Bull Tokyo Dent Coll (2015) 56(3): 185–188

Short Communication

Additional Glucose-PTS Induction in *Streptococcus mutans* Mutant Deficient in Mannose- and Cellobiose-PTS

Yutaka Sato[1)], Kazuko Okamoto-Shibayama[2)] and Toshifumi Azuma[1,3)]

[1)] *Department of Biochemistry, Tokyo Dental College, 2-9-18 Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan*
[2)] *Department of Microbiology, Tokyo Dental College, 2-1-14 Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan*
[3)] *Oral Health Science Center, Tokyo Dental College, 2-9-18 Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan*

Received 19 February, 2015/Accepted for publication 13 April, 2015

Abstract

Streptococcus mutans utilizes maltooligosaccharides, including maltose derived from human dietary starch. We recently reported that the glucose-phosphotransferase system (Glc-PTS) was also involved in the metabolism of glucose derived from intracellular maltooligosaccharides in *S. mutans*. The activity of the Glc-PTS was mediated by the mannose-(*manLMN*) and cellobiose-PTSs (*celABRCD*) in this organism. The purpose of this study was to identify which kind of glucose transporter was involved in this process. A *celD, manLM,* and *glk* triple mutant, cm6vU1, was constructed and its growth in maltose or glucose broth measured. When cm6vU1 cells were inoculated into a fresh glucose broth following prolonged incubation with glucose, their growth rate was greater than that in the initial inoculum. This suggested that an additional Glc-PTS was induced in these cells. To investigate this possibility, permeabilized *S. mutans* cells were constructed and Glc-PTS activity examined by photometrical assay method. Activity in the cells was higher in the secondary inocula than in the initial inocula. These results suggest that *S. mutans* possesses an additional as yet uncharacterized PTS transporter for glucose in addition to the mannose- and cellobiose-PTSs.

Key words: *Streptococcus mutans* — Glucokinase — Maltose metabolism — Glucose-PTS — 4-alpha-glucanotransferase

Streptococcus mutans, a major etiologic agent of human dental caries, utilizes maltose and maltooligosaccharides derived from human dietary starch in the presence of saliva or amylase[6)]. These oligosaccharides are taken up as intracellular constituents following transportation utilizing two specific carrier systems[4,9,10)]. Intracellular oligosaccharides are then metabolized by 4-alpha-glucanotransferase encoded by the *malQ* gene, giving rise to intracellular glucose moieties[6)]. Glucose is the first substrate in the Embden-Meyerhof pathway, the first enzymatic reaction of which is phosphorylation of glucose by glucokinase

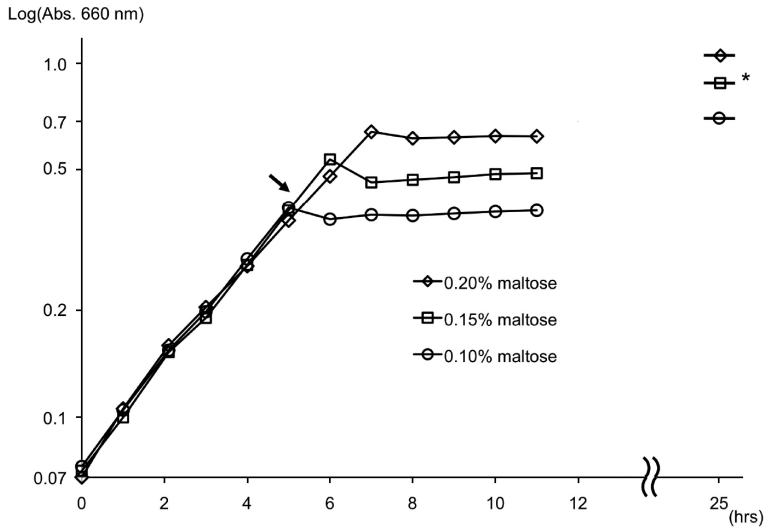

Fig. 1 Growth curves of *celD*, *manLM*, and *glk* triple mutant, cm6vU1

Growth curves are representative of 3 independent experiments.
Growth of mutant in BTR-maltose broth was measured at absorbance (Abs.) of 660 nm with Ultrospec 500 pro spectrophotometer (GE Healthcare Life Sciences). Values of Abs.660 nm were recorded at 1-hr intervals following inoculation of cultures into screw-capped glass tubes containing 11 ml culture. Six-ml samples for Glc-PTS assay were harvested in late exponential phase of first growth phase (indicated by an arrow) from BTR-0.15% maltose cultures. Growth was intermittently measured with remaining 5 ml culture following harvesting. An asterisk indicates 25-hr BTR-0.15% maltose culture.

encoded by the *glk* gene. This suggests that this enzyme is essential in intracellular metabolism of glucose derived from maltose within *S. mutans* cells. However, the Glk protein is not essential in the metabolism of glucose in *S. mutans* as this organism possesses a glucose-phosphotransferase system (Glc-PTS), which transports extracellular glucose into cells with concomitant phosphorylation. The PTS is a widely distributed major sugar transport system in numerous bacteria. It consists of non-sugar-specific cytoplasmic proteins enzyme I and HPr, and a sugar-specific membrane protein, enzyme II. The PTSs in various bacteria involve orthologous proteins, and each orthologous protein family is usually classified by the name of a specific sugar as a prefix, glucose-PTS, for example. However, actual sugar-specificity sometimes differs among species. The *S. mutans* PTS protein encoded by the gene previously symbolized as *ptsG* (meaning glucose-PTS) actually encodes maltose-PTS activity, and has therefore recently been renamed *malT*[9]. The activity of the Glc-PTS in *S. mutans* is reported to be mediated by the mannose-(*manLMN*)[1] and cellobiose-PTSs (*celABRCD*)[12].

