovery of a promoter-like gene between ptsI and crr (18).

Factor III has been isolated from E. coli and purified to "apparent homogeneity" as shown in Table III. The term "apparent" is used because the final preparation of Factor III exhibits, in addition to phosphoryl transfer activity, a potent phosphatase activity specific for hexose 6-phosphate esters of the D-gluco and D-manno configuration. The phosphatase activity associated with Factor III will be discussed in detail in a later paragraph.

The properties of the reactions in which Factor III is involved are shown in Fig. 8, and they indicate that III, because of the phosphatase activity associated with the molecule, has a dual function. III is involved in the formation of sugar phosphate (this reaction can be considered the forward reaction), but Factor III can also catalyze the hydrolysis of sugar phosphates (backward reaction). Phosphoryl transfer to sugar from P~HPr (forward reaction) requires the presence of the membrane protein II-B′, whereas the phosphatase activity (backward reaction) occurs independently of II-B′. As will be discussed later, the PTS activity of Factor III is heat stable and KF insensitive, while the phosphatase activity is heat labile and abolished in the presence of KF.

P– Transfer **Phosphatase**



III Glc

1. Mol·wt. (app) ~ 20,000; 3–4 subunits
2. $P{\sim}III^{Glc}$ = acyl~P
3. Transferase, heat stable; phosphatase, heat labile

Fig. 8. Factor III^{Glc} (E. coli).

TABLE IV. Phosphate-Acceptor Specificity of the III^{Glc}-II-B′ System of E. coli*

Phosphate Acceptor	6-P-Ester Formed (μmole/30′ min)
D-Glucose	0.192
Methyl α-D-glucoside	0.218
D-Mannose	0.109
Methyl α-D-mannoside	0.103
Methyl thio-β-D-galactoside	0.046

*Inactive: N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, D-glucosamine, D-mannosamine, mannitol, D-galactose, fructose. The incubations contained a P ~ HPr generating system.

THE PHOSPHORYL TRANSFER REACTION CATALYZED BY FACTOR III FROM E. COLI

As indicated in Fig. 1, phosphoryl transfer from P~HPr to sugar via the high-affinity PTS of E. coli requires the cytoplasmic protein Factor III, as well as the membrane-bound protein II-B′. The phosphoryl acceptor specificity of this system is not as stringent as it is in the II-A/II-B system where distinctly separate systems for glucose, mannose, and fructose exist. The Factor III/II-B′ system will effect phosphoryl transfer to glucose and mannose as indicated in Table IV. For some unknown reason phosphoryl transfer will also occur to thiomethyl β-galactoside, while other phosphate acceptors of the D-galacto configuration are completely inactive.

As indicated in Figs. 1 and 8, a direct transfer of the phosphoryl moiety exists between P~HPr and Factor III, giving rise to a phosphorylated form of Factor III. When ^{32}P~HPr is incubated with Factor III without the presence of any other proteins ^{32}P is transferred to III, and ^{32}P~III can be isolated by Sephadex gel filtration as illustrated in Fig. 9. The phosphate exchange between HPr and Factor III seems to be an equilibrium reaction with the equilibrium state heavily favoring the formation of P~III, but the formation of P~HPr from P~III is demonstrable. Saturation studies indicated that there is one phosphate group incorporated per molecule of Factor III of 20,000 daltons molecular weight.

The phosphoryl linkage in P~III has been intensively studied and clearly identified as acylphosphate by the following criteria: (a) pH profile of the stability of the P-linkage (as shown in Fig. 10), (b) the lability of the P-linkage toward hydroxylamine, and (c) demonstration of the formation of ^{3}H-labeled protein-bound n-propyl-hydroxamic acid (21) as a result of the reaction of P~III with (2, 3 ^{3}H) N-(n-propyl) hydroxylamine. Preliminary experiments following the procedures developed by Suzuki et al. (22) for citrate lyase have indicated that the phosphoryl moiety in P~III is linked to an aspartyl residue.

