APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1993, p. 2844–2850
0099-2240/93/092844-07$02.00/0

Vol. 59, No. 9

Glucose Toxicity in *Prevotella ruminicola*: Methylglyoxal Accumulation and Its Effect on Membrane Physiology

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Received 22 March 1993/Accepted 26 June 1993

When the ruminal bacterium *Prevotella ruminicola* B_14-M was grown in a defined medium with an excess of glucose (3.6 mM ammonia and 50 mM glucose), the cells accumulated large amounts of cellular polysaccharide and the viable cell number decreased at least 1,000-fold. This decrease in viability was correlated with an accumulation of methylglyoxal in the supernatant (3 to 4 mM). Other genetically distinct strains of *P. ruminicola* produced methylglyoxal, but methylglyoxal production was not ubiquitous among the strains. When *P. ruminicola* B_14-M was grown in continuous culture (dilution rate, 0.1 h^{-1}) with an excess of glucose, there was an oscillating pattern of growth and cell death which was correlated with the accumulation and washout of methylglyoxal from the culture vessel. Mutants which resisted an excess of glucose took up glucose at a slower rate and produced less methylglyoxal than the wild type. These mutants were, however, not stable. There was always a long lag time, and the mutants could only be maintained with a daily transfer schedule. When the mutants were transferred less frequently, methylglyoxal eventually accumulated and the cultures died. The mutants transported glucose at a threefold-slower rate than the wild type, and in each case the carrier had more than one binding site for glucose. Because glucose transport could not be driven by phosphoenolpyruvate or ATP, the glucose carrier of *P. ruminicola* is probably a proton symport system. When *P. ruminicola* B_14-M cultures were treated with 4 mM methylglyoxal, the $\Delta\psi$ decreased even though intracellular ATP concentrations were high. The decrease in $\Delta\psi$ was associated with a decline in the intracellular potassium level and an inhibition of high-affinity glucose transport. Dicyclohexylcarbodiimide, an inhibitor of the F_1F_0 ATPase, had a similar effect on $\Delta\psi$, intracellular potassium level, and high-affinity glucose transport.

In ruminant animals, foodstuffs are fermented in the rumen prior to gastric and intestinal digestion, and the animal must depend on the fermentation end products (volatile fatty acids and microbial protein) for much of its nutrition. The study of rumen fermentation has been confounded by the diversity of ruminal microorganisms. Enumeration studies indicated that the numbers of individual bacteria vary with diet and during the course of a feeding cycle, but these trends have never been quantitated in a systematic fashion (2, 3, 8, 18).

When Costerton et al. (4) examined ruminal bacteria with an electron microscope, they noted that *Prevotella ruminicola* accumulated large amounts of "electron-light carbohydrate material" and that the cytoplasm of some cells was "nearly filled by this substance." Howlett et al. (7) reported that anthrone-reactive material accounted for approximately 30% of the dry weight of *P. ruminicola* B_14 and that half of the polysaccharide was present as intracellular granules. The relationship between this intracellular "storage" and viability was, however, not considered.

When *P. ruminicola* was grown in a nitrogen-limited medium which contained an excess of glucose, the cells accumulated very large amounts of polysaccharide, and the viable-cell count decreased dramatically (15). The mechanism of this "glucose toxicity," however, was not clear. The results presented here indicated that cultures which had accumulated large amounts of intracellular polysaccharide (i) had very high concentrations of ATP, (ii) could not maintain a membrane potential or concentration gradient of potassium across the cell membrane, and (iii) secreted methylglyoxal into the culture medium. Mutants which resisted glucose toxicity had a lower rate of glucose transport, accumulated less polysaccharide, and produced methylglyoxal at a slower rate.

