Utilization and Transport of Hexoses by Mutant Strains of Salmonella typhimurium Lacking Enzyme I of the Phosphoenolpyruvate-dependent Phosphotransferase System*

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SUMMARY

Mutant strains of Salmonella typhimurium that lack Enzyme I of the phosphoenolpyruvate-dependent phosphotransferase system cannot take up several sugars either by group translocation or by facilitated diffusion. A second mutation, which resulted in a 25-fold enhancement of the ATP-dependent manno(fructo)kinase activity, partially restored the ability of the cells to take up the sugars that this enzyme phosphorylates. It is suggested that phosphorylation of these sugars is required for their release from a membrane carrier into the cytoplasm.

Preliminary evidence indicates that the mutation affecting the constitutively synthesized manno(fructo)kinase results in increased levels of the protein, rather than in a structural modification. This suggested that phosphorylation of these sugars is required for their release from a membrane carrier into the cytoplasm.

A bacterial phosphotransferase system catalyzes the following reactions:

\[ \text{P} \text{enolpyruvate} + \text{HPr} \xrightarrow{\text{Enzyme I}} \text{phospho-HPr} + \text{pyruvate} \]  
\[ \text{Phospho-HPr} + \text{sugar} \xrightarrow{\text{Enzyme II complex}} \text{sugar-P} + \text{HPr} \]

Enzyme I and HPr\(^1\) are the energy-coupling proteins of the system, common to all sugars phosphorylated by this P-enolpyruvate-dependent mechanism. Enzyme II constitutes a family of sugar-specific enzyme complexes, each of which is involved in the transport and phosphorylation of one or a few sugars (1–3). In addition to (or as a consequence of) its role in sugar transport, the PTS is involved in the repression of enzyme synthesis. For example, in mutants deficient in Enzyme I or HPr, the synthesis of a number of inducible enzymes is hypersensitive to repression by several sugars (4, 5).

Mutants resistant to repression were isolated (6) in order to study the role of the PTS in sugar transport independently of its function in controlling enzyme synthesis. The genetic defect in these strains was designated crr for carbohydrate repression resistance. Introduction of the crr mutation into Salmonella typhimurium or Escherichia coli strains lacking Enzyme I or HPr of the PTS (ptsI or ptsII mutants, respectively (7)) permitted them to utilize certain carbohydrates.\(^2\)

Enzyme I mutants contain normal or increased levels of the sugar-specific proteins of the PTS, the components of the Enzyme II complexes that are the presumed carriers for transport of the sugars across the cell membrane (3). Thus, such mutants might be expected to be capable of transporting sugars across the membrane by the process of facilitated diffusion. If the rate of this process is adequate, the sugars should be phosphorylated and metabolized by intracellular enzymes, such as ATP-dependent kinases. In fact, the S. typhimurium ptsI mutant first described was shown to grow more rapidly in high concentrations of glucose than in low concentrations, or when glucose entered the cell via an inducible galactose permease (8). Growth dependent on phosphorylation of the sugar by glucokinase. Subsequent attempts to demonstrate growth on fructose, mannose, or N-acetylglucosamine using high concentrations of these sugars in the growth medium were not successful, even though the cells contained kinases capable of phosphorylating these sugars. This suggested that the membrane carriers for these sugars were immobilized by the Enzyme I mutation so that they could not transport sugars.

This communication describes several mutant strains, derived from an S. typhimurium ptsI crr double mutant, which varied with respect to their ability to utilize and take up glucose, mannose, and fructose, but which could not take up or metabolize N-acetylglucosamine or mannitol. Extracts derived from the mutants showed differences either in glucokinase activity or in manno(fructo)kinase activity, providing an explanation for the different abilities of the strains to grow on the hexoses. One of the mutants exhibited greatly elevated manno(fructo)kinase activity; preliminary results suggested that the mutation did not affect the catalytic properties of the enzyme. The increased activity in the cell may therefore result from mutation of a regulatory gene controlling mannokinase synthesis. Until recently, there has been no evidence to suggest that constitutively synthesized enzymes are under regulatory control.

ptsII8 crr-3 guk-2 (SB1191) is a guokase-less mutant derived from ptsI8 crr-3 (SB1798) (6). The properties described below are typical of eight separately isolated guk mutants. ptsII8 crr-3 mak-1 (SB2127), also derived from ptsII8 crr-3, was isolated as a strain which could grow on fructose. ptsII8 crr-3 guk-2 mak-1 (SB2268) was constructed by transfer of the mak-1 gene into ptsI18 crr-3 guk-2 by transduction.

