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Glycogen Formation by the Ruminal Bacterium *Prevotella ruminicola*†

J. LOU, K. A. DAWSON, AND H. J. STROBEL*

Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546-0215

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***Prevotella ruminicola* is an important ruminal bacteria. In maltose-grown cells, nearly 60% of cell dry weight consisted of high-molecular-weight (>2 × 10^6) glycogen. The ratio of glycogen to protein (grams per gram) was relatively low (1.3) during exponential growth, but when cell growth slowed during the transition to the stationary phase, the ratio increased to 1.8. As much as 40% of the maltose was converted to glycogen during cell growth. Glycogen accumulation in glucose-grown cells was threefold lower than that in maltose-grown cells. In continuous cultures provided with maltose, much less glycogen was synthesized at high (>0.2 per h) than at low dilution rates, where maltose was limiting (28 versus 60% of dry weight, respectively). These results indicated that glycogen synthesis was stimulated at low growth rates and was also influenced by the growth substrate. In permeabilized cells, glycogen was synthesized from [^{14}C]glucose-1-phosphate but not radiolabelled glucose, indicating that glucose-1-phosphate is the initial precursor of glycogen formation. Glycogen accumulation may provide a survival mechanism for *P. ruminicola* during periods of carbon starvation and may have a role in controlling starch fermentation in the rumen.**

Many bacteria have the capacity to synthesize energy storage compounds such as glycogen (23), polyphosphate (18), and triacylglycerols (19). Glycogen is a glucose polymer with α-1,4 and α-1,6 linkages and is the most common form of stored carbohydrate in bacteria. Because it is readily metabolized, glycogen is usually considered a stored energy and carbon source and may play an important role in bacterial survival in natural environments (21, 23). Microorganisms typically synthesize glycogen in response to carbon excess, depletion of nutrients such as nitrogen (31), sulfur, or phosphate, or environmental stress such as unfavorable pH (21, 23). Therefore, glycogen accumulation is often observed during periods of slow growth or the stationary phase. However, synthesis during rapid exponential growth does occur in several bacterial species, including *Fibrobacter succinogenes* (8) and *Streptococcus mitis* (10).

Prevotella ruminicola is one of the most common bacteria found in the rumen, comprising up to 19% of total bacterial counts (3). The predominance of this particular organism is partially explained by its ability to use a variety of carbon and nitrogen sources and its highly efficient energy metabolism (11). Previous work showed that *P. ruminicola* accumulated large amounts of intracellular polysaccharide granules which disappeared several hours later after reaching the stationary phase (5, 11). Recent studies (26, 27) demonstrated that the organism accumulated polysaccharide under nitrogen-limiting and glucose-excess conditions in batch as well as continuous cultures. However, the exact nature of the accumulated polysaccharide was unclear. In preliminary experiments, we found that *P. ruminicola* synthesized anthrone-reactive material to a greater degree when grown on maltose- than on glucose-grown cultures (13). This study investigated the nature of this polysaccharide and the regulation of its synthesis in this important ruminal bacterium.

* Corresponding author. Mailing address: 212 W. P. Garrigus Building, Department of Animal Sciences, University of Kentucky, Lexington, KY 40546-0215. E-mail: strobel@pop.uky.edu.

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MATERIALS AND METHODS

Bacterium and growth. *P. ruminicola* B_14 was obtained from J. B. Russell, Cornell University, Ithaca, N.Y. The bacterium was cultured anaerobically at 39°C in a semidefined medium (14). Acetate, propionate, and butyrate were omitted from the medium used in continuous culture. Continuous culture studies were conducted with New Brunswick MultiGen fermentors (345-ml working volume). Dilution rates were changed in a random fashion, and samples were taken after at least 98% turnover of original culture volume. Samples were centrifuged (15,000 × *g*, 10 min, 4°C), and the supernatant was stored at −20°C until analysis. Cell pellets were washed twice with 0.9% NaCl, resuspended, and frozen.

