CARBOHYDRATE TRANSPORT IN STAPHYLOCOCCUS AUREUS IV.

MALTOSE ACCUMULATION AND METABOLISM

D.K. Button[1], J. Barry Egan[2], W. Hengstenberg[3], and M.L. Morse

Webb-Waring Lung Institute and the

Department of Biophysics and Genetics, University of Colorado Medical Center

Denver, Colorado 80220

[1]Institute of Marine Science
University of Alaska
College, Alaska 99701

[2]Department of Biochemistry
University of Adelaide
Adelaide, South Australia 5001

[3]Max-Planck-Institut
Fur Medizinische Forschur
Abteilung fur
Molekulare Biologie
69 Heidelberg 1
Jahnstrasse 29
West Germany

Received April 13,1973

SUMMARY

Staphylococcus aureus transports and metabolizes maltose by a pathway that does not involve the phosphoenolpyruvate dependent phosphotransferase system (PTS). Maltose appears to enter the cell by diffusion, is hydrolyzed to glucose, which is then metabolized chiefly by the PTS, but a second minor pathway of glucose metabolism is also present.

INTRODUCTION

A phosphoenol pyruvate dependent phosphotransferase system (PTS) which translocates carbohydrates and phosphorylates them has been discovered in Staphylococcus aureus (1, 2, 3). It has been speculated (4) that all carbohydrates are transported and phosphorylated by this system in S. aureus, and we have shown that phosphate derivatives of several carbohydrates are formed (5). We wish to show here that maltose is metabolized principally via diffusion and hydrolysis of the non-phosphorylated molecule.

MATERIALS AND METHODS

The cultures employed are derivatives of Staphylococcus aureus NCTC 8511 and have been described in earlier papers (2,5). Characterization of mutants and growth procedures have been described (7). Biochemical procedures, chromatography, sources of chemicals are described in reference (5). The procedure for the study of p-nitrophenyl-α-D-glucoside hydrolysis was as for o-nitrophenyl-β-D-galactopyranoside (7).

RESULTS

Wild type S. aureus and ptsI mutants of it lacking enzyme I of the PTS produce acid from maltose and glucose in broth cultures containing these carbohydrates. The mutants, however, do so only after 3-5 days at 37 C. The acid produced from glucose by ptsI mutants can be owing to a leaky ptsI mutation or to a second pathway of glucose utilization. Further mutations to make the ptsI unable to produce acid from glucose can be induced with mutagens. Transductants selected on glucose media from the ptsI glucose negative strains, formed from phage from the wild type, are of two types: complete wild type, and only glucose positive, in about equal numbers. The first of these represents restoration of enzyme I function and the second, restoration of a second glucose pathway. The nature of the second pathway is unknown but may be an ATP dependent kinase. The principal pathway appears to be via the PTS, since the second pathway (glu A^+) produced only weakly positive colonies on glucose indicator agar.

Maltose utilization- The delayed utilization of maltose cannot be explained on a second glucose pathway unless maltose is first cleaved to glucose. Evidence that S. aureus possesses an enzyme that splits maltose to two molecules of glucose has been presented (1). It has also been reported that the wild type forms a derivative of maltose, a presumed phosphate (5). In an attempt to clarify the pathways of maltose utilization by S. aureus, we have studied the hydrolysis of p-nitrophenyl-α-D-glucoside (PNPαG), an analogue of maltose and also a colorimetric substrate of the staphylococcal maltase. Whole cells as well as extracts were studied.

In figure 1 the velocity of hydrolysis of PNPαG by whole cells, as a function of PNPαG concentration, is shown. Two wild type cultures, and three independently derived ptsI mutants were studied. There is no significant difference between the wild type and the mutants in hydrolysis rate. This result indicates that the phosphorylation step, missing in the ptsI mutant, is not important in the entry of PNPαG into the cell, and that the maltase cleaves the non-phosphorylated analogue.