Growth was conducted and followed as described previously (5) and kinase activities (both P-enolpyruvate- and ATP-dependent) were measured at pH 7.4 in the presence of the fluoromagnesium phosphate complex to inhibit phosphatase activity

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\(^1\) The abbreviations used are: HPr, histidine-containing phosphate carrier protein of the phosphoenolpyruvate-dependent phosphotransferase system; cyclic AMP, adenosine 3',5'-monophosphate.

\(^2\) Enzymes systems sensitive to PTS-mediated repression in Salmonella typhimurium include those involved in the catabolism of maltose, glucose, and fructose; these sugars are not phosphorylated by the PTS. The crr mutation permits Enzyme I and HPr-deficient strains to use this class of sugars for growth, and also permits rapid utilization of glucose. However, pts crr double mutants are unable to metabolize most sugars which are taken up via the PTS (mannose, fructose, glucose, galactose, N-acetylglucosamine, mannitol, etc.). Repression of enzyme synthesis is therefore not considered responsible for defective metabolism of these sugars.
as did extracts from the parental strain. Mannose, fructose, and N-acetylglucosamine at the same rates were not phosphorylated unless the wild type strain and showed increased rates of growth on glucose and fructose.

Glucokinase, which phosphorylates glucose and glucosamine, was depressed at least 25-fold increased kinase activity with mannose and fructose as phosphate acceptors, and slightly increased kinase activity with glucose. Phosphorylation of N-acetylglucosamine was not altered. The ratio of specific activity for the phosphorylation of mannose to that of fructose was the same in both extracts. (c) Mannokinase activity in both extracts was about 60,000. The ratio of the phosphorylating activities toward mannose to that of fructose was the same in both extracts.

Mutant strain

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Fructose</th>
<th>N-Acetylglucosamine</th>
<th>Man-</th>
<th>Doubling time on</th>
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<td>$\infty$</td>
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<tr>
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<td>10</td>
<td>17</td>
<td>5</td>
</tr>
</tbody>
</table>

(a) The ratio of specific activity for the phosphorylation of mannose to that of fructose was the same. (b) Activities from both extracts eluted from a Sephadex G-75 column as a protein with a molecular weight of about 60,000. The ratio of the phosphorylating activities toward mannose and fructose was constant across the activity peak for both extracts. (c) Mannokinase activity in both extracts was stable at 60° (10), and the rates of denaturation at 67° were the same. (d) As in the parental strain, the level of mannonokinase activity in mak-1 strains was not appreciably affected by the carbon source used for growth. Thus the enzyme appeared to be constitutively synthesized.

### Table I

Growth rates of mutant strains of *S. typhimurium* on sugars

Table I summarizes growth properties of the pertinent strains. *pta18 err-3*, which lacks Enzyme I of the PTS, and is resistant to repression of enzyme synthesis by carbohydrates, grows slowly on glucose and glucosamine, but not on the other sugars listed. Loss of glucokinase activity (guk-2) resulted in an inability to grow on glucosamine, and a dramatic decrease in the growth rate on glucose. The mutant containing mak-1 gained the ability to utilize mannose at rates comparable with the wild type strain and showed increased rates of growth on glucose and fructose.

As shown in Table II, ATP-dependent kinase activity toward all of the sugars listed, except mannitol, was demonstrable in an extract derived from *pta18 err-3*. Glucokinase, which phosphorylates glucose and glucosamine, was depressed at least 10-fold in strain guk-2, whereas the extracts phosphorylated mannose, fructose, and N-acetylglucosamine at the same rates as did extracts from the parental strain.

Extracts from strains containing the mak-1 mutation showed 25-fold enhanced kinase activity with mannose and fructose as phosphate acceptors, and slightly increased kinase activity with glucose. Phosphorylation of N-acetylglucosamine was not altered. Increased kinase activity generally correlated with enhanced growth rates (Table I).

