JOURNAL OF BACTERIOLOGY, Dec. 1979, p. 1102-1104
0021-9193/79/12-1102/03$02.00/0

Vol. 140, No. 3

Lactose Metabolism by *Streptococcus mutans*: Evidence for Induction of the Tagatose 6-Phosphate Pathway

IAN R. HAMILTON* AND HILDA LEBTAG

Department of Oral Biology, Faculty of Dentistry, The University of Manitoba, Winnipeg, Canada R3E OW3

Received for publication 5 September 1979

Growth on lactose by strains of *Streptococcus mutans* resulted in the induction of the lactose-phosphoenolpyruvate-phosphotransferase system, phospho-β-galactosidase, and the enzymes of the tagatose 6-phosphate pathway.

We have previously demonstrated that strains of *Streptococcus mutans* and *S. sanguis* 10556 transport lactose via an inducible lactose-phosphoenolpyruvate (PEP)-phosphotransferase (PT) system (8), which results in the transport and phosphorylation of the lactose molecule in the 6 position of the galactose moiety. Subsequent action by the intracellular, inducible enzyme phospho-β-galactosidase results in the formation of glucose and galactose 6-phosphate (4, 8). However, another oral isolate, *S. salivarius* 25975, utilizes both the lactose-PT system and the enzymes in the *lac* operon, since β-galactosidase is also induced to high levels after growth on lactose (8). In the latter case, the products of the initial reaction are glucose and galactose, with the latter hexose being converted to glucose 6-phosphate via the Leloir pathway (2): D-galactose → D-galactose 1-phosphate → D-glucose 1-phosphate → D-glucose 6-phosphate. On the other hand, results from work with *Staphylococcus aureus* (1) and group N streptococci (2, 12) have demonstrated that the galactose 6-phosphate formed from the action of the lactose-PT system is metabolized to triose phosphates of the glycolytic pathway via the tagatose 6-phosphate pathway (1), D-galactose 6-phosphate → D-tagatose 6-phosphate → D-tagatose 1,6-diphosphate → D-glyceraldehyde 3-phosphate plus dihydroxyacetone phosphate, and involves the enzymes galactose 6-phosphate isomerase, tagatose 6-phosphate kinase, and tagatose 1,6-diphosphate aldolase, respectively. Since strains of *S. mutans* induce the lactose-PT system and phospho-β-galactosidase during growth on lactose (3, 4, 8), we have undertaken to determine whether strains of this organism also possess the enzymes for the tagatose pathway. As a comparison, we have also measured the activity of β-galactosidase and enzymes of the Leloir pathway in the same strains, since both pathways are known to be present in group N streptococci (2).

Strains AHT, FA1, Ingbritt, B13, and OMZ65 of *S. mutans*, representing serogroups *a*, *b*, *c*, *d*, and *g* (11), respectively, were grown anaerobically in tryptone-yeast extract broth (8) supplemented with the appropriate carbon source at a concentration of 0.3%. Cells were harvested in the exponential phase of growth, washed twice in Tris-hydrochloride buffer (pH 7.5, 50 mM), and resuspended in the same buffer. A portion of the suspension was used to assay for PEP-PT activity after toluene treatment, as previously described (7). Cell extracts were prepared from the remainder of the cell suspension by sonic disruption in a Branson sonifier for 5 min (9) followed by centrifugation at 10,000 × *g* for 10 min and overnight dialysis against 50 mM Tris buffer (pH 7.5).

β-Galactosidase (EC 3.2.1.23) and phospho-β-galactosidase were assayed with *o*-nitrophenyl-β-D-galactoside and *o*-nitrophenyl-β-D-galactoside 6-phosphate, respectively, as outlined previously (8). Galactokinase (EC 2.7.1.6), galactose 1-phosphate uridyl transferase (hexose-1-phosphate uridylyltransferase, EC 2.7.7.12), and UDP-glucose 4-epimerase (EC 5.1.3.2) were assayed by published methods (2), whereas tagatose 6-phosphate kinase and tagatose 1,6-diphosphate aldolase were assayed essentially by the methods of Bissett and Anderson (1). The above-mentioned procedures were altered to include a reaction volume of 1.0 ml with Tris buffer (pH 7.5, 10 mM) and, where appropriate, a tagatose 6-phosphate concentration of 1.0 mM (final concentration). Galactose 6-phosphate isomerase was assayed by a three-step procedure involving (i) a primary assay, which involved the conversion of tagatose 6-phosphate to galactose 6-phosphate (1); (ii) dephosphorylation of the galactose 6-phosphate formed; and finally (iii) assay of the galactose formed with galactose dehydrogenase. The primary assay solution (i) contained the following: 10 mM Tris buffer (pH 7.5), 10 mM $MgCl_2$, 10