MATERIALS AND METHODS

Organisms. Preliminary experiments indicated that our stock culture of *P.* (*Bacteroides*) *ruminicola* B_14 (now designated B_14-R) had threefold less β-glucosidase than was previously reported (16), and we obtained another culture from Terry Miller, New York State Health Department, Albany, N.Y. Because the Miller subculture (now designated B_14-M) had a β-glucosidase activity comparable to that in the previous report (16), we decided to the use this bacterium. As the work progressed, it became apparent that B_14-M had a somewhat lower affinity for glucose than B_14-R (15), but the maximum velocities of glucose transport and growth rates were similar. B_14-R and B_14-M both showed glucose toxicity (discussed below). *P. ruminicola* GA33, 20-63, and M384 were obtained from Michael Cotta, U.S. Department of Agriculture, Peoria, Ill. Strains 23, 118B, and GA33 were obtained from Terry Miller.

Cell growth. *P. ruminicola* B_14-M was grown anaerobically in a medium containing (per liter) 292 mg of K_2HPO_4, 292 mg of KH_2PO_4, 240 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4,000 mg of Na_2CO_3, 300 mg of cysteine hydrochloride, 1 mg of hemin, 0.3 mmol each of acetate, isobutyrate, isovalerate, 2-methylbutyrate, and valerate, and vitamins and microminerals as described previously (14). Culture supernatants and concentrated cell suspensions were stored at −15°C until they were analyzed.

ATP. Cells from 1 ml of culture were extracted for 20 min with 0.5 ml of ice-cold 14% perchloric acid which contained

9 mM EDTA. After centrifugation (13,000 × *g*, 5 min, 22°C), the supernatant fluid (1 ml) was neutralized with 0.5 ml of KOH–$KHCO_3$ (1 M each) at 0°C. Potassium perchlorate was removed by centrifugation (13,000 × *g*, 5 min, 22°C), and the supernatant fluid was assayed for ATP by the firefly luciferin-luciferase method (11). Neutralized extracts were diluted 50-fold with 40 mM Tris containing 2 mM EDTA, 10 mM $MgCl_2$, and 0.1% bovine serum albumin (pH 7.75). The luciferase reaction was initiated by adding 100 μl of a purified luciferin-luciferase mix to 100 μl of diluted extract according to the supplier's recommendations (Sigma Chemical Co., St. Louis, Mo.). Light output was measured immediately with a luminometer (model 1250; LKB Instruments, Inc., Gaithersburg, Md.), with ATP as a standard.

Viability. Cultures were serially diluted in duplicate (96-well polystyrene boxes, model no. 25860; Corning) (30 μl into 270 μl) in basal medium which was supplemented with 10 mmol of glucose, 1.0 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), and 0.5 g of yeast extract per liter and incubated in an anaerobic glove box at 39°C. Growth was monitored visually by turbidity.

Membrane potential (Δψ). Cultures were anaerobically transferred with a hypodermic syringe (2.0 ml) to tubes (13 by 100 mm) which contained ^3H-labeled tetraphenylphosphonium ion (0.5 μCi, 23 mCi/mmol), ^3H_2O (4 μCi), or [U-^{14}C]taurine (0.5 μCi, 115 mCi/mmol). After 5 min of incubation at 39°C, the cultures were centrifuged (13,000 × *g*, 5 min) through silicon oil (50:50 mixture of Hysol 550 and 560 [Hysol Co., Olean, N.Y.], incubated overnight in an anaerobic glove box to remove (O_2). Supernatant samples (20 μl) were removed, and the remaining sample was frozen (−15°C). The pellets at the bottom of the tubes were removed with a pair of dog nail clippers. The radioactivity in supernatant samples and cell pellets was counted by liquid scintillation. The intracellular volume (4.6 μl/mg of protein) was estimated from the relative uptake of ^3H_2O (4 μCi) and [U-^{14}C]taurine (0.5 μCi, 115 mCi/mmol). The Δψ was calculated from the uptake of tetraphenylphosphonium ion with the Nernst equation (62 mV times the logarithm of the intracellular concentration divided by the extracellular concentration, and the nonspecific binding of tetraphenylphosphoniomion ion was estimated with cells which had been treated with toluene (1%).