We recently reported that *S. mutans* released intracellular glucose derived from maltose and maltooligosaccharides, and that this extracellular glucose was transported back into the cells by the Glc-PTS[5] with concomitant phosphorylation. It was concluded, therefore, that glucokinase was not essential in the metabolism of maltose in this organism. This suggests that the activity of the Glc-PTS plays an important role in the metabolism of maltose in *S. mutans*. The purpose of this study was to identify which kind of glucose transporter was involved in this process. A *celD*, *manLM*, and *glk* triple mutant, cm6vU1, was constructed and its growth in the presence of maltose or glucose as the sole carbon source and Glc-PTS activity investigated.

The mutant cm6vU1 was constructed by employing a markerless mutagenesis method[11]

Table 1 Glucose-PTS activities of cm6vU1 and UA159

cm6vU1 (maltose)	cm6vU1 (adapted)	UA159 (glucose)
ND*	4.03 ± 0.59	10.62 ± 4.62

(nmoles/min/mg dry weight)

*ND: Not Detected. (mean ± SD, n = 5)

as described previously. Growth of *S. mutans* strains UA159 or cm6vU1 in BTR (modified Berman's basal medium)-maltose or -glucose broth[7] was measured at an absorbance (Abs.) of 660 nm with the Ultrospec 500 pro spectrophotometer (GE Healthcare Life Sciences, Uppsala, Sweden). The mutant cm6vU1 exhibited diauxic growth (Fig. 1) in the presence of maltose as the sole carbon source, as was previously reported in the *manLM* and *glk* double mutant, cmvU1[5]. However, growth cessation before the 2nd growth phase in cm6vU1 was extremely prolonged, and the growth yield of the initial growth phase appeared to depend on the concentration of maltose (Fig. 1). In addition, when cm6vU1 cells (cultured for 25 hr; indicated by an asterisk in Fig. 1) designated adapted cells were inoculated into fresh BTR-glucose broth, their growth rate was markedly higher than that in the initial inoculum (data not shown). This suggested that another Glc-PTS was active in these cells. The cm6vU1 cells grown in BTR-0.15% maltose and harvested during the exponential growth phase (indicated by an arrow in Fig. 1), adapted cm6vU1 cells grown in BTR-glucose, and parental strain UA159 cells grown in BTR-glucose were permeabilized according to the method reported by Vadeboncoeur and Trahan[8]. These cells were then assayed for Glc-PTS activity using NADH-linked lactate dehydrogenase enzymatic reaction. Permeabilized cells were incubated for 5 min at 37°C in the reaction mixture. The reactions were started by the addition of phosphoenolpyruvate (PEP) or H_2O as a PEP-independent NADH oxidase control[3]. The reaction mixture was then centrifuged and the supernatant prepared following filtration through a disposable membrane filter unit, Dismic-03CP045AN (Advantec, Tokyo, Japan), for spectrophotometric assay. Specific activities were expressed as nanomoles of PEP-dependent NADH oxidized per minute per milligram (dry weight) in the cells.

Table 1 shows Glc-PTS activity in these cells. The cm6vU1 cells grown in BTR-maltose broth showed no Glc-PTS activity under these experimental conditions, whereas the adapted cm6vU1 cells did, at a level corresponding to approximately 40% of that of the parental strain, UA159. In addition, no glucokinase activity was detected in cm6vU1, even in cells adapted to glucose (data not shown). This finding is incompatible with the hypothesis that non-PTS transport systems support the growth of adapted cm6vU1 cells. In a recent report[5], we hypothesized that the second growth phase of cmvU1 (*manLM*, *glk*) represented a contribution from the Glc-PTS designated cellobiose-PTS. However, the present results appear to contradict this hypothesis, rather suggesting that *S. mutans* possesses another PTS transporter for glucose in addition to mannose-PTS and cellobiose-PTS. This as yet uncharacterized Glc-PTS was likely repressed in the presence of maltose but may be induced under famine conditions such as an insufficiency of extracellular glucose, for example. Genome sequence data[2] will help to identify the candidate gene(s) for this novel Glc-PTS activity.

Acknowledgements

We wish to thank J. Merritt (Oregon Health & Science University, School of Dentistry, Portland, OR) for providing the IFDC2 cassette. We thank H.K. Kuramitsu (State

University of New York at Buffalo, NY) for critically reading this article.

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Correspondence:
Dr. Yutaka Sato
Department of Biochemistry,
Tokyo Dental College,
2-9-18 Misaki-cho, Chiyoda-ku,
Tokyo 101-0061, Japan
E-mail: yusato@tdc.ac.jp