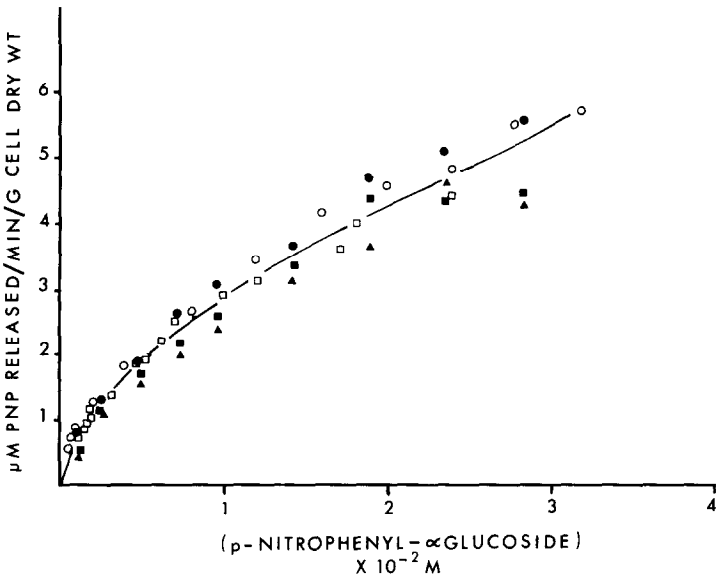

Figure 1
The hydrolysis of p-nitrophenyl-α-D-glucoside by suspensions of the wild type and a ptsI mutants of Staphylococcus aureus as a function of external concentration. Open symbols, wild type cultures. Closed symbols, three independent ptsI mutants.

The concentration range in the plot in figure 1 is between 10^{-3} and 3×10^{-2}M because of the sensitivity of the assay. This plot is similar to that observed earlier (6, 7) in studies of hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG) by intact cells. The lack of a plateau again indicates that the hydrolysis of the nitrophenyl carbohydrate is not the limiting step in uptake and hydrolysis by intact cells. Disrupted cells yield a linear response to PNPαG concentration from the lowest concentrations employable up to the solubility of the substrate.

Exit from the cells of glucose does not require enzyme I of the PTS. When a suspension of cells of the ptsI mutant, also deficient for the second glucose pathway (ptsI gluA) are exposed to ^{14}C-maltose, the maltose enters the cells and is hydrolyzed to glucose which leaks from the cells into the medium. The leached glucose can be separated chromatographically from maltose and quantitated with a scintillation counter. From this information the hydrolysis rate can be shown to be proportional to external maltose concentration over a 10^{4}-

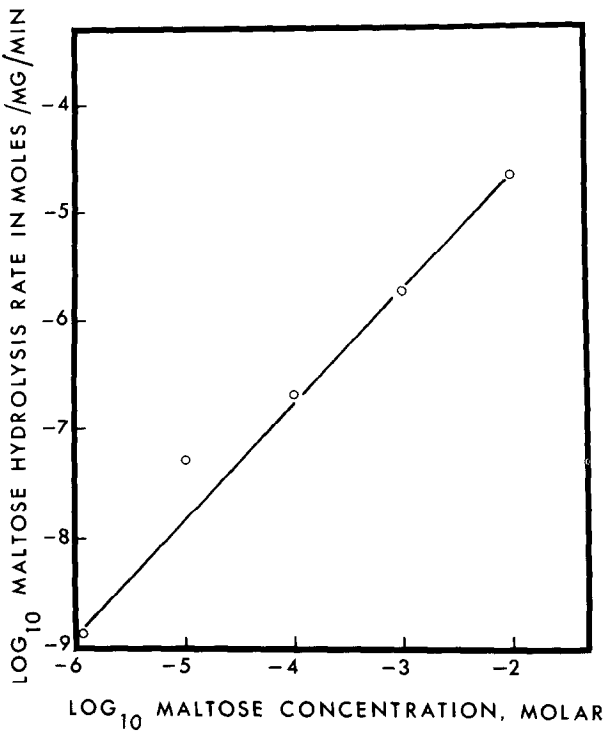

Figure 2
Rate of hydrolysis of maltose by cell suspensions of a ptsI gluA mutant as a function of external maltose concentration, detected by chromatography of the cell culture and measuring the concentration of glucose produced.

fold range (figure 2), another indication that maltase is not the limiting step in uptake and hydrolysis by the intact cell.