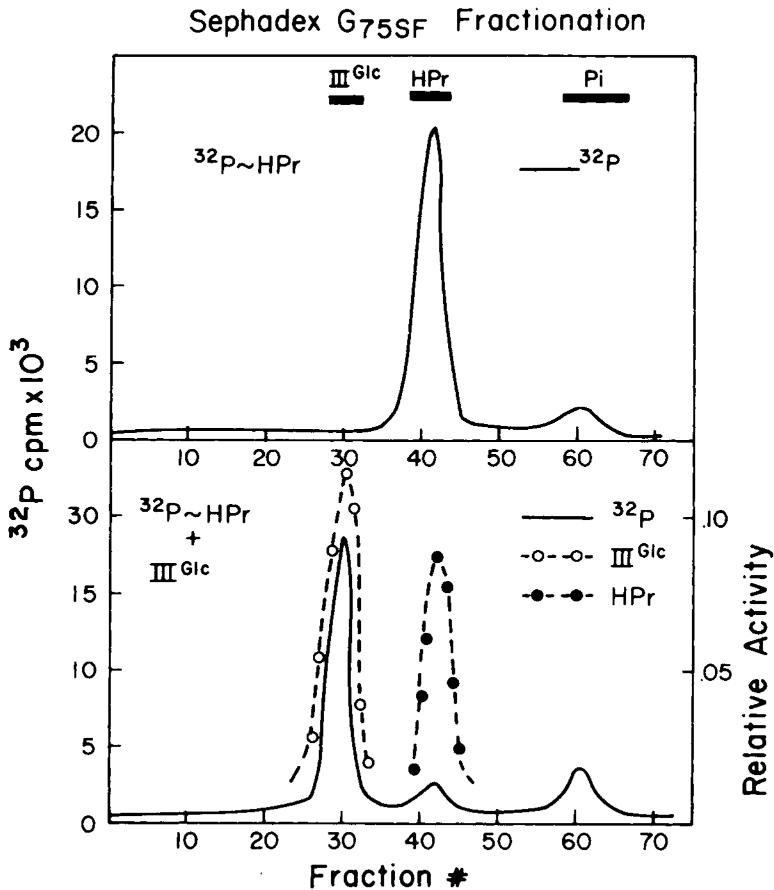


Fig. 9. Transfer of phosphate from $^{32}P \sim HPr$ to III^{Glc}.

THE PHOSPHATASE ACTIVITY ASSOCIATED WITH FACTOR III FROM E. COLI

As indicated earlier the phosphatase activity associated with Factor III (backward reaction, see Fig. 8) occurs independently of the membrane-bound protein II-B′. The phosphatase has a pH optimum of approximately pH 7.2 and is specific for 6-phosphate esters of the D-gluco and D-manno configuration as indicated in Table V. The specificity for the glycose moiety resembles closely that of the phosphoryl transfer reaction (forward reaction) catalyzed by Factor III. The phosphatase also has a very stringent requirement for a divalent metal ion, with Mg^{2+} being the most active and Mn^{2+} giving approximately 50% of the activity as compared to Mg^{2+}. The phosphatase activity associated with Factor III differs from other phosphatases in E. coli (23) by its substrate specificity (only 6-P esters of D-gluco and D-manno configuration are hydrolyzed with no hydrolysis of 1-P esters), by its metal ion requirement, and by its cellular location in the cytoplasm. Most phosphatases seem to be located in the periplasmic space of E. coli and can be removed from the cells by cold shock (23), while Factor III is not released from the cells by this procedure.

