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Uptake and Incorporation of Glucose and Mannose by Whole Cells of *Bacteroides thetaiotaomicron*

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Glucose uptake by whole-cell suspensions of the obligate anaerobe *Bacteroides thetaiotaomicron* was two- to fourfold higher under aerobic conditions than during incubation under atmospheres of N_2 or H_2 gas. The O_2-stimulated uptake activity was lost rapidly (>70% in 5 h) when cell suspensions were incubated aerobically, but this loss was prevented by the addition of crude catalase. Catalase had no apparent effect on cell viability during these incubations. Glucose uptake activity was strongly inhibited by a 10-fold excess of mannose or galactose but not by methyl-α-D-glucoside, fructose, or lactose. Both glucose and mannose were rapidly incorporated into polyglucose after uptake. The O_2-stimulated glucose uptake was not inhibited by cyanide, azide, 2,4-dinitrophenol, or 2-*N*-heptyl-4-hydroxyquinoline-*N*-oxide. However, *p*-chloromercuribenzoate, menadione, and sodium fluoride inhibited uptake by 88, 67, and 55%, respectively. All attempts to detect phosphoenolpyruvate-phosphotransferase activity for glucose, methyl-α-D-glucoside, and 2-deoxyglucose were negative. The bacteria contained hexokinase activity and a complete glycolytic Embden-Meyerhof pathway.

Certain species of the genus *Bacteroides* are the most frequently isolated intestinal anaerobic bacteria (3, 14). Several investigators have reported that *B. vulgatus, B. thetaiotaomicron,* and *B. distasonis* are found in fecal samples in the range of 1×10^{10} to 5×10^{10} viable organisms per gram (wet weight) of human feces (13, 14). Moreover, the intestinal bacteroides consist of a heterogenous group comprising a number of genetically distinct species (9, 15). However, all of the intestinal *Bacteroides* species are superficially very similar (4). Intestinal *Bacteroides* species can ferment a variety of simple sugars (4), mucin, and plant polysaccharides (20), and usually generate succinate, acetate, propionate, and formate as fermentative end products (4, 13).

In view of the obvious ecological importance of these bacteria to man, there is a paucity of published information regarding how these bacteria transport and metabolize nutrients and how they are able to compete and survive so successfully in the anaerobic environment of the human gastrointestinal tract. In this communication, we report selected data on the uptake and metabolism of glucose and mannose by whole cells of *B. thetaiotaomicron.*

MATERIALS AND METHODS

Growth of bacteria. Stock cultures of *B. thetaiotaomicron* were maintained in chopped-meat medium under anaerobic conditions as described by Holdeman and Moore (4). For uptake experiments, *B. thetaiotaomicron* NCTC 10852 was cultured in brain heart infusion-hemin medium or in a chemically defined growth medium as described previously (7).

Cell viability. Cell suspensions were serially diluted in 50 mM potassium phosphate (pH 7.0), and 0.1 ml of each of the diluted suspensions was spread evenly on the surface of duplicate blood agar plates. The inoculated plates were placed in GasPak anaerobic jars (BBL, Cockeysville, Md.); anaerobic conditions were established with H_2 and CO_2 gas generator envelopes. The inoculated plates were incubated at 37°C for 48 h before colony counts were made.

Uptake of [^{14}C]glucose and [^{14}C]mannose by whole-cell suspensions. Cells were harvested by centrifugation at 13,700 × *g* for 15 min at 22°C from a culture sample taken during late log phase of growth. Cells were suspended in a sufficient volume of 50 mM potassium phosphate buffer (pH 7.0) to yield a turbidity of 280 Klett units (no. 66 filter, 1.0 to 1.1 mg of cell protein per ml). Crude beef liver catalase (4,000 IU/ml of cell suspension; Sigma Chemical Co., St. Louis, Mo.) was added to cell suspensions and incubated aerobically for 3 h at 37°C, unless otherwise indicated. Portions of cell suspensions (0.75 ml) were transferred to anaerobe tubes (18 by 142 mm) containing 0.45 ml of 50 mM potassium phosphate buffer (pH 7.0) at 37°C. The uptake reaction was initiated by adding 0.30 ml of prewarmed (37°C) D-[*U*-^{14}C]glucose (1.55 Ci/mol), D-[*U*-^{14}C]mannose (100 Ci/mol), or other selected ^{14}C-labeled sugars. The final concentration of all sugars was 100 μM unless otherwise indicated. Cell suspensions were incubated at 37°C either aerobically, by slowly bubbling air through the cell suspension

