Preferential Utilization of Glucose over Melibiose, and *vice Versa*, in a *pts* Mutant of *Salmonella typhimurium*

Masayuki KURODA, Nobuko OSAKI, Masaaki TSUDA, and Tomofusa TSUCHIYA*

Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan. *Received December 9, 1991*

Preferential utilization of glucose and melibiose was investigated in wild type cells and in *pts* mutant (*ptsI*-leaky) cells of *Salmonella typhimurium*. A typical diauxic growth and preferential utilization of glucose over melibiose were observed in wild type cells when these two sugars were added as carbon source. Similar results were obtained with a *pts* mutant (SB1476) although utilization of glucose was slow. When cyclic adenosine 3′,5′-monophosphate (cAMP) was added to the culture medium to release the catabolite repression, preferential utilization of glucose was still observed in wild type cells. With glucose-induced mutant cells, preferential utilization of glucose was observed in the presence of cAMP. Gradual utilization of melibiose took place when glucose concentration in the medium decreased. Surprisingly, preferential utilization of melibiose over glucose was observed with melibiose-induced and glucose-uninduced mutant cells in the presence of cAMP.

Keywords sugar utilization; glucose; melibiose; catabolite repression; *pts* mutant; *Salmonella typhimurium*

Diauxie is a classic and famous phenomenon in the growth of microorganisms such as *Escherichia coli* and *Salmonella typhimurium*. When cells are grown in the presence of two carbohydrates, such as glucose and lactose (or melibiose; *S. typhimurium* can't utilize lactose), the growth curve exhibits two successive growth cycles, separated by a period of lag.[1,2] This type of growth behavior has been termed diauxie. It is now known that there are two classes of carbohydrates which result in diauxie, the first is represented by glucose and the second by lactose, melibiose and glycerol. Addition of both a first class carbohydrate and a second class one to the medium as carbon sources causes the diauxic growth. In 1972 it was established that the first class carbohydrates were taken up by cells *via* the phosphoenolpyruvate: sugar phosphotransferase system (PTS) (PTS carbohydrates), while those in the second class (non-PTS carbohydrates) were not taken up by the PTS,[3] instead being incorporated either by active transport or by facilitated diffusion.

There are two control mechanisms behind the diauxic growth, catabolite repression and inducer exclusion.[4,5] When glucose (or other PTS carbohydrate) is present in the growth medium, intracellular cyclic adenosine 3′,5′-monophosphate (cAMP) concentration is reduced, and expression of cAMP-dependent operons, such as the lactose operon and the melibiose operon and others, is repressed.[6,7] This phenomenon is called catabolite repression. The mechanism of catabolite repression has been elucidated in detail, and the PTS is involved in it.[8] The second control mechanism, inducer exclusion, works on the transport process. Again, the PTS is involved[8]: glucose or other PTS carbohydrates indirectly inhibit transport of non-PTS carbohydrates, such as melibiose, through the PTS.

It has not been clear whether or not catabolite repression alone or inducer exclusion alone is enough to regulate utilization of non-PTS carbohydrates. Here, we report the contribution of inducer exclusion in preferential utilization of glucose over melibiose, and the effects of cAMP on the utilization of sugars in wild type and a *pts* mutant of *S. typhimurium*.

Experimental

Bacterium, Medium and Growth *S. typhimurium* strains LT2 (wild type), SB1476 (enzyme I-leaky mutant derived from LT2)[9] were used. This mutant was generously provided by Dr. M. Saier of University of California, La Jolla. Cells were grown in a minimal medium[10] (Na^+ salts were replaced with K^+ salts) supplemented with 2 mM glucose and 5 mM melibiose, unless otherwise indicated. cAMP was added at 5 mM where indicated. Cells were grown under aerobic conditions at 37 °C, and the growth was monitored turbidimetrically at 650 nm.

Measurement of Glucose and Melibiose in the Culture Medium Samples (2.0 ml) were taken at intervals, and filtered through a membrane filter (pore size, 0.45 μm) to remove cells. Glucose in the filtrate was determined by glucose oxidase method (Glucose test, Wako Pure Chemicals, Osaka), as suggested by the manufacturer. To determine melibiose in the presence of glucose, the procedure outlined by Asensio *et al.*[11] for the measurement of lactose was modified. The sample (0.1 ml) was diluted with 0.9 ml of water. To this was added 0.1 ml of 5% solution of $NaBH_4$, and the mixture was kept at room temperature for 2 h to reduce glucose and melibiose. The remaining D-galactopyranosyl-α-D-glucitol was determined by the anthron method.[12]