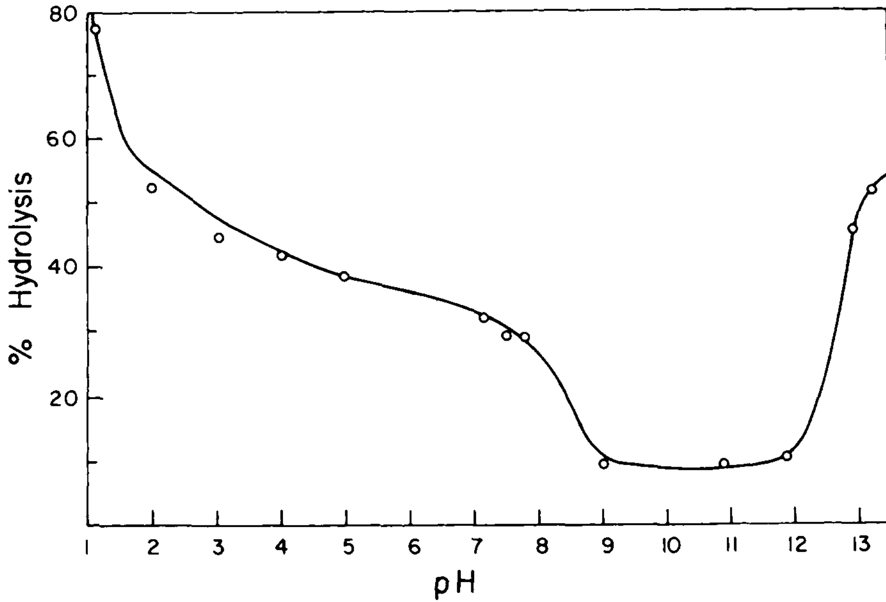


Fig. 10. Hydrolysis of $^{32}P \sim III^{Glc}$ (20 min, 46°C).

As discussed earlier, the phosphatase activity associated with Factor III is extremely heat labile, while the phosphoryl transfer activity is not. The heat lability of the phosphatase and the heat stability of the phosphoryl transferase activity at 70°C and pH 7.2 are illustrated in Fig. 11. An indication as to what is happening to the Factor III molecule during heating was obtained when the unheated and heated fractions were examined by gel filtration on Sephadex G-75 as shown in Fig. 12. The patterns that develop are quite clear: heating effects a dissociation of the 20,000 molecular weight form of Factor III into smaller molecules, probably subunits. Only the original 20,000

TABLE V. Substrate Specificity of III^{Glc} Associated Phosphatase

Substrate	Relative Rate* of Hydrolysis
Glucose-6-P	100
Methyl α-glucopyranoside-6-P	154
α-Glucose-1-P	5
2-Deoxyglucose-6-P	112
Glucosamine-6-P	42
Mannose-6-P	52
N-Ac-mannosamine-6-P	53
Fructose-6-P	51
Fructose-1-P	6

*Glucose-6-P (100) = 1.15 μmole/15 min. Inactive: Fructose-1, 6-di P, Gal-6-P, Gal-1-P, TMG-6-P, P-gluconate, gylcerol-P, acetyl-P, PEP, TPN AMP, nitrophenyl-P.