Cellular polysaccharide isolation and component analysis. Polysaccharide was isolated from bacterial cells by a method described previously (9). The isolated polysaccharide was dissolved in water and desalted by the addition of mixed-bed resin (Sigma TMD-8) until the pH of the solution decreased to approximately 6.5. More than 95% of a commercial glycogen preparation was recovered after treatment in this manner.

Isolated polysaccharide (75 μg) was digested by amyloglucosidase (15 U) at 55°C in sodium acetate buffer (100 mM, pH 4.5) for 2 h, and sugar residues were separated on thin-layer chromatography plates (LK6DF Silica 60 Å; Whatman) by developing in ethyl acetate-water-methanol (8:3:4) for 90 min. Sugars were visualized by spraying with 50% H_2SO_4 after oven drying (20 min, 120°C). Polysaccharide components were also analyzed by gas chromatography. Isolated cellular polysaccharide (2 mg) was boiled in 1 ml of 4 M HCl for 6 h, and sugar residues were then converted into alditol acetate derivatives (34). The derivatives were separated by gas chromatography with a Hewlett-Packard chromatograph (model 5890) and a Supelco column (GP 3% SP2340 on 100/120 Supelcoport). Derivatives of rhamnose, ribose, arabinose, xylose, mannose, fructose, galactose, and glucose were well separated from each other.

Enzyme digestion. Polysaccharide samples (5 mg) were digested with 32 U of α-amylase (Sigma A6380) in a solution (5 ml) containing 14 mM NaCl and 20 mM K phosphate (pH 7.0) at 25°C or with 30 U of β-amylase (Sigma A7130) in 5 ml of 50 mM sodium acetate (pH 4.8) at 25°C. Polysaccharide (12.5 mg) was digested with 4,000 U of isoamylase (Sigma I2758) in 5 ml of 50 mM sodium acetate (pH 3.5) at 40°C. All enzyme incubation conditions were performed as recommended by the supplier. The reducing sugars released during enzyme digestion were then measured with dinitrosalicylic acid (20).

Molecular weight and average chain length determination. The molecular weight of the isolated cellular polysaccharide was estimated by separation of the material (1 mg) on a Sepharose CL-4B column (0.7 by 50 cm) and elution with distilled water (0.7 ml/h; 0.35-ml fractions). Dextrans of known molecular weight (Sigma) were used to estimate the molecular weight of the bacterial polysaccharide. The average chain length was determined by a periodate oxidation method (7).

Enzyme assays. Crude extracts were prepared as described previously (14). The activities of maltose and sucrose phosphorylase were determined spectrophotometrically by monitoring NADPH formation at 340 nm. The reaction mixture contained 50 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); pH 6.8], 33 mM sodium-potassium-phosphate, 3 mM $MgCl_2$, 1 mM NADP, 8 U

of phosphoglucomutase, 5 U of glucose-6-phosphate dehydrogenase, a 20 mM concentration of the respective disaccharide, and aliquots of crude extracts containing approximately 120 μg of protein. Maltase and sucrase activities were measured by an NADPH-linked assay for determining β-glucosidase (14) except that maltose or sucrose was substituted for cellobiose. Phosphoglucomutase activities were determined as described by Kotze (12).

Glycogen synthesis by permeabilized cells. Cells were anaerobically harvested during the late logarithmic phase and washed with anaerobic Tris buffer (50 mM, pH 7.2; prepared under nitrogen). Cells (3 mg of protein/ml) were permeabilized by a method described by Strobel (32). The reaction mixture (600 μl) for glycogen synthesis included 5 mM [^{14}C]glucose-1-phosphate (4.4×10^5 dpm/μmol) or [^{14}C]glucose (4.4×10^5 dpm/μmol), 5 mM ATP, 5 mM $MgCl_2$, 2 mM fructose-1,6-phosphate, and 30 mM Tris (pH 7.2). The reaction was initiated by adding permeabilized cells (0.15 mg of protein). Samples (100 μl) were withdrawn at various times and immediately added to 300 μl of 67% KOH to stop the reaction. Radiolabelled glycogen was isolated by the method described above and resuspended in 150 μl of H_2O, and radioactivity was determined by liquid scintillation. Isolated radiolabelled glycogen was also digested by amyloglucosidase and then separated by thin-layer chromatography as described above. Nearly 100% of radioactivity was found in a glucose band after digestion, demonstrating that the radioactivity was essentially from glycogen.