with a Pasteur pipette, or anaerobically, by using O_2-free nitrogen or hydrogen gas. Samples (100 μl) were removed at 1, 2, 3, 5, and 7 min and applied to prewashed membrane filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.). Samples were filtered and washed with 2 ml of 37°C, 20 mM potassium phosphate buffer (pH 7.0). Radioactivity of filters bearing washed cells was determined by a previously described procedure (16). Uptake of ^{14}C-labeled sugars was recorded as nanomoles per milligram of whole-cell protein.

Incorporation of [^{14}C]glucose and [^{14}C]mannose by whole-cell suspensions. Incorporation of [^{14}C]glucose and [^{14}C]mannose into cold trichloroacetic acid-precipitable material was measured by using the same reaction mixture and incubation conditions as for uptake studies. Samples (100 μl) were removed at various time intervals, placed in 2 ml of ice-cold 10% trichloroacetic acid (4°C) and allowed to incubate for approximately 30 min. Trichloroacetic acid-treated samples were filtered (0.45-μm pore size; Millipore Corp.), washed with an additional 2 ml of ice-cold 10% trichloroacetic acid, placed into scintillation vials, and counted by a previously described procedure (16).

Purification and characterization of cold trichloroacetic acid-precipitable ^{14}C-labeled material. A 30-ml volume of cell suspension was prepared in the same manner as for [^{14}C]glucose or [^{14}C]mannose uptake studies. Either [^{14}C]glucose or [^{14}C]mannose was added, and the cell suspensions were allowed to incubate for 30 min at 37°C. The cells were harvested by centrifugation at 5,900 × *g* for 10 min at 22°C, and the cell sediments were suspended in 10 ml of 50 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.5) containing 0.4 M sucrose and 1 mM ethylenediaminetetraacetic acid. Lysozyme (50 μg/ml) was added to the cell suspensions and incubated for 15 min at 37°C. Sodium dodecyl sulfate was added (1.5% final concentration) to gently lyse cells. Two volumes of liquified phenol were added to the lysed-cell suspensions, and the mixture was shaken for 15 min at 22°C. The phenol-treated cell suspensions were subjected to centrifugation at 5,900 × *g* for 10 min at 4°C. The bulk of the radioactivity was found in the aqueous supernatant fluid for both glucose and mannose cell suspensions. The aqueous layer was removed, and 2 volumes of cold (4°C) 95% ethanol were slowly added to it. This mixture was centrifuged at 5,900 × *g* for 10 min at 4°C, and the precipitated material, containing more than 80% of the total radioactivity from the aqueous layer, was dissolved in 10 ml of 20 mM sodium phosphate buffer (pH 7.5). Ribonuclease (40 μg/ml) and deoxyribonuclease (40 μg/ml) were added and incubated for 1 h at 37°C, and the ^{14}C-labeled material was dialyzed for 12 h in 1 liter of 20 mM sodium phosphate buffer (pH 7.5) at 4°C. Over 98% of the total radioactivity remained in the dialysis bag for both glucose and mannose. The dialyzed ^{14}C-labeled material was then subjected to acid hydrolysis (0.5 N HCl under nitrogen gas, 95°C for 18 h), and the acid-treated sample was concentrated 2.5-fold by lyophilization. A portion of this sample was then spotted onto Whatman no. 1 paper, and the chromatogram was developed (descending) for 18 h in a solvent system containing ethyl acetate, pyridine, acetic acid, and distilled water (5:5:1:3 by volume). The chromatograms were cut into strips and scanned for radioactivity by a packard radiochromatogram model 7201 scanner (Packard Instrument Co., Downers Grove, Ill.). The remaining portion of the acid-hydrolyzed sample was neutralized to pH 7.0 with 1 N NaOH, and was treated for 1 h with glucose oxidase reagent (Worthington Biochemicals Corp., Freehold, N.J.). The glucose oxidase-treated samples were then chromatographed in the same solvent system described above and scanned for radioactivity.