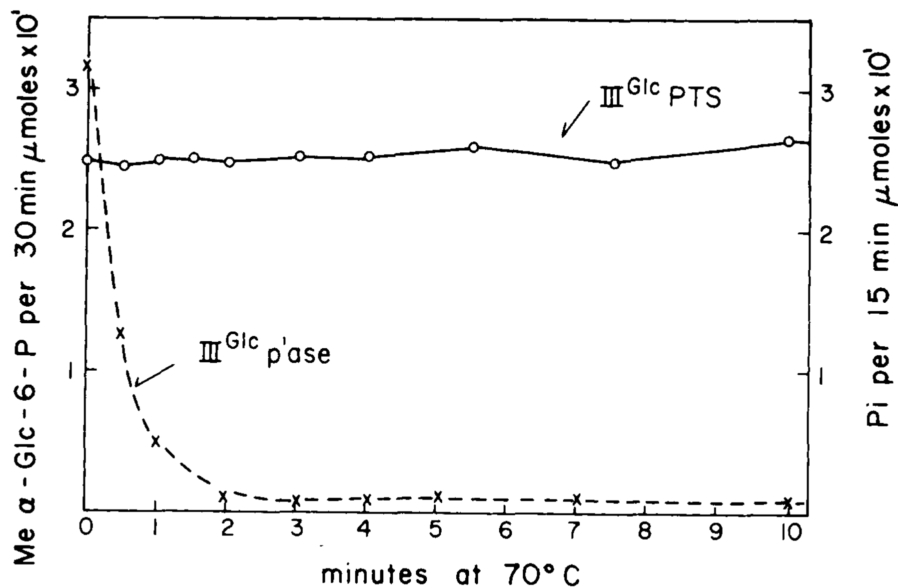


Fig. 11. Heat treatment of III^{Glc} (70°C).

dalton molecule of Factor III is capable of catalyzing the hydrolysis of phosphate esters, while all other forms of Factor III including the probable monomeric form (approximately 5,000 daltons) are still capable of effecting the phosphoryl transfer from P~HPr to sugar in the presence of the membrane protein II-B′. At present we do not know whether the subunits of Factor III are identical or not.

The dissociation of Factor III and the concomitant disappearance of phosphatase activity were also obtained by treating Factor III with chelating agents such as EDTA, dipyridyl, and Chelex-resin (Biorad). The results obtained with the chelating agents indicate that a divalent metal ion is necessary for the structural integrity of the oligomeric form of Factor III.

Unfortunately experiments to reassociate a functional oligomeric form of Factor III from the dissociated forms have so far met with only very limited success.

ISOLATION AND CHARACTERIZATION OF II-B′

As indicated at the outset of the discussion the membrane protein (II-B′) involved in the high-affinity glucose PTS is different from the II-B protein involved in the low-affinity II-A/II-B system. II-B′ is clearly distinguishable from II-B inasmuch as enzymatically active II-B′ proteins depends on an SH-function, whereas II-B does not. The lipid specificity of the isolated II-B and II-B′ proteins, as well as the characteristics of their association with the lipids, are different.

Active II-B′ has been solubilized from purified membranes using chaotropic agents (specifically $NaClO_4$) and more recently by the use of detergents with the scheme shown

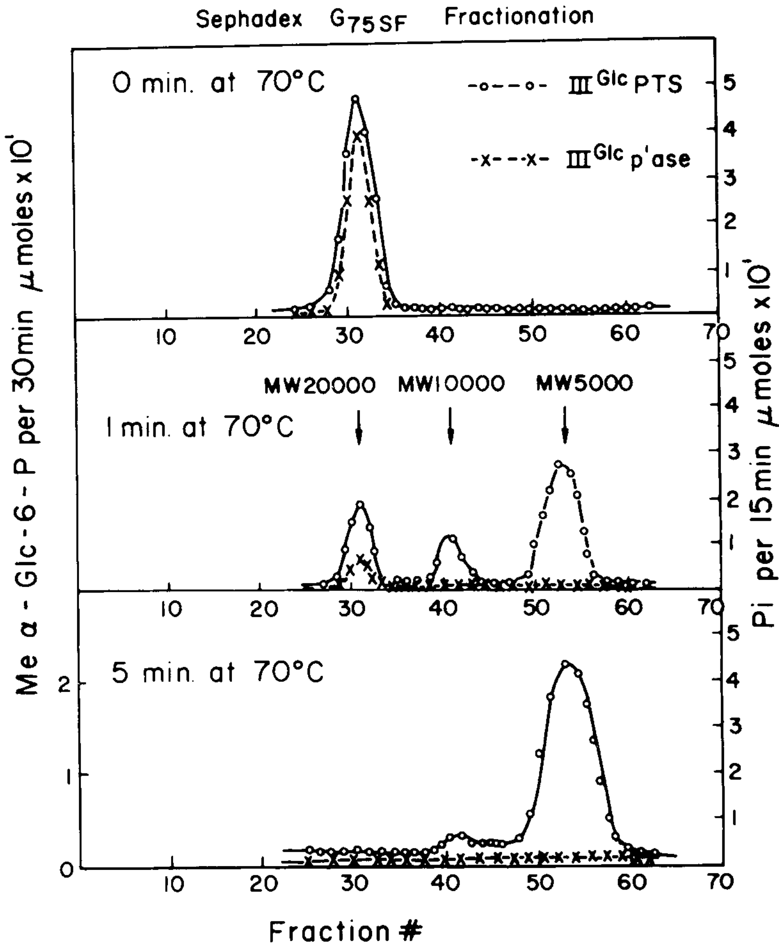


Fig. 12. Fractionation of heated III^{Glc} on Sephadex G 75SF.