Sodium and potassium determinations. Cultures (4 ml) were centrifuged through 0.3 ml of silicon oil as described above. The cell pellets and supernatant samples (20 μl) were digested at room temperature for 24 h in 3 N HNO_3, and insoluble cell debris was removed by centrifugation (33,000 × *g*, 15 min). Sodium and potassium concentrations were determined by flame photometry (Cole-Parmer 2655-00 digital flame analyzer; Cole-Parmer Instrument Co., Chicago, Ill.). Values for the cell pellets were corrected for extracellular contamination.

Sugar transport. Exponentially growing cells were harvested by centrifugation (1,800 × *g*, 15 min, 5°C) and washed anaerobically in basal medium. The cells (approximately 80 μg of protein per 0.2 ml) were then dispensed anaerobically into tubes containing ^{14}C-labeled glucose (typically 0.3 μCi, 3 mCi/mmol). Uptake of sugar was measured from 0 to 120 s, and preliminary experiments indicated that the uptake rate was linear for 60 s. Transport assays (30 s) were terminated by adding 2 ml of ice-cold 100 mM LiCl to the assay tube and filtering through cellulose-nitrate membrane filters (pore size, 0.45 μm). The filters were washed once with 2 ml of 100 mM LiCl and dried for 20 min at 105°C. The radioactivity on filters was then counted by liquid scintillation.

Other analyses. Glucose was analyzed by an enzymatic method with hexokinase and glucose-6-phosphate dehydrogenase (14). Protein from NaOH-hydrolyzed cells (0.2 N NaOH, 100°C, 15 min) was assayed by the method of Lowry et al. (10). The ratio of protein to optical density was 160 μg of protein per ml per optical density unit (1-cm cuvettes, measured at 600 nm). Cellular polysaccharide was measured by the anthrone method with glucose as a standard (7). Succinate, lactate, acetate, and methylglyoxal in supernatant samples were analyzed with a Beckman 334 liquid chromatograph (model 156 refractive index detector and a Bio-Rad HPX-87H organic acid column). The sample size was 20 μl, the eluant was 0.013 N H_2SO_4, the flow rate was 0.5 ml/min, and the column temperature was 65°C.

Reagents. Biochemicals were obtained from Sigma Chemical Co. Radioactive isotopes were obtained from Amersham, Arlington Heights, Ill. All other chemicals were reagent grade.

RESULTS

Wild type and mutants. Wild-type *P. ruminicola* $B_1$4-M grew well (0.50 h^{-1}) in basal medium which contained 3.6 mM ammonia as long as the glucose concentration was less than 20 mM. When the glucose concentration was greater than 20 mM, there was initially no growth in the subsequent transfer. After a long lag (>12 h), growth was observed, but the glucose-resistant cells were highly unstable. The glucose-resistant mutants always had a long lag time (>8 h), could only be maintained if the culture was transferred daily, and never persisted for more than 10 transfers in basal medium containing an excess of glucose (data not shown).

Kinetics of glucose transport. When wild-type cells were washed in basal medium lacking glucose and incubated with increasing concentrations of glucose, the rate of transport increased, but the kinetics did not follow a typical pattern of saturation (Fig. 1a). At low glucose concentrations, the velocity was abnormally low, and *V/S* increased as the glucose concentration was increased (Fig. 1a). *V/S* was maximal at a glucose concentration of approximately 1,000 μM, and the decline in *V/S* indicated that the V_{max} was 155 nmol/mg of protein/min. Because the slope of the Hill plot (n_{app}) was significantly greater than 1.0, it appeared that the transport system had more than one binding site for glucose. The glucose-resistant mutant took up glucose at a slower rate, and the V_{max} was threefold lower (Fig. 1a). When the glucose concentration was increased, *V/S* initially increased, and there was no decline in *V/S* until the extracellular glucose concentration was greater than 1,000 μM (Fig. 1b). The Hill plot once again had a slope (n_{app}) which was significantly greater than 1.0 (Fig. 1c).