Enzyme assays. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), glucose 6-phosphate isomerase (EC 5.3.1.9), and hexokinase (EC 2.7.1.1) were determined as previously described (6, 8). Fructose 1,6-bisphosphate aldolase (EC 4.1.2.13) activity was assayed as described by Rutter and Hunsley (19). Phosphofructokinase (EC 2.7.1.11) was assayed spectrophotometrically by coupling its activity to endogenous fructose 1,6-bisphosphate aldolase. Enzyme reactions were initiated by the addition of crude cell extracts. The initial rates of oxidation or reduction of pyridine nucleotides were determined in a Gilford model 2400-S recording spectrophotometer at a wavelength of 340 nm. Assays were performed at room temperature (23 to 25°C), and specific activities were expressed as nanomoles of substrate converted per minute per milligram of extract protein. All enzyme activities were determined in the 105,000 × *g* supernatant fraction of crude extracts prepared as previously described (6).

RESULTS

Initial studies of apparent glucose uptake by freshly prepared whole-cell suspensions of *B. thetaiotaomicron* cultured in brain heart infusion-hemin medium showed that the ability of cells to take up glucose decreased rapidly over a time course of 5 h (Fig. 1). However, the exogenous addition of crude catalase to cell suspensions stabilized and surprisingly increased (50%) the rate of glucose uptake by aerobically incubated cell suspensions. Additional studies showed no detectable differences in cell viability between cell suspensions containing catalase and those not containing catalase over a time course of 5 h. Moreover, crude catalase heated to 95°C for 10 min retained the ability to stabilize and enhance glucose uptake activity. More purified preparations of catalase (Sigma type C-30) failed to stabilize and enhance glucose uptake activity.

The data presented in Fig. 2 show the uptake rates of [^{14}C]glucose, [^{14}C]mannose, [^{14}C]fructose, methyl-α-D-[^{14}C]glucoside and methyl-β-D-[^{14}C]thiogalactoside by whole-cell suspensions of *B. thetaiotaomicron* incubated aerobically in catalase. The apparent rates of glucose and mannose uptakes were much greater than for other carbohydrates tested. However, the uptake of both [^{14}C]glucose and [^{14}C]mannose varied somewhat from experiment to experiment. For

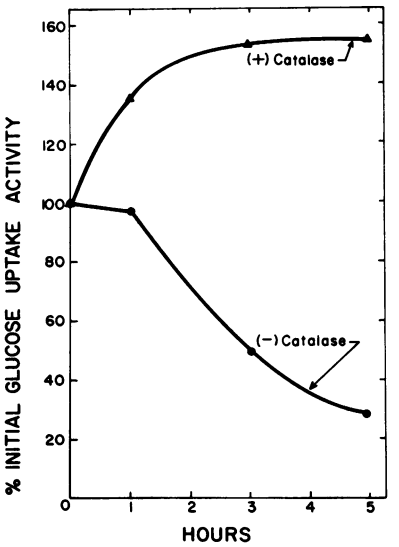

FIG. 1. *Effect of exogenous crude catalase on glucose uptake activity after aerobic incubation of whole-cell suspensions of B. thetaiotaomicron in 50 mM sodium phosphate buffer (pH 7.0) at 37°C. Glucose uptake activity was determined from a 5-min time course.*

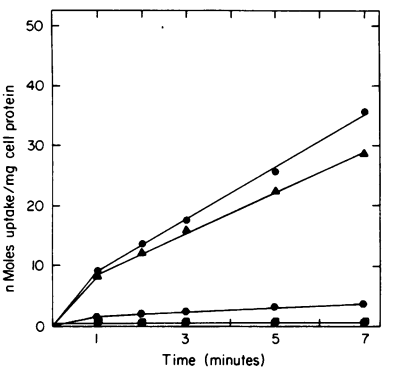

FIG. 2. *Uptake of glucose (●), mannose (▲), fructose (◆), methyl-α-D-glucoside (■), and methyl-β-D-thiogalactoside (□) by aerobically incubated whole-cell suspensions of B. thetaiotaomicron.*

example, the specific activity of glucose uptake varied from 11 to 45 nmol/mg of cell protein over 5 min, for 18 independent experiments, with an average uptake activity of 19.5 nmol/mg of cell protein over 5 min. Similar results were obtained for mannose uptake activity.