Analyses. Total carbohydrate was measured by an anthrone method (1). Glucose was measured by an enzymatic method using hexokinase and glucose-6-phosphate dehydrogenase (25). Glycogen and maltose were specifically determined by measuring glucose after incubation with either amyloglucosidase (8) or 5 U of α-glucosidase in 50 mM sodium acetate (pH 6.0), respectively. Glucose-1-phosphate was measured with phosphoglucomutase as described by Schimz et al. (28), and its intracellular concentration was calculated on the basis that an average cell volume was approximately 3 μl/mg of protein (33). The reaction mixtures were first incubated without phosphoglucomutase to correct for endogenous glucose-6-phosphate. Volatile fatty acids and methylated succinate were determined by gas chromatography with a Supelco 1000 column (1% H_3PO_4, 100/120 mesh). Protein was determined by the Lowry method after cells were boiled in 0.2 M NaOH for 15 min (15). Cellular dry weight was measured after cell suspensions were dried at 105°C overnight in aluminum pans.

RESULTS

Polysaccharide composition and characterization. When *P. ruminicola* cells grown on maltose were harvested during late exponential growth, anthrone-reactive material accounted for approximately 60% of cell dry weight. This material was isolated by a KOH-ethanol extraction method and digested in 4 M HCl, and the resulting monosaccharides were converted to alditol acetate derivatives. Glucitol, the alditol acetate derivative of glucose, was the only derivative detected from the isolated polysaccharide after separation by gas chromatography (data not shown). Thin-layer chromatography confirmed that glucose was the sole sugar component; only glucose appeared on a thin-layer chromatography plate after the polysaccharide was digested with amyloglucosidase (data not shown). Similar results were obtained with commercially prepared glycogen from bovine or slipper limpets. Polysaccharide isolated from glucose-, sucrose-, or cellobiose-grown *P. ruminicola* cells also contained only glucose. These results indicated that the anthrone-reactive material accumulated by *P. ruminicola* was a glucose polymer.

The molecular weight of the isolated polysaccharide was estimated by gel filtration. It eluted before blue dextran (2×10^6 Da) and coeluted with rabbit liver glycogen, which has a molecular weight of greater than 3×10^6. The glucose polymer obtained after KOH-ethanol extraction was also characterized by use of enzymatic digestion and comparison to several commercial preparations. Little reducing capacity was detected in the isolated glucose polymer, but reducing sugars were released after incubation with α-amylase, β-amylase, or isoamylase (data not shown). As much as 66 and 72% of the total carbohydrate was converted to reducing sugar by α- and β-amylases, respectively. These values were similar to those of bovine liver glycogen but twofold higher than those of slipper limpet glycogen. Since both enzymes only cleave α-1,4 linkages, the results indicated the presence of α-1,4 linkages. The glucose polymer from *P. ruminicola* was also cleaved by isoamylase, indicating the presence of α-1,6 bonds (data not shown).