in Table VI. The isolation of II-B′ was most successful when purified cytoplasmic membranes (24) were used as starting material. Cytoplasmic membranes were obtained either by the Osborn procedure (24) or by the isolation of phage-induced intracellular membrane vesicles formed in certain phage-infected E. coli strains (25). The E. coli strain used for most of our studies, E. coli K235, forms intracellular vesicles when grown to the stationary phase of growth (Fig. 13). These intracellular vesicles represent pure cytoplasmic membrane with no detectable traces of either lipopolysaccharide or cell wall components. They contain all cytoplasmic membrane markers as well as the membrane components of the PTS (II-A, II-B, II-B′). The vesicles were isolated from cells ruptured in a "Manton-Gaulin" press and used for the isolation of II-B′ as indicated in Table VI. As was the case with II-B, purified II-B′ had to be associated with phospholipids in order to form enzymatically active complexes. However, the phospholipid specificity was somewhat different from that of the II-B complexes; again phosphatidylglycerol was the most

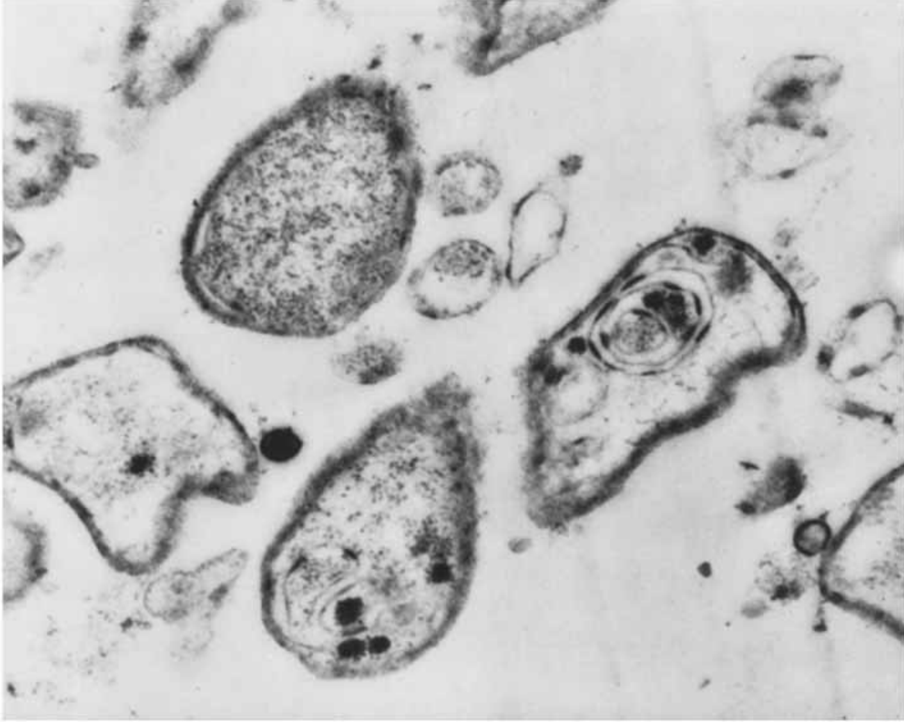


Fig. 13. Electron micrograph of E. coli K 235 grown to stationary phase in mineral salts medium and glucose.