The cultural conditions affected the ability of whole cells to take up [^{14}C]glucose (Fig. 3). For example, whole-cell suspensions prepared from cells cultured in brain heart infusion-hemin medium took up [^{14}C]glucose at a much faster rate than those prepared from cells grown in a chemically defined glucose-hemin medium (Fig. 3). The effects of different incubation conditions on the rate of apparent glucose uptake were investigated by using whole-cell suspensions derived from bacteria cultured on brain heart infusion-hemin medium and treated for 3 h with exogenous crude catalase. The extent of glucose uptake was 1.5- to 2-fold greater in cell suspensions incubated aerobically than on the same cell suspensions when incubated anaerobically under N_2 gas (Fig. 3 and 4). The exogenous addition of 10 mM fumarate to cell suspensions incubated

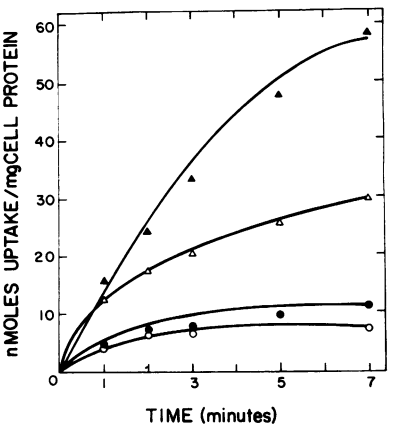

FIG. 3. *Glucose uptake activity of cells cultured in chemically defined glucose medium (○, ●) or brain heart infusion medium (△, ▲) and incubated either aerobically (●, ▲) or anaerobically (○, △) under N_2 gas atmosphere.*

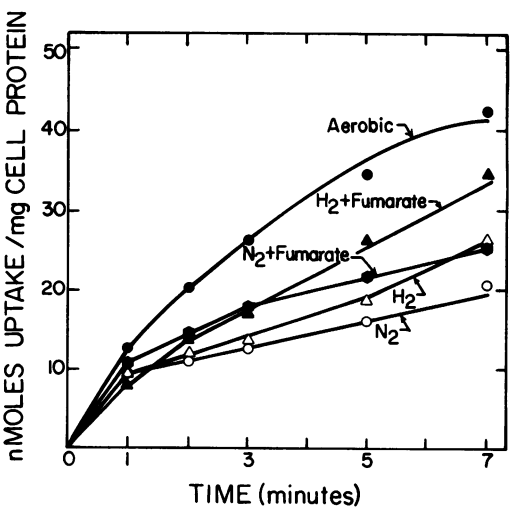

FIG. 4. *Effects of different incubation conditions on glucose uptake activity. Sodium fumarate (10 mM) was added to the reaction mixture 10 min before glucose addition.*

anaerobically under N_2 or H_2 gas always increased the apparent rate of glucose uptake activity when compared with incubation under N_2 or H_2 alone (Fig. 4). The addition of 10 mM fumarate to aerobic cell suspensions did not stimulate further the rate of glucose uptake.

Additional experiments were performed to gain some insight into the specificity of the glucose uptake system by comparing the ability of different unlabeled hexoses and hexose analogs to compete for [^{14}C]glucose uptake. After 1.5 min of incubation in 0.1 mM [^{14}C]glucose, 10-fold excess unlabeled 1.0 mM hexose was added and the apparent rate of [^{14}C]glucose uptake was determined. Only D-mannose and D-galactose effectively competed for glucose uptake (Fig. 5). In other experiments, it was shown that 2-deoxy-D-glucose (at 10-fold excess concentration) was not an effective inhibitor (<10% decrease) of glucose uptake by whole-cell suspensions of *B. thetaiotaomicron*.

Experiments were carried out to determine the effects of selected metabolic and chemical inhibitors on the extent of [^{14}C]glucose uptake. Only NaF, menadione, and *p*-chloromercuribenzoate were effective in inhibiting uptake (Table 1).