Results

Diauxic Growth and Preferential Utilization of Glucose over Melibiose When wild type cells of *S. typhimurium* LT2 were grown in a minimal medium containing 2 mM glucose and 5 mM melibiose as carbon sources, a typical diauxic growth was observed (Fig. 1). The first growth phase continued for about 3 h, and after a brief cessation of growth, the second growth phase began. Generation

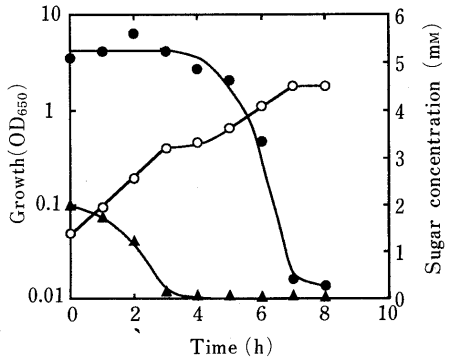

Fig. 1. Diauxic Growth and Sugar Utilization in Wild Type LT2

Cells of *S. typhimurium* LT2 were grown in a minimal medium containing 2 mM glucose and 5 mM melibiose at 37 °C under aerobic conditions. Cell growth (○) was monitored turbidimetrically at 650 nm. Concentration of glucose (▲) and melibiose (●) in the medium was determined colorimetrically.

time of the first phase was 58 min and that of the second was 79 min. It seemed that during the first growth phase both catabolite repression and inducer exclusion were operating because of the presence of glucose in the culture medium, and melibiose utilization was inhibited. We measured the contents of glucose and melibiose in the culture medium during the growth. As shown in Fig. 1, glucose was consumed in the first growth phase, as expected; after it was exhausted, cell growth stopped for a while (about 60 min). As previously shown in *E. coli*,[13] induction of the melibiose operon occurred after this short lag time (data not shown); cells then began consuming melibiose and growth resumed. Thus preferential utilization of glucose over melibiose was confirmed in *S. typhimurium*. After the exhaustion of melibiose, growth of cells stopped.

Strain SB1476 is a mutant possessing very low activity of enzyme I (about 0.5% of the parent)[9] which is a member of the PTS. This mutant grew on glucose (and other PTS carbohydrates) very slowly because of the defect in the PTS (Fig. 2). The generation time of SB1476 cells was 220 min when glucose was the carbon source, compared with 50 min in the wild type. However, SB1476 cells grew normally on melibiose. The generation time was 63 min with SB1476 and 57 min with the wild type. Thus is seems that melibiose utilizing ability is almost the same in the mutant and the wild type.

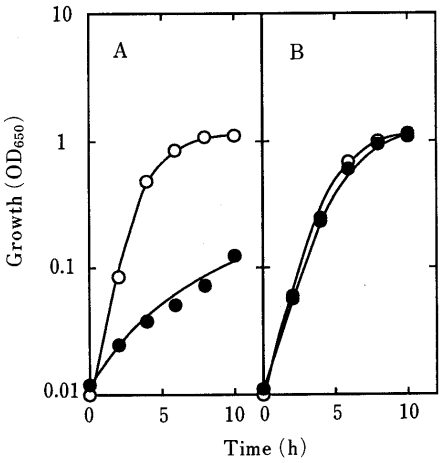

Fig. 2. Growth of Wild Type (LT2) Cells and *pts*-Mutant (SB1476) Cells on Glucose or Melibiose

Cells of LT2 (○) or SB1476 (●) were grown in a minimal medium containing either glucose (A) or melibiose (B).

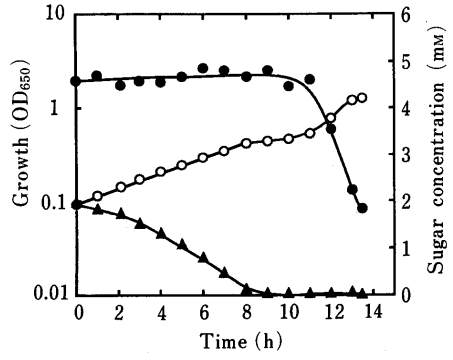

Fig. 3. Diauxic Growth and Sugar Utilization in SB1476

Growth of cells (○), and concentration of glucose (▲) and melibiose (●) in the medium were measured as in the legend for Fig. 1.

We observed diauxic growth and preferential utilization of glucose over melibiose in this mutant, too (Fig. 3). When cells of SB1476 were grown in a medium containing glucose and melibiose as carbon source, we observed a slow growth phase, transient cessation of the growth and then a fast growth phase. In the first slow growth phase (generation time was 225 min), a slow utilization of glucose was observed. In the second growth phase, however, the growth rate became higher with the consumption of melibiose. Generation time during this second phase was 90 min which was comparable to that observed in Fig. 2.