TABLE VI. Isolation of II-B′ from E. coli

A. **Isolation of cytoplasmic membrane vesicles**

Cells ruptured in "Manton-Gaulin" press

Removal of cell walls and large membranes by centrifugation at 180,000 × g (90 min)

Ammonium sulfate fractionation (30% to 80% saturation) precipitate dissolved and dialyzed

Isolation of small cytoplasmic membrane vesicles by centrifugation at 350,000 × g (5 hr)

B. **Solubilization of II-B′ from E. coli Membranes***

Extraction of membrane vesicles with 1% sarkosyl (30 min, 37°C)

Removal of insoluble material by centrifugation at 200,000 × g (45 min)

Precipitation of II-B′ with ammonium sulfate (35% saturation), precipitate dissolved and dialyzed

Fractionation of II-B′ on DEAE-cellulose

*The conditions for the solubilization of II-B′ from membranes using sarkosyl have been worked out in our laboratory by Dr. Konrad Sandhoff and Dr. Norman D. Meadow.

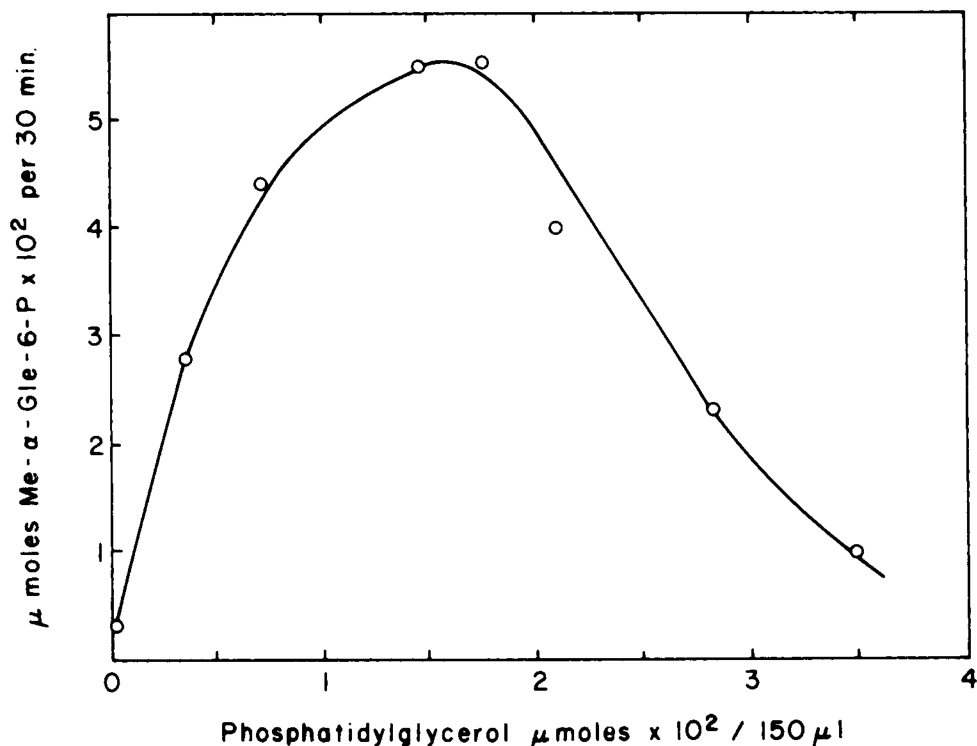


Fig. 14. Interaction of phosphatidylglycerol with II-B′.

effective phospholipid in restoring active complexes with II-B′, but cardiolipin was also effective, with approximately 90% of the activity of PG (Table VII). Careful analyses showed that cardiolipin itself was active and was not first transformed to PG and phosphatidic acid by a specific phospholipase (26). The formation of enzymatically active complexes between II-B′ and PG is indicated in Fig. 14. A comparison with the II-B′/PG complexes in Fig. 5 clearly reveals that the association of the II-B′ protein with PG follows different criteria. In the case of the II-B/PG association (Fig. 14) the formation of active complexes passes through a very narrow plateau region of maximum activity and then drops very rapidly upon further addition of PG. Intensive studies on the association of phospholipids with II-B′ are at present in progress and they may help clarify the differences that exist between the molecular arrangements in the II-B/PG and II-B′/PG lipid-protein complexes.