Preliminary studies showed that both [^{14}C]-glucose and [^{14}C]mannose were rapidly incorporated into cold 10% trichloroacetic acid-precipitable macromolecules (Fig. 6). The rapid rate of [^{14}C]glucose and [^{14}C]mannose incorporation appears to account for a large portion of glucose (50 to 80%) and mannose (30 to 50%) uptake activity (Fig. 6). Moreover, the apparent uptake and incorporation of [^{14}C]glucose were not inhibited (<10%) by 100 μg of clindamycin per ml or 100 μg of rifampin per ml (unpublished data).

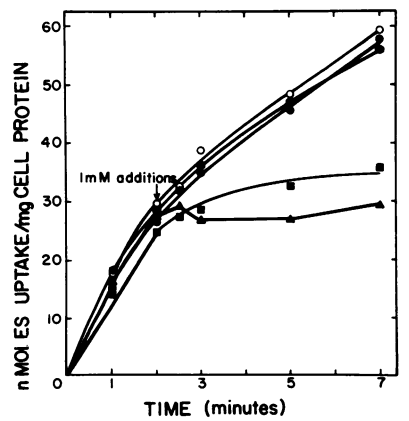

FIG. 5. *Inhibition of aerobic glucose uptake (●) by selected carbohydrates. Fructose (◉), mannose (▲), galactose (■), or methyl-α-D-glucoside (○) was added individually at point indicated by arrow.*

TABLE 1. *Inhibition of aerobic glucose uptake in B. thetaiotaomicron NCTC 10852 by selected compounds*

Compound	Concn (mM)	Uptake activity[a] (%)
Control		100
Azide	10	89
NaF	50	55
KCN	10	87
2,4-DNP[b]	0.1	96
HOQNO[c]	0.1	103
Dicumerol	0.1	97
	1.0	78
Menadione	0.1	53
	1.0	33
	4.0	9
PCMB[d]	0.1	93
	1.0	12

[a] Percent uptake was calculated at 5 min from time course uptake experiments.
[b] 2,4-DNP, 2,4-Dinitrophenol.
[c] HOQNO, 2-*N*-heptyl-4-hydroxyquinoline-*N*-oxide.
[d] PCMB, *p*-Chloromercuribenzoate.

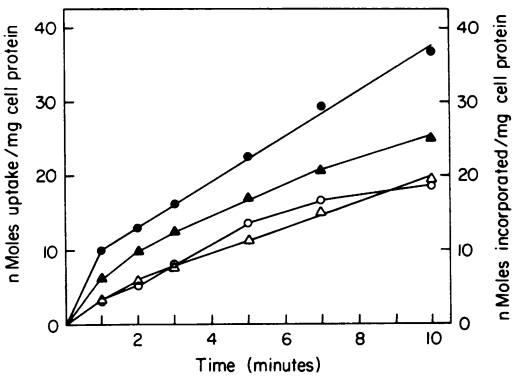

FIG. 6. *Rate of uptake (●, ▲) and incorporation (○, △) of either glucose (△, ▲) or mannose (○, ●) into cold 10% trichloroacetic acid-precipitable material by whole-cell suspensions of B. thetaiotaomicron incubated aerobically at 37°C.*

Additional studies were carried out to partially purify and characterize the rapidly labeled macromolecule(s). All evidence to date indicates that the rapidly labeled macromolecules from both glucose and mannose incorporation experiments behave as polysaccharide, as described above. Acid hydrolysis and paper chromatography of purified polysaccharide from cells allowed to

take up either [^{14}C]glucose or [^{14}C]mannose showed that the product(s) migrated as a single peak identical to free glucose. Treatment of the ^{14}C-labeled acid hydrolysate with glucose oxidase reagent converted all detectable ^{14}C-labeled material to a product that chromatographed with approximately the same R_f value as that of gluconic acid (Table 2). In control experiments, treatment of D-[^{14}C]fructose or D-[^{14}C]mannose with glucose oxidase reagent did not generate any products.