The following preliminary evidence suggests that the mak-1 mutant and the parental strain contain different quantities of the same mannokinase (rather than different proteins), similar to the one described in *E. coli* by Sebastián and Asensio (10). (a) The ratio of specific activity for the phosphorylation of mannose to that of fructose was the same in both extracts (Table II). (b) Activities from both extracts eluted from a Sephadex G-75 column as a protein with a molecular weight of about 60,000. The ratio of the phosphorylating activities toward mannose and fructose was constant across the activity peak for both extracts. (c) Mannokinase activity in both extracts was stable at 60° (10), and the rates of denaturation at 67° were the same. (d) As in the parental strain, the level of mannonokinase activity in mak-1 strains was not appreciably affected by the carbon source used for growth. Thus the enzyme appeared to be constitutively synthesized.
Mutants for three such proteins have been isolated suggests that evidence for a genetic control mechanism responsible for the transport of sugars (12). After the completion of this work, dehydrogenase activity appeared (13). In these two cases, mutation has been reported in E. coli (11). Although the mutation did not alter the constitutivity of the mannokinase, the level of mannokinase, which phosphorylated mannose and fructose. Nevertheless, only glucose and glucosamine supported growth. Growth of the mutants on glucose required transport of the sugar across the cell membrane and subsequent phosphorylation by glucose kinase. Mutants defective in this enzyme utilized glucose at extremely low rates (20-hour generation time). In contrast, the cells could not utilize fructose, mannose, and N-acetylglucosamine for growth, although kinases that phosphorylate these sugars were present. This suggested that the membrane carriers responsible for the transport of these sugars into the bacterial cell could not mediate facilitated diffusion at rates sufficient to support growth. Uptake experiments confirmed this conclusion. However, a large increase in the mannokinase activity of the cell resulted in enhanced uptake rates for mannose and glucosone. It therefore appears that entry of sugar into the intracellular space requires phosphorylation, although not necessarily by the P-enolpyruvate-dependent phosphotransferase system which is coupled to sugar transport in the wild type cell.

Another class of mutants, exhibiting low to negligible levels of mannokinase activity, has recently been derived from the mak-l strain. These mutants take up fructose and mannose at rates comparable to or lower than the parental strain, ptsH18 con-3 (data not shown). If at least some of these mutants are defective in the structural gene for mannokinase, these results provide further evidence to support the conclusion offered above.

The (mak-l) mutation in ptsH18 con-3 permitted utilization of mannose and fructose in addition to glucose, but not of N-acetylglucosamine or mannitol. A similar mutant phenotype has been reported in E. coli (11). Although the mutation did not alter the constitutivity of the mannokinase, the level of this enzyme activity was elevated 25-fold over that of the parental strain. Possibly, the mutation is in a regulatory gene controlling the constitutive synthesis of mannokinase. A mutation that enhances the constitutive synthesis of the lactose repressor (the product of the I gene) has been reported (12), and, after the completion of this work, a report describing a mutant with enhanced glucose 6-phosphate dehydrogenase activity appeared (13). In these two cases mutation of the promoter genes controlling the synthesis of the proteins was suggested. The isolation of these mutants provides evidence for a genetic control mechanism responsible for the synthesis of constitutive enzymes. The fact that comparable mutants for three such proteins have been isolated suggests that the mechanism may be of general importance in the synthesis of many proteins.

**REFERENCES**


**On the Equilibrium of the Adenylate Cyclase Reaction**

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**SUMMARY**

With a partially purified enzyme from Brevibacterium liquefaciens, the adenylate cyclase reaction, ATP → cyclic AMP + PPi, was demonstrated to be readily reversible. Pyruvate, which was known to activate the reaction in the forward direction, had a similar stimulatory effect on the reverse reaction. The equilibrium constant at pH 7.3 and 25°C was approximately 0.065 M at the magnesium concentrations used. This result indicates a high free energy of hydrolysis (ΔG°) for the 3′ ester bond of cyclic adenosine 3′,5′-monophosphate (cyclic AMP), estimated at −11.9 kcal mol−1 under these conditions.

Since the reaction involves one reactant and two products, the equilibrium is concentration-dependent, i.e., high concentrations of cyclic AMP and PPi are required to show a high percentage of conversion to ATP; high enzyme concentrations and long incubation periods are therefore needed. The recent report by others of failure to confirm the reversibility of the reaction with the same enzyme system is attributed to failure to observe these requirements.

The availability of a partially purified adenylate cyclase from Brevibacterium liquefaciens (1) made feasible the study of the reversibility and the estimation of the equilibrium constant of the reaction:

\[ \text{ATP} \rightarrow \text{cyclic AMP} + \text{pyrophosphate} \]

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The abbreviation used is: cyclic AMP, cyclic adenosine 3′,5′-monophosphate.
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