mM tagatose 6-phosphate, and enzyme in a volume of 0.1 ml. After incubation for 30 min, the galactose 6-phosphate formed was dephosphorylated (ii) by addition of the following to the primary assay solution: 10 μmol of glycine-sodium hydroxide buffer (pH 10.5) and 3 U of alkaline phosphatase to a final combined volume of 0.5 ml. Incubation was for 60 min, at which time the galactose formed was measured with (iii) a Lactose/Galactose Kit of Boehringer Mannheim Ltd., Montreal. Standard curves were linear to 100 μg of galactose 6-phosphate. All spectrophotometric assays were carried out at 37°C in a Pye-Unicam 1800 recording spectrophotometer, employing cuvettes with a light pathway at 10 mm. Protein was determined by the method of Lowry et al. (10), whereas cell dry weight estimations were obtained from optical density/dry weight standard curves (5).

The inducible nature of the lactose-PT system in the five strains of *S. mutans* can be seen by comparing the activities in glucose- and lactose-grown cells when lactose was the substrate in the PT assay (Table 1). Specific activity increases ranged from 3.6 (strain FA1) to 88-fold (strain B13) higher in the lactose-grown cells compared with the same cells grown with glucose. As reported previously (6), considerable variation is observed in the specific activities of the PT system between strains after growth on either glucose or lactose (e.g., B13 versus OMZ65 with glucose) and, in all cases except with B13, the glucose-PT system was repressed after growth in lactose. Induction of the lactose-PT system in these strains is accompanied by the induction of phospho-β-galactosidase after growth on lactose, confirming earlier observations (4, 8). In the three cases in which it was tested, growth on galactose induced higher levels of the enzyme. All strains possessed low β-galactosidase activity, but only with strains B13 and OMZ65 was β-galactosidase induced to any extent after growth on lactose. Growth on galactose resulted in a small increase in activity over that seen with glucose-grown cells.

Whereas activity was observed for all of the enzymes in the Leloir pathway in the test strains (except for OMZ65, which possessed no UDP-glucose 4-epimerase activity), growth in lactose had little effect on enzyme activity (Table 2). Only with galactokinase and galactose 1-phosphate uridylyl transferase was there any increase in specific activity after growth on lactose, and this was less than threefold. Growth on galactose, on the other hand, resulted in significant induction of this pathway in the three strains tested.

Contrary to this are the data for the tagatose pathway enzymes. Except possibly for strains B13 and OMZ65, this pathway would appear to be partially constitutive, since significant activity was seen in preparations of glucose-grown cells. However, growth on lactose resulted in significant induction of the enzymes of the tagatose pathway, although the increases over the glucose-grown activity for galactose 6-phosphate isomerase were not dramatic in three of the strains (AHT, B13, and OMZ65). Thus, it is apparent that *S. mutans* strains employ the tagatose pathway for degradation of the galac-

TABLE 1. *PEP-PT, phospho-β-galactosidase, and β-galactosidase activities in S. mutans strains growth on glucose, lactose, or galactose*

Carbon source for growth	Substrate in assay	Activity				
		AHT[a]	FA1	Ingbritt	B13	OMZ65
PEP-PT system						
Glucose	Glucose	36.7[b]	38.8	21.8	101.4	2.1
	Lactose	4.2	5.6	0.0	0.7	1.8
Lactose	Glucose	28.5	25.8	2.2	101.4	0.0
	Lactose	34.1	20.2	16.8	61.6	55.4
Phospho-β-galactosidase						
Glucose	ONPG-6-P	63.4[c]	23.4	41.1	10.1	18.0
Lactose	ONPG-6-P	322.7	118.0	2109.2	133.8	144.7
Galactose	ONPG-6-P	397.8	2415.4	3479.3	—[d]	—
β-Galactosidase						
Glucose	ONPG	42.6[e]	50.4	51.5	4.1	3.5
Lactose	ONPG	30.7	54.1	34.3	13.7	11.0
Galactose	ONPG	47.8	82.7	54.1	—	—

[a] *S. mutans* strain.

[b] Nanomoles of pyruvate formed per milligram (dry weight) of cells per minute.

[c] Nanomoles of *o*-nitrophenol liberated from *o*-nitrophenyl-β-D-galactopyranoside 6-phosphate (ONPG-6-P) per milligram of protein per minute.

[d] Not done.

[e] Nanomoles of *o*-nitrophenol liberated from *o*-nitrophenyl-β-D-galactopyranoside (ONPG) per milligram of protein per minute.