Efforts were made to detect the phosphoenolpyruvate-hexose phosphotransferase system (10, 12, 21) in *B. thetaiotaomicron* by using either toluenized whole-cell suspensions as described by Gachelin (2) or crude sonically treated broken-cell suspensions as described previously (16). Phosphoenolpyruvate-dependent phosphorylation of methyl-α-D-[^{14}C]glucoside was not detected in extracts of *B. thetaiotaomicron*, although the activity was readily demonstrated in extracts of *Escherichia coli* (Table 3). Phosphoenolpyruvate-dependent phosphorylation of [^{14}C]glucose also was not detected in extracts of *B. thetaiotaomicron*, and the results were confirmed in experiments with toluenized whole-cell suspensions (unpublished data). In the absence of positive results for the phosphoenolpyruvate-hexose phosphotransferase system in *B. thetaiotaomicron*, efforts were made to determine alternative mechanisms of sugar phosphorylation.

TABLE 2. *Identification of hexoses in partially purified polysaccharide isolated from B. thetaiotaomicron NCTC 10852*[a]

Hexose	Migration distance (cm)[b]
Glucose standard	29.8
Glucose oxidase-treated standard	16.2
Gluconate standard[c]	17.7
Fructose standard[c]	30.8
Mannose standard[c]	27.8
Acid-treated polysaccharide isolated from cells allowed to incorporate [^{14}C]glucose	29.6
Glucose oxidase-treated acid hydrolysate from cells allowed to incorporate [^{14}C]glucose	16.6
Acid-treated polysaccharide isolated from cells allowed to incorporate [^{14}C]mannose	29.6
Glucose oxidase-treated acid hydrolysate from cells allowed to incorporate [^{14}C]mannose	15.8

[a] For details of polysaccharide isolation, acid hydrolysis and glucose oxidase treatment, see the text.

[b] Descending chromatography (18 h) in a solvent system of pyridine-ethyl acetate-acetic acid-water (5:5:1:3 by volume).

[c] Not substrates for glucose oxidase enzyme.

TABLE 3. *Phosphorylation of methyl-α-D-glucoside by crude extracts of B. thetaiotaomicron and Escherichia coli*[a]

Organism	Additions (mM)	Radioactivity absorbed on Dowex column (cpm)[b]
B. thetaiotaomicron NCTC 10852	None	34
	PEP (5.0)	46
	ATP (5.0)	42
	ATP + PEP (5.0 each)	53
E. coli K-12	None	1,851
	PEP (5.0)	13,457
	ATP (5.0)	1,162
	ATP + PEP (5.0 each)	22,262

[a] Details of method described in reference 16.

[b] Methyl-α-D-[^{14}C]glucoside (approximately 3,000 cpm/nmol) was used as the phosphoryl acceptor in these experiments.

Enzyme assays carried out by using crude cell extracts showed high adenosine 5′-triphosphate-linked hexose phosphorylating activity (Table 4). Surprisingly, cells cultured in peptone-yeast (PY)-galactose had very low levels of fructose 1,6-bisphosphate aldolase activity (Table 4).

DISCUSSION

The stimulation of glucose uptake and incorporation by whole-cell suspensions of *B. thetaiotaomicron* incubated aerobically was surprising in view of the anaerobic nature of this bacterium. However, the enhanced rate of glucose uptake and incorporation by whole-cell suspensions incubated aerobically or anerobically (N_2 or H_2 atmosphere) in the presence of fumarate may be attributed to the ability of O_2 and fumarate to serve as terminal oxidants for an electron transport system. We have shown that whole-cell suspensions of *B. thetaiotaomicron* rapidly take up molecular oxygen although it probably is a non-physiological electron acceptor. Moreover, Macy et al. (13) reported that membranes prepared from *B. fragilis* cultured in medium containing hemin showed an oxidized-reduced absorption spectrum characteristic of cytochrome *b*. These same membrane preparations also had high fumarate reductase activity, and evidence was presented indicating adenosine 5′-triphosphate generation via cytochrome-linked electron transfer to fumarate.

Differences in the rate of glucose uptake and incorporation by whole cells incubated under different conditions might be caused by changes in the intracellular concentrations of molecules known to regulate polyglucose biosynthesis. For example, 50 to 80% of the glucose taken up by whole cells of *B. thetaiotaomicron* was incorporated into glucose polysaccharide material

TABLE 4. *Specific activities of carbohydrate catabolic enzymes in cell extracts of B. thetaiotaomicron NCTC 10852 when cultured on selected carbon and energy sources*[a]

Enzyme	Carbon source for growth	
	PY-glucose (30 mM)	PY-galactose (30 mM)
Hexokinase		
Fructose	77[b]	51
Glucose	160	38
Glucose 6-phosphate dehydrogenase (oxidized nicotinamide adenine dinucleotide phosphate)	28	21
6-Phosphogluconate dehydrogenase (oxidized nicotinamide adenine dinucleotide phosphate)	4	6
Glucose 6-phosphate isomerase	634	462
Phosphofructokinase	23	ND[c]
Fructose 1,6-bisphosphate aldolase	83	7

[a] Enzyme assays performed on the 105,000 × *g* supernatant fluid of sonically treated extracts.