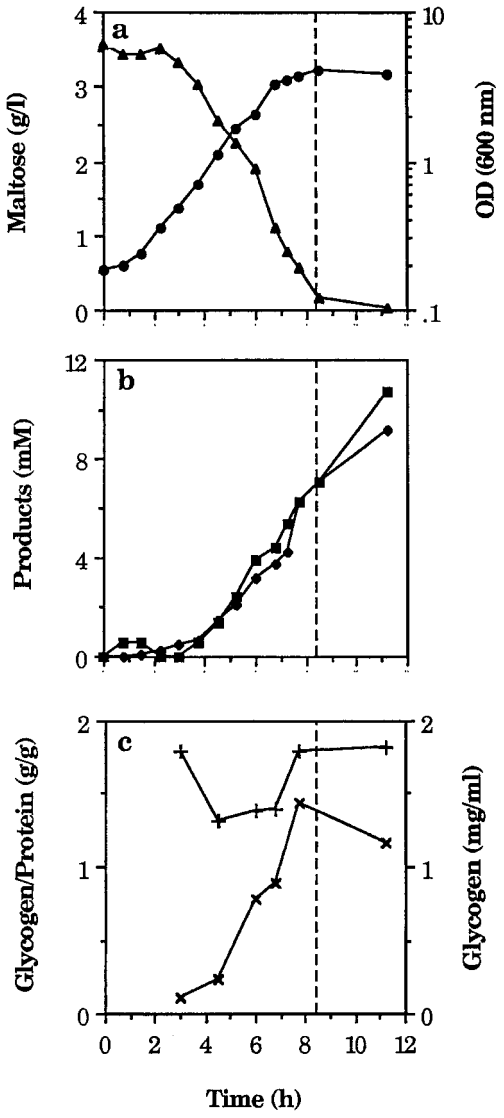

FIG. 1. Glycogen accumulation in *P. ruminicola* cells grown on maltose. (a) Symbols: ●, optical density (OD); ▲, maltose concentration in the culture fluid. (b) Symbols: ◆, succinate; ■, acetate. (c) Symbols: ✚, ratio of glycogen to protein; ×, cellular glycogen level. Glycogen was isolated by a KOH-ethanol extraction method. The dotted line indicates the cessation of growth.

The linear chain length of the polysaccharide, determined by a periodate oxidation method (7), was an average of 8 glucosyl units from each branch point. This value was smaller than those determined for rabbit liver glycogen (15 glucosyl units) or bovine liver glycogen (13 glucosyl units); the estimates for the commercial glycogens were close to previously reported values (7). Based on the chemical, enzymatic, and molecular weight analyses, it appeared that the polysaccharide accumulated by *P. ruminicola* was glycogen.

Glycogen synthesis in batch cultures. When *P. ruminicola* grew on maltose, glycogen was synthesized during the entire period of cell growth but the rate of glycogen synthesis varied (Fig. 1). The ratio of glycogen to protein at the beginning of exponential growth was 1.8 and decreased approximately 40% during the exponential phase (growth rate, 0.52 per h). As the

TABLE 1. Glycogen accumulation on different substrates during batch culture growth of *P. ruminicola*[a]

Growth substrate	Growth rate (h^{-1})	Glycogen/protein ratio (g/g)	Glycogen[b] (%)
Maltose	0.46 ± 0.02	1.8 ± 0.3	58 ± 3
Cellobiose	0.67 ± 0.03	1.2 ± 0.2	47 ± 4
Sucrose	0.73 ± 0.03	0.6 ± 0.2	26 ± 3
Glucose	0.90 ± 0.04	0.4 ± 0.2	19 ± 3

[a] Cultures were provided with 4 g of carbohydrate per liter and harvested during the early stationary phase. All results represent means of duplicate culture ± ranges.
[b] As a percentage of dry weight.

growth of the culture slowed (growth rate, 0.11 per h), the ratio of glycogen to protein increased to 1.8. As much as 40% of the maltose was incorporated into glycogen by the stationary phase. However, several hours after cells entered the stationary phase, the amount of glycogen decreased and the production of acetate and succinate during this time could account for greater than 90% of this glycogen disappearance.

Similar growth phase-dependent changes in glycogen accumulations were observed with glucose-grown cells (data not shown), but the ratio of glycogen to protein was approximately threefold lower than that of maltose-grown cultures (Table 1). Glycogen was also synthesized when cells were grown on sucrose and cellobiose, with substantially more synthesis in the presence of the latter disaccharide. In general, higher growth rates were associated with lower glycogen accumulation.

Glycogen synthesis in continuous cultures. Continuous cultures provided a useful tool for investigating glycogen synthesis at submaximal growth rates. In a continuous culture provided with maltose, cell growth was maltose limited at low dilution rates (<0.2 per h), and the ratio of glycogen to protein was as high as 2.1 (Fig. 2). When the dilution rate was increased to greater than 0.2 per h, maltose accumulated in the culture fluid but much less glycogen was synthesized. Glycogen as a proportion of cellular dry weight decreased from 60 to 28% as dilution rate increased, and the proportion of maltose incorporated in glycogen also declined from 39 to 18%. If glucose was provided, a similar inverse relationship between dilution rate and glycogen-to-protein ratio was observed (data not shown). However, the glycogen-to-protein ratio, percentage of glycogen in dry weight, and proportion of sugar incorporation into glycogen were at least twofold lower than those in maltose-grown cultures.