Utilization of glucose produced from maltose by a $PtsI^+$ Glu_2^- Mal^+ mutant in the presence of an external glucose oxidase prep- The phosphotransferase has been implicated in membrane passage in several microorganisms (4). The evidence presented above indicates that maltose enters the staphylococcal cell by diffusion and is then hydrolyzed to glucose. What is the route for the utilization of the glucose produced? Must it exit the cell to be phosphorylated and translocated by the phosphotransferase system?

A gluA $ptsI^+$ mutant has been used to test these questions, by exposing suspensions of this mutant to ^{14}C-maltose in the presence of an external glucose trap (glucose oxidase plus catalase), checking for glucose utilization

with a pH indicator and chromatographing the culture medium in systems that resolve glucose, maltose, and gluconic acid. This strain of Staphylococcus aureus does not utilize gluconic acid. The results were: acid production within 18 hours at 37 C. The chromatograms of the fluid of this culture showed no glucose, or gluconic acid, but two small peaks, neither of which corresponds to lactate. Continued incubation of the suspension to 72 hours resulted in increased acidity (over 18 hours) and the formation of a peak corresponding to the gluconic acid.

These results indicate that glucose formed from maltose internally in the staphylococcal cell can be utilized without exiting the cell. Since glucose utilization in this mutant must be via the phosphotransferase system (the second glucose pathway is defective), the results indicate that at least the later steps of the PTS are available to internal glucose.

Action of the PTS on maltose- It has been reported that the wild type strains of S. aureus produce a derivative of maltose, suspected to be phosphate(s) (5). This derivative is produced by wild type, but not ptsI strains. This derivative has been investigated further: (1) There is no enzyme in extracts of wild type cells that can convert this material to products with chromatographic properties different from the untreated compound. This in contrast with the case of lactose-phosphate which was split to galactose-6-phosphate and glucose (8). (2) Both alkaline and acid phosphatase produce no chromatographic change in this material. The nature of this material remains obscure, but it may be a polysaccharide or phosphorous containing maltose polymer.

DISCUSSION

The hydrolysis of PNPαG by intact staphylococcal cells, both by wild type and mutants lacking enzyme I of the PTS is identical and indicates that the analogue enters the cell by diffusion and is hydrolyzed as the free derivative. This is presumably true for maltose as well. Although a derivative, presumed phosphate, is formed by wild type cells, this derivative is

only seen at low maltose concentrations and probably represents a minor pathway, since further metabolism of it was not detected. Perhaps it is a product of a low specificity of the glucose metabolic pathway via the PTS, with the glucose enzyme II phosphorylating maltose. In any case, at high concentrations, a non PTS pathway appears to predominate.

Acknowledgements:

This study was supported by National Science Foundation Grant GB 2726 and by a grant from the Life Insurance Medical Research Fund. This is publication number 500 from the Department of Biophysics and Genetics.

Literature Cited

1. Egan, J. Barry and Morse, M.L., Biochimica et Biophysica Acta, 97, 310 (1965)
2. Hengstenberg, Wolfgang, Penberthy, W.K., Hill Katherine L., and Morse, M.L., Journal of Bacteriology, 99, 383 (1969)
3. Simoni, R.D., Smith, M.F., and Roseman, S., Biochemical - Biophysical Research Communications, 31, 804 (1968)
4. Roseman, Saul (1972), "Carbohydrate Transport in Bacterial Cells", In Metabolic Transport (Ed., Lowell E. Hokin), Volume 6 of Metabolic Pathways
5. Hengstenberg, Wolfgang, Egan, J. Barry, and Morse, M.L., Journal of Biological Chemistry, 243, No. 8, 1881 (1968)
6. Egan, J. Barry and Morse, M.L., Biochimica et Biophysica Acta, 112, 63 (1966)
7. Egan, J. Barry and Morse, M.L., Biochimica et Biophysica Acta, 109, 172 (1965)
8. Hengstenberg, Wolfgang, Egan, J. Barry, Morse, M.L., Proceedings of the National Academy of Sciences, 58, No. 1, 274 (1967)