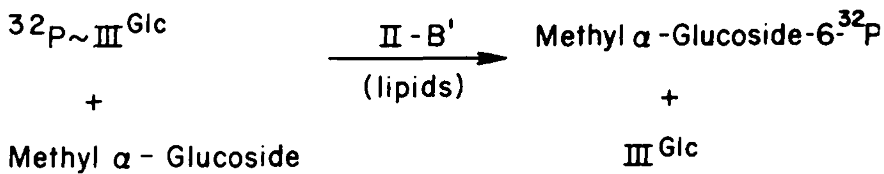
PHOSPHORYL TRANSFER FROM P~III TO SUGARS

The transfer of the phosphoryl moiety from P~III to methyl α-glucoside is summarized in Fig. 15. As mentioned earlier this phosphoryl transfer requires the presence of the II-B′/PG complex. Extensive studies have indicated that the transfer of the phosphate moiety from P~III to sugar is a direct reaction with no preceding formation of other phosphorylated intermediates.

TABLE VII. Lipid Specificity of II-B′

Lipid*	Me α-Glc-6-P (μmoles/30 min)
None	0.004
Phosphatidylglycerol	0.154
Cardiolipin	0.145
Sarkosyl	0.018
Deoxycholate	0.011

*Lipids and detergents tested at individual optimal concentrations. Inactive: phosphatidylethanolamine; phosphatidylserine; phosphatidylcholine; phosphatidic acid; Triton X-100.



Incubation Mixture	$^{32}P \sim III^{Glc}$ remaining cpm	Products formed: Me α-Glc-6-^{32}P cpm	Products formed: $^{32}P_i$ cpm
complete	0	6153	229
− KF	0	5222	1359
− II-B'	6121	98	158

Incubations (0.14 ml): $^{32}P \sim III^{Glc}$, 6400 cpm;
Me α-Glc, 0.5 μm; $MgCl_2$, 1.0 μm; KF, 1.0 μm; Bicine buffer, pH 8.0, 10 μm; 10 min, 37°

Fig. 15. Transfer of phosphate from $^{32}P \sim III^{Glc}$ to methyl α-glucoside.

PHYSIOLOGICAL FUNCTIONS OF THE LOW-AND HIGH-AFFINITY PTS

Considerable information has been accumulated during the past few years about the physiological functions of the PTS by isolation and study of specific mutants. Summaries of these studies have been published (9, 27). The physiological role of the low-affinity PTS (II-A/II-B system) seems primarily to be one of translocating sugars across the bacterial cell membrane. The physiological functions of the high-affinity PTS (III/II-B′ system) in E. coli and S. typhimurium are not yet clear. It seems likely that the high-affinity system is involved in the translocation of sugars across the cell membrane when only very low concentrations of substrates are available, but besides these transport functions the system has been implicated in various regulatory functions (9). Strains of E. coli or S. typhimurium with mutations in either the structural gene for enzyme I or HPr are hypersensitive to catabolite repression (28), but this hypersensitivity can be corrected by the addition of cyclic AMP. When catabolite repression-resistant mutants in the enzyme I negative or HPr, negative background were isolated they were shown to be devoid of Factor III^{Glc}. The gene locus for the mutation which makes the cells resistant to catabolite repression (crr mutation) maps adjacent to the PTS operon in S. typhimurium (see Fig. 3) (17). Based on studies with mutants of the type described above the high-affinity PTS in E. coli and S. typhimurium has also been implicated in taking part in the regulation of the transport of sugars that are not phosphorylated and in the regulation of the synthesis of inducible enzymes (29). It is intriguing to speculate about the molecular mechanisms that are involved in these regulatory functions of the high affinity glucose PTS, but conclusive answers will be obtained only through careful studies involving the homogeneous components of the system.

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