Phosphorylase activities and glucose-1-phosphate incorporation. Both maltase and sucrase were detected when cells were grown on cellobiose, maltose, sucrose, and glucose (Table 2). However, maltose phosphorylase activity was found only in maltose- and cellobiose-grown cultures, and sucrose phosphorylase could not be detected in cells grown on any of these four carbohydrates (data not shown). Cellobiose phosphorylase and β-glucosidase activities were described previously (14). In addition, phosphoglucomutase activities were also found when cells were grown on different substrates.

Maltose- and cellobiose-grown cells had approximately 1.9 and 0.3 mM intracellular glucose-1-phosphate, respectively, while cells grown on sucrose or glucose had no detectable glucose-1-phosphate. Based on these results, it appeared that intracellular glucose-1-phosphate may have been important in glycogen synthesis. Experiments were then conducted to investigate glycogen synthesis from glucose-1-phosphate compared with that from glucose. Permeabilized cells synthesized glycogen from [^{14}C]glucose-1-phosphate but not from radiolabelled

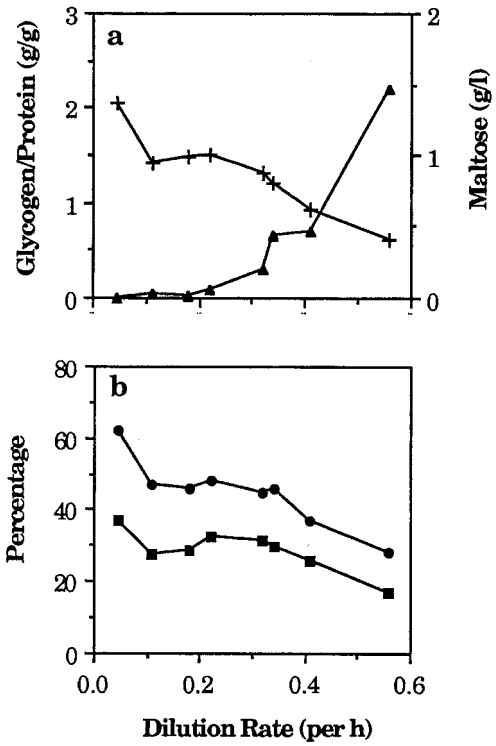

FIG. 2. Glycogen accumulation in a continuous culture provided with 4 g of maltose per liter. (a) Symbols: ✚, ratio of glycogen to protein; ▲, residual maltose concentration in culture fluid. (b) Symbols: ●, percentage of glycogen in cell dry weight; ■, percentage of maltose incorporated into glycogen. Maltose incorporation assumes that all glycogen was derived from maltose, and the percentage is calculated as (glycogen formation/maltose consumption) × 100. Glycogen was isolated by a KOH-ethanol extraction method.

glucose, indicating that glucose-1-phosphate served as a precursor for glycogen synthesis (Fig. 3). The rate of glycogen synthesis in maltose-grown cells (53.8 nmol of hexose incorporated/min/mg of protein) was 2.2-fold greater than that of glucose-grown cultures, and this was consistent with results obtained with growing cells (Fig. 1).

Cell morphology. There were dramatic differences in the shape and size of maltose- versus glucose-grown cells (Fig. 4). The shape of maltose-grown cells was not homogeneous, and cells were two- to eightfold longer than those grown on glucose. The long cells were not observed during growth on cellobiose or sucrose. In a continuous culture provided with maltose, cell size decreased as dilution rate decreased and the size of cells at the lowest dilution rate was similar to that of glucose-

TABLE 2. Enzymatic activities in cells grown on different substrates

Growth substrate	Sp act (nmol/min/mg of protein)[a]			
	PGM	Maltase	Maltose Pase	Sucrase
Maltose	305 ± 45	43 ± 1	8 ± 1	43 ± 1
Cellobiose	512 ± 67	27 ± 1	2 ± 1	40 ± 7
Sucrose	563 ± 23	18 ± 1	ND[b]	83 ± 1
Glucose	190 ± 10	32 ± 2	ND	36 ± 1

[a] Phosphoglucomutase (PGM) is measured in nanomoles of NADPH per minute per milligram of protein; other enzymes are measured in nanomoles of substrate cleaved per minute per milligram of protein. All results represent means of duplicate cultures ± ranges. Pase, phosphorylase.
[b] ND, not detected.