[b] Specific activities are expressed as nanomoles per minute per milligram of extract protein.

[c] ND, Not determined.

(Fig. 6 and Table 2). Any alterations in the levels of intracellular molecules known to regulate polyglucose biosynthesis should change the rate of incorporation and could also alter the rate of apparent glucose uptake. Most bacteria that synthesize polyglucose as a reserve carbon source utilize the sugar nucleotide adenosine 5'-diphosphate-glucose as the glucosyl donor for its biosynthesis (11). In vitro regulation of bacterial polyglucose synthesis is observed at the level of adenosine 5'-diphosphate-glucose pyrophosphorylase (EC 2.7.7.27), and glycolytic intermediates (i.e., fructose diphosphate) have been shown to be activators of the reaction, whereas adenosine 5'-monophosphate, adenosine 5'-diphosphate, or inorganic orthophosphate have been shown to be inhibitors of adenosine 5'-diphosphate-glucose synthesis (17).

It is not immediately obvious why *Bacteroides* species are the most predominant members of the microbial population in the gastrointestinal tract, which contains so many other carbohydrate-utilizing, organic acid-producing bacteria. One reason for the quantitative predominance of these bacteria may be the result of their great versatility toward carbohydrate substrates utilized (4, 20). Moreover, the ability of intestinal *Bacteroides* species to take up and accumulate hexoses into polyglucose might give these organisms a competitive advantage over other intestinal bacteria. In this regard, several of the predominant anaerobic, gram-negative rumen bacteria form polyglucose (1, 5).

Discussion of the mechanism of glucose and mannose transport in *B. thetaiotaomicron* must be somewhat speculative because of an inability to separate substrate transport from subsequent metabolism. For example, we have been unable to show any significant uptake of the non-metabolizable sugar analog such as methyl-α-D-[^{14}C]glucoside or methyl-β-D-[^{14}C]thiogalactoside at concentrations as high as 3.0 mM, attempts to make active membrane vesicles have not yet been successful, and *B. thetaiotaomicron* mutants blocked in the initial stages of glucose or mannose metabolism have not been isolated. However, the present results provide evidence that the uptake of glucose and mannose is a carrier-meditated reaction. For instance, we have obtained evidence of substrate specificity in that certain hexoses inhibited the rates of both glucose uptake and incorporation (Fig. 5).

We also demonstrated that the rate of glucose uptake was sensitive to changes in the pH of cell suspensions with an optimum of approximately 7.0 (unpublished data), and —SH-binding compounds such as *p*-chloromercuribenzoate strongly inhibited glucose uptake (Table 1).

A number of inhibitors of active transport systems in other bacteria showed little effect on glucose uptake activity in *B. thetaiotaomicron*. These results may constitute suggestive evidence for hexose uptake by facilitated diffusion in this bacterium, but we have no direct evidence for such a mode of transport.

We have been unable to detect phosphoenolpyruvate-dependent phosphorylation of glucose or methyl-α-D-[^{14}C]glucoside in cell extracts (Table 3) or in assays with toluene-treated cell suspensions by the method of Gachelin (2). Negative results also were obtained when phosphoenolpyruvate-phosphotransferase activity for glucose and 2-deoxyglucose was assayed in cells of *B. thetaiotaomicron* by the methods of Romano et al. (18; A. H. Romano, personal communication). These results may provide the first evidence for the absence of a phosphoenolpyruvate:glucose-phosphotransferase system in a bacterium that ferments glucose via the Embden-Meyerhof pathway. To the best of our knowledge, however, those bacteria known to contain glucose-phosphotransferase activity also contain a functional Embden-Meyerhoff pathway for glucose catabolism.

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