Effects of cAMP on Utilization of Sugars in Wild Type and *pts* Mutant It is well known that cAMP releases catabolite repression.[7] We tested the effect of cAMP on the diauxic growth and the preferential utilization of glucose over melibiose in wild type cells (Fig. 4). When 5 mM cAMP was added to the culture medium containing glucose and melibiose, the transient cessation of the growth observed in the absence of cAMP disappeared; instead, the growth curve became biphasic. Generation time of the first phase was 60 min and that of the second phase was 75 min, both of which corresponded to generation time of the first and the second, respectively, of the diauxic growth described above (Fig. 1). Thus, it still seemed that glucose utilization occurred preferentially over melibiose; glucose was consumed during the first growth phase, and melibiose during the second phase (Fig. 4). Also, it seemed that the melibiose operon which consists of *melA* (α-galactosidase gene) and *melB* (melibiose carrier gene) was induced during the first growth phase (glucose is present), because of the presence of both cAMP and inducer (melibiose). In fact, we observed α-galactosidase activity during the first growth phase (data not shown).

We also tested the effects of cAMP on the sugar utilization and on growth in SB1476. Two different types of growth curve was seen. When cells were pre-grown on glucose, or glucose plus melibiose, and then inoculated into the medium containing glucose, melibiose and cAMP, there were two consecutive growth phases, a slow phase (generation time; 170 min) and then a fast phase (generation time; 100 min) (Fig. 5). During the growth, glucose was utilized first, and then utilization of melibiose started as the glucose concentration in the medium decreased. As melibiose was consumed and the cells grew, the growth rate increased.

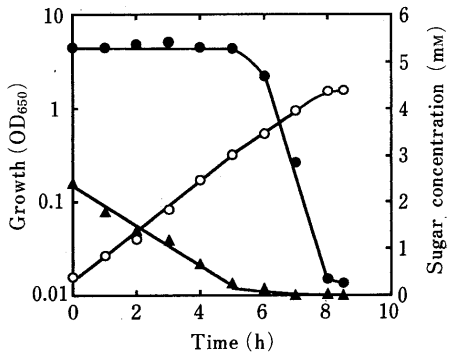

Fig. 4. Effect of cAMP on Diauxic Growth and Sugar Utilization in LT2

Cells were grown in a minimal medium containing 2 mM glucose, 5 mM melibiose and 5 mM cAMP. Cell growth (○), and concentration of glucose (▲) and melibiose (●) in the medium were measured as in the legend for Fig. 1.

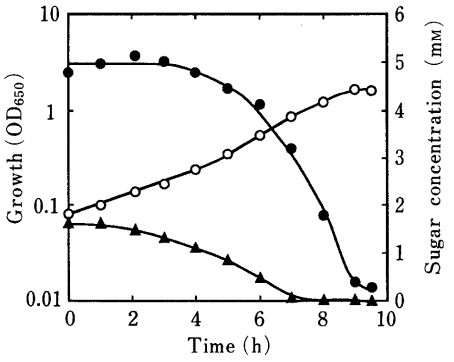

Fig. 5. Effect of cAMP on Diauxic Growth and Sugar Utilization in SB1476

Cells of SB1476 pre-grown on glucose, or on glucose plus melibiose, were inoculated into a minimal medium containing 2 mM glucose, 5 mM melibiose and 5 mM cAMP. Cell growth (○) and concentration of glucose (▲) or melibiose (●) were measured as in the legend for Fig. 1.

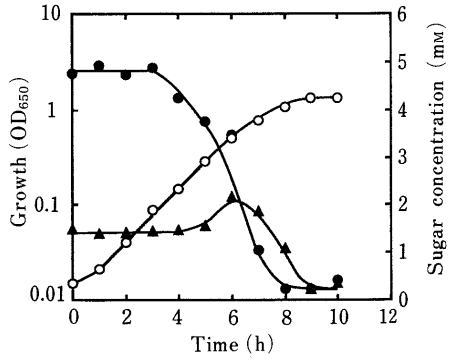

Fig. 6. Preferential Utilization of Melibiose over Glucose in SB1476

Cells were pre-grown on melibiose and inoculated into a minimal medium containing 2 mM glucose, 5 mM melibiose and 5 mM cAMP. Cell growth (○), and consumption of glucose (▲) and melibiose (●) were measured.

On the other hand, when cells were pre-grown on melibiose, and inoculated into a medium containing glucose, melibiose and cAMP, a monophasic growth pattern was obtained (Fig. 6). The generation time was 74 min. Measurement of the utilization of glucose and melibiose showed, surprisingly, that melibiose was utilized first and then glucose was utilized in the presence of cAMP. Some increase in medium glucose was observed just before the exhaustion of medium melibiose. It seemed that this increase in medium glucose was derived from melibiose which was cleaved to glucose and galactose by α-galactosidase in cells. To test this possibility we measured medium glucose with SB1476 grown on melibiose in the presence of cAMP. As expected, glucose was found in the culture medium just prior to the exhaustion of melibiose, and the excreted glucose was consumed afterward (data not shown).