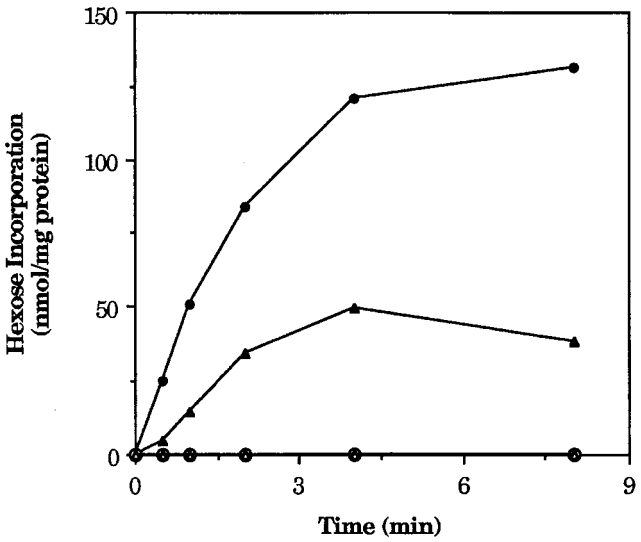

FIG. 3. Radiolabelled glucose-1-phosphate or glucose incorporation into glycogen by permeabilized cells. The reaction mixture (600 μl) included 5 mM [^{14}C]glucose-1-phosphate (closed symbols) (4.4×10^5 dpm/μmol) or [^{14}C]glucose (open symbols) (4.4×10^5 dpm/μmol), 5 mM ATP, 5 mM $MgCl_2$, 2 mM fructose-1,6-phosphate, 30 mM Tris (pH 7.2), and either maltose- (circles) or glucose-grown (triangles) permeabilized cells (0.15 mg of protein each).

grown cells (data not shown). The morphology of glucose-grown cells did not change with dilution rate.

DISCUSSION

Past examination by electron microscopy revealed that *P. ruminicola* accumulated large amounts of electron-light carbohydrate in its cytoplasm and some cells was nearly completely filled with this material (5). Based on chemical and enzymatic characterization, the present study showed that the polysaccharide produced was glycogen with a molecular weight of over 2×10^6 and an average chain length of 8 glucosyl units, both of which were in the range for glycogen found in mammalian and other bacterial cells (2, 7, 36). Glycogen has been detected in more than 40 bacterial species and is the most common form of stored carbohydrate in bacteria (21).

Initially, it appeared that the cell morphology of maltose-grown cells in batch culture might be directly related to high glycogen accumulation. However, the inverse relationship between cell size and glycogen content in continuous culture indicated that the presence of large cells was not directly linked to high glycogen levels. Also, the absence of large cells in cellobiose cultures suggested that other factors were contributing to cell size. Sjoberg and Hahn-Hagerdal (30) observed that maltose-grown cells of *Lactococcus lactis* were more spherical and rigid than their glucose-grown counterparts and attributed this to the availability of cell wall precursor metabolites. However, the mechanisms responsible for the morphology changes in maltose-grown *P. ruminicola* cells is unclear and requires further examination.

In contrast to many bacterial species which synthesize glycogen only during limited growth (21, 23), *P. ruminicola* produced glycogen during exponential growth as well as in the transition to the stationary phase. It was quite surprising that as much as 40% of the maltose provided to cultures was converted to glycogen rather than being used for generation of metabolic energy and subsequent protein formation. It is also worth noting that this accumulation occurred in medium which was not limited for nutrients such as nitrogen. Glycogen accumulation during exponential growth has been observed in several other microorganisms, including the ruminal organism *F. succinogenes* (8) and *S. mitis* (10). However, the rate of glycogen synthesis in *P. ruminicola* was not constant and increased during the transition from exponential growth to the stationary phase as cell growth slowed. Also, because there was an inverse relationship between glycogen accumulation and the growth rate supported by a particular sugar (Table 1), it appeared that glycogen synthesis in *P. ruminicola* was at least partially growth rate dependent.