TABLE 2. *Activity[a] of enzymes in the Leloir pathway and the tagatose 6-phosphate pathways in five strains of S. mutans*

Strain	Serotype	Carbon source for growth	Activity in the Leloir pathway			Activity in the tagatose 6-phosphate pathway		
			Galactokinase	Galactose 1-phosphate uridylyl transferase	UDP-glucose 4-epimerase	Galactose 6-phosphate isomerase	Tagatose 6-phosphate kinase	Tagatose 1,6-diphosphate aldolase
AHT	*a*	Glucose	76.1	0.53	0.39	9.7	19.7	27.6
		Lactose	85.7	0.52	0.42	16.8	373.5	301.0
		Galactose	396.2	2.2	0.91	13.3	117.4	152.5
FA1	*b*	Glucose	11.3	1.2	1.2	14.4	44.7	13.4
		Lactose	27.7	3.3	1.7	37.5	246.9	269.2
		Galactose	23.8	4.0	1.6	23.8	477.4	428.3
Ingbritt	*c*	Glucose	45.0	0.97	0.45	14.8	43.4	7.2
		Lactose	94.7	1.45	0.42	69.3	240.8	17.8
		Galactose	311.2	9.66	5.08	49.0	157.5	38.2
B13	*d*	Glucose	3.5	3.1	0.39	13.7	0.0	0.0
		Lactose	2.4	0.75	0.30	16.7	29.8	2.7
OMZ65	*g*	Glucose	4.6	0.0	0.0	9.8	0.0	0.0
		Lactose	2.5	2.3	0.0	14.3	49.7	23.1

[a] Nanomoles of product formed per milligram of protein per minute.

tose 6-phosphate generated during lactose metabolism through the action of the lactose-PEP-PT system and phospho-β-galactosidase. The presence of β-galactosidase activity in some of these strains indicates that, as in the group N streptococci (2), both the Leloir pathway and the tagatose pathway are operable, with the latter probably of greater physiological significance.

Furthermore, it is clear from the results (Tables 1 and 2) that, in those strains tested (i.e., AHT, FA1, and Ingbritt), galactose metabolism follows similar routes. This hexose can be transported via the PEP-PT system (8) and consequently utilizes the tagatose pathway to degrade galactose 6-phosphate (Table 2). However, the induction of the Leloir pathway after growth on galactose indicates that both pathways are involved in galactose metabolism in these strains.

We thank T. D. Thomas, New Zealand Dairy Research Institute, for his kind gift of tagatose 6-phosphate.

This research was supported by a grant (MT-3546) from the Medical Research Council of Canada.

LITERATURE CITED

1. **Bissett, D. L., and R. L. Anderson.** 1973. Lactose and D-galactose metabolism in *Staphylococcus aureus*: pathway of D-galactose 6-phosphate degradation. Biochem. Biophys. Res. Commun. **52**:641-647.
2. **Bissett, D. L., and R. L. Anderson.** 1974. Lactose and D-galactose metabolism in group N streptococci: presence of enzymes for both the D-galactose 1-phosphate and D-tagatose 6-phosphate pathways. J. Bacteriol. **117**:318-320.
3. **Calmes, R.** 1978. Involvement of phosphoenolpyruvate in the catabolism of caries-conducive disaccharides by *Streptococcus mutans*: lactose transport. Infect. Immun. **19**:934-942.
4. **Calmes, R., and A. T. Brown.** 1979. Regulation of lactose catabolism in *Streptococcus mutans*: purification and regulatory properties of phospho-beta-galactosidase. Infect. Immun. **23**:68-79.
5. **Hamilton, I. R.** 1968. Synthesis and degradation of intracellular polyglucose in *Streptococcus salivarius*. Can. J. Microbiol. **14**:65-77.
6. **Hamilton, I. R.** 1977. Effects of fluoride on enzymatic regulation of bacterial carbohydrate metabolism. *In* Cariostatic mechanisms of fluoride. Caries Res. **11**(Suppl. 1):262-278.
7. **Hamilton, I. R., and D. C. Ellwood.** 1978. Effects of fluoride on carbohydrate metabolism by washed cells of *Streptococcus mutans* grown at various pH values in a chemostat. Infect. Immun. **19**:434-442.
8. **Hamilton, I. R., and G. C. Y. Lo.** 1978. Co-induction of β-galactosidase and the lactose-P-enolpyruvate phosphotransferase system in *Streptococcus salivarius* and *Streptococcus mutans*. J. Bacteriol. **136**:900-908.
9. **Khandelwal, R. L., and I. R. Hamilton.** 1971. Purification and properties of adenyl cyclase from *Streptococcus salivarius*. J. Biol. Chem. **246**:3297-3304.
10. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
11. **Perch, B., E. Kjems, and T. Raun.** 1974. Biochemical and serological properties of *Streptococcus mutans* from various human and animal sources. Acta Pathol. Microbiol. Scand. Sect. B **82**:357-370.
12. **Thomas, T. D.** 1976. Regulation of lactose metabolism in group N streptococci. Appl. Environ. Microbiol. **32**:474-478.