Effects of cAMP on the Growth of SB1476 Cells of SB1476 can't grow when melibiose is added as a sole carbon source to the culture medium in the presence of methyl-α-glucoside, an unmetabolizable PTS-sugar.[9] To evaluate the contributions of catabolite repression and inducer exclusion in the growth inhibition, we tested the effect of cAMP on the growth of SB1476 on melibiose in the presence of methyl-α-glucoside. An addition of cAMP (5 mM) to the culture medium did not have a significant effect on the growth of SB1476 (data not shown). Although cAMP releases the catabolite repression, the inducer exclusion is not affected. Thus the major cause of the observed growth inhibition by methyl-α-glucoside in SB1476 is inducer exclusion, the inhibition of melibiose uptake.

Discussion

When wild type cells of *S. typhimurium* were grown on glucose and melibiose, glucose was utilized first and then melibiose, regardless of the presence or absence of added cAMP. In the absence of cAMP, both catabolite repression and inducer exclusion are functional, and the utilization system for melibiose (non-PTS sugar) is repressed and its transport inhibited by glucose (PTS sugar). In the presence of cAMP, catabolite repression is released and only inducer exclusion is operative. Preferential utilization of glucose over melibiose was still observed under such conditions. This means that inducer exclusion is sufficient for assuring preferential utilization of glucose over melibiose in wild type cells.

It is not clear whether catabolite repression alone is adequate for the preferential utilization of PTS carbohydrates over non-PTS carbohydrates. An inducer exclusion-resistant mutant is necessary to test this idea.

In the *pts* mutant SB1476, although rate of utilization of glucose was slow compared with that in wild type, preferential utilization of glucose over melibiose was observed in the absence of added cAMP. When SB1476 cells pre-grown in the presence of glucose were used, preferential utilization of glucose was still observed in the presence of cAMP. However, when cells not induced with glucose but induced with melibiose were used, surprisingly, melibiose was utilized preferentially in the presence of cAMP. This suggests that if the glucose PTS is not induced in the mutant, then the inducer exclusion does not function well and there is sufficient transport of melibiose to support cell growth. The mutant cells thus grow with consumption of melibiose even in the presence of glucose. Another possibility is that an unknown regulatory mechanism(s) is involved in glucose utilization. For example, when melibiose is taken up, glucose is produced from melibiose in cells. Glucose might be accumulated in cells of SB1476 perhaps because of its slow metabolism in this mutant and leaks from cells, as suggested by the fact that glucose was detected in the culture medium of SB1476 when melibiose was added. The high intracellular glucose might repress or inhibit glucose utilization in the presence of cAMP in SB1476. On the other hand, glucose was not detected in the culture medium with wild type cells when melibiose was added (data not shown), suggesting that glucose concentration in wild type cells is not high. Thus such repression or inhibition would not work in wild type cells under such conditions, even if such mechanisms exist. It should be noted that cAMP alone did not inhibit glucose utilization in SB1476.

Acknowledgment This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan to T. T.

References

1) J. Monod, *C. R. Acad. Sci., Ser. D*, **212**, 934 (1941).
2) J. Monod, *Growth Symposium*, **11**, 223 (1947)

3) S. Roseman, "Metabolic Transport," Academic Press, New York, 1972, p. 41.
4) B. Magasanik, *Cold Spring Harbor Symp. Quant. Biol.*, **26**, 249 (1961).
5) B. Magasanik, "The Lactose Operon," Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1970, p. 189.
6) R. S. Makman and E. W. Sutherland, *J. Biol. Chem.*, **240**, 1309 (1965).
7) R. Perlman and I. Pastan, *J. Biol. Chem.*, **243**, 5420 (1968).
8) M. H. Saier, *Microbiol. Rev.*, **53**, 109 (1989).
9) M. H. Saier and S. Roseman, *J. Biol. Chem.*, **251**, 6584 (1976).
10) S. Tanaka, S. A. Lerner, and E. C. C. Lin, *J. Bacteriol.*, **93**, 642 (1967).
11) C. Asensio, G. Avigado, and B. L. Horecker, *Arch. Biochem. Biophys.*, **103**, 299 (1963).
12) J. H. Roe, *J. Biol. Chem.*, **208**, 889 (1954).
13) T. Okada, K. Ueyama, S. Niiya, H. Kanazawa, M. Futai, and T. Tsuchiya, *J. Bacteriol.*, **146**, 1030 (1981).