Consistent with results from batch cultures, there was an inverse relationship between growth (dilution) rate and glycogen accumulation in continuous cultures. In general, carbon substrates can either enter glycolysis to generate energy and support cell growth or be stored as compounds such as glycogen (Fig. 5). In continuous culture under substrate-limited conditions, *P. ruminicola* used a larger portion of the maltose for growth and less for glycogen formation as the dilution rate was increased (Fig. 2). Preiss and Romeo (23) have proposed that several intracellular metabolites such as pyrophosphate (22) and guanosine tetraphosphate (24) may be responsible for greater glycogen synthesis during slow growth or when bacterial cell growth ceases (i.e., in the stationary phase). At the present time, it is not known whether similar phenomena occur in *P. ruminicola*. It should be noted that relatively few studies

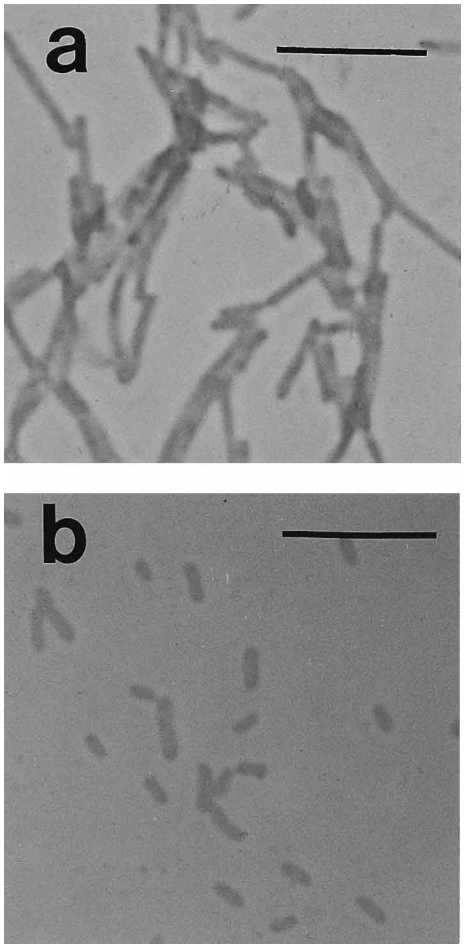

FIG. 4. *P. ruminicola* cells grown in batch culture on either maltose (a) or glucose (b). Bar, 10 μm.

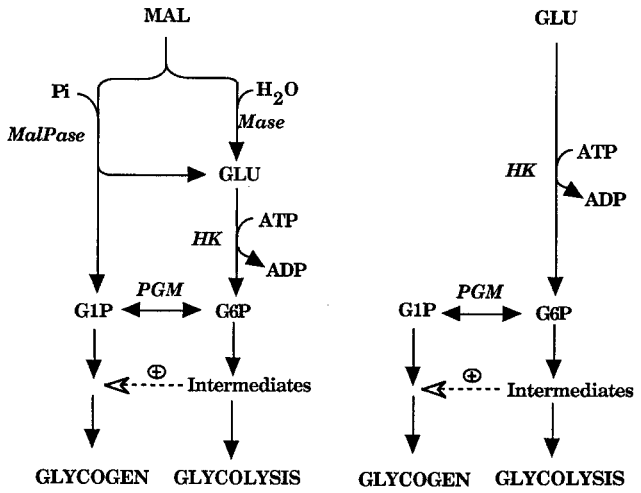

FIG. 5. Model for glycogen synthesis in maltose- versus glucose-grown cells. Abbreviations: MAL, maltose; GLU, glucose; MalPase, maltose phosphorylase; Mase, maltase; HK, hexokinase, PGM, phosphoglucomutase, G1P, glucose-1-phosphate; G6P, glucose-6-phosphate.

have examined the effect of dilution rate on glycogen accumulation in ruminal bacteria. Unlike the case with *P. ruminicola*, dilution rate did not affect the carbohydrate-to-protein ratio in *F. succinogenes* (37).

If glycogen synthesis responded only to growth rate, glycogen accumulations should be similar in cells grown on different substrates but at similar growth rates in continuous culture. However, glycogen levels in the glucose continuous culture were at least twofold lower than that in maltose-grown cells at all dilution rates. This observation indicated that glycogen accumulation was also regulated by mechanisms other than growth rate. Experiments in which glycogen was synthesized by permeabilized cells demonstrated that glucose-1-phosphate apparently was the precursor of glycogen formation. The presence of glucose-1-phosphate in maltose- and cellobiose-grown cells was consistent with the activity of disaccharide phosphorylases (14) and the high accumulation of glycogen in these cells. Sucrose phosphorylase activity was not detected, and this result agrees with the much lower glycogen level in sucrose-grown cells (Table 1). In addition, phosphoglucomutase activities were present when cells were grown on different carbohydrates. Based on these results, glucose-1-phosphate could be formed either from disaccharide phosphorolysis or from glucose-6-phosphate through phosphoglucomutase in maltose- or cellobiose-grown cells while sucrose- or glucose-grown cells might use only the latter pathway (Fig. 5).

In natural environments where growth substrates are typically only transiently available, bacteria shift between states of growth and nongrowth. Many bacteria have developed mechanisms that allow them to survive during starvation and resume growth rapidly when nutrients become available (29); glycogen appears to play such a role in some bacteria. In *Escherichia coli* (31) and *S. mitis* (35), glycogen-containing cells have prolonged viability under starvation conditions compared to cells which lack the polysaccharide. Since energy sources are often transiently available in the rumen, it is not surprising that several important ruminal microorganisms such as *Megasphaera elsdenii* (4), *F. succinogenes* (8), and *Selenomonas ruminantium* (36) store intracellular glycogen. Some ruminal microorganisms were very sensitive to even brief periods of energy starvation (16, 17), and glycogen may serve a role in resisting energy deprivation. However, the exact physiological and ecological significance of glycogen accumulation by these organisms and *P. ruminicola* is uncertain. Recent work suggested that endogenous polysaccharide utilization by *P. ruminicola* may not prevent losses in cell viability during starvation (26, 27). However, the greatest losses in viability were observed under nitrogen-limited conditions. It is not known whether similar viability losses occur under the nitrogen-rich condition of our study. Gaudet et al. (8) observed by use of in situ nuclear magnetic resonance spectroscopy that glycogen in *F. succinogenes* was simultaneously synthesized and degraded during cell growth. Whether a similar process occurs in *P. ruminicola* needs to be investigated.

Although the importance and function of glycogen formation in *P. ruminicola* are still unclear, the large accumulations in growing cells raise some interesting possibilities. *P. ruminicola* is a major amylolytic microorganism in the rumen and produces high amylase activity, which cleaves starch into maltose and longer maltodextrins (6). Starch is an important component of many ruminant diets, but rapid starch fermentation often causes digestive disorders, namely, lactic acidosis. Some ruminal protozoa are known to sequester starch granules in specialized internal organelles and prevent the rapid fermentation associated with acidosis (38). *P. ruminicola* may similarly influence the starch fermentation rate by storing starch in the form of intracellular glycogen. Cheng et al. (4) reported that a number of bacterial cells from the rumen contained polysaccharide granules, suggesting that glycogen accumulation is not necessarily an artifact of laboratory cultivation conditions. In addition, the fact that up to 40% of maltose was converted into glycogen by *P. ruminicola* during cell growth rather than directly to fermentation acids and microbial protein may have important implications in terms of the composition of digesta presented to the host animal and, ultimately, ruminant nutrition. Further experiments are needed to delineate carbon flux in *P. ruminicola*; such information will help to elucidate factors which limit the ability of the organism to generate metabolic energy from maltose and subsequently synthesize bacterial protein.

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