Growth kinetics of the wild type in batch culture. When wild-type *P. ruminicola* $B_1$4-M was inoculated into basal medium that contained 5 mM glucose, the cells grew rapidly (0.50 h^{-1}) until the glucose was exhausted (Fig. 2). Ammonia was never depleted, and the ratio of cellular polysaccharide to protein remained more or less constant (0.5 mg/mg). Exponentially growing cells had approximately 3 mM ATP, and there was only a small decline in the ATP level after the glucose was depleted. The cells maintained a Δψ of 120 mV for at least 24 h, and the intracellular potassium level did not decline. Even starved (24 h) cells took up [^{14}C]glucose (external concentration, 500 μM) at a rate of approximately 30 nmol/mg of protein/min. When the cells were treated with dicyclohexylcarbodiimide (DCCD), growth and glucose uti-

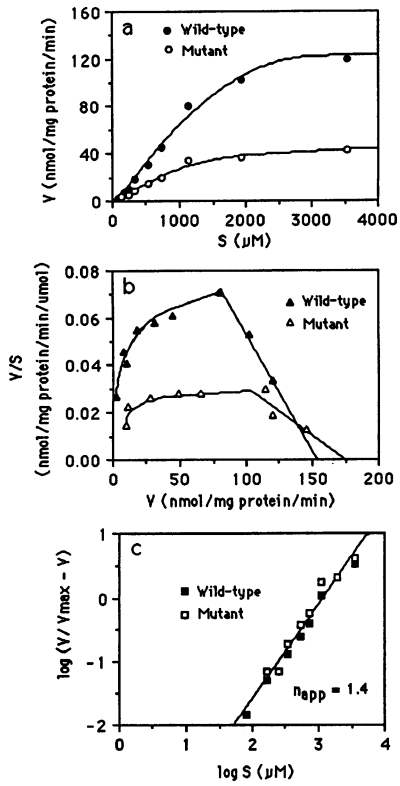

FIG. 1. Michaelis-Menten (a), Eadie-Hofstee (b), and Hill (c) plots of glucose transport (60 s) by wild-type *P. ruminicola* $B_1$4-M cells which were provided with increasing concentrations of glucose (pH 6.5).

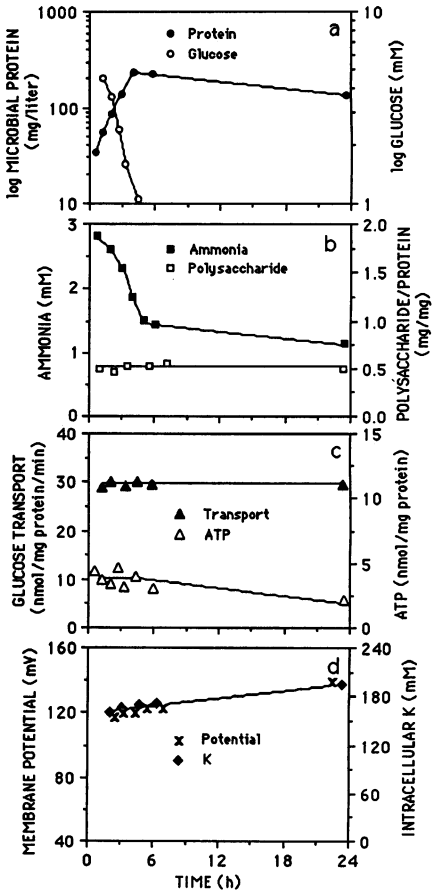

FIG. 2. Growth and glucose utilization (a), ammonia utilization and polysaccharide production (b), high-affinity glucose transport and intracellular ATP levels (c), and membrane potential ($\Delta\psi$) and intracellular potassium levels (d) of wild-type *P. ruminicola* $B_1$4-M in medium which contained 5 mM glucose.

lization ceased (Fig. 3). DCCD-treated cells could not maintain a membrane potential or retain intracellular potassium. DCCD had relatively little effect on intracellular ATP, but high-affinity glucose transport was abolished.

When wild-type *P. ruminicola* $B_1$4-M was inoculated into basal medium which contained 50 mM glucose, the cells grew exponentially (0.50 h^{-1}) until the ammonia was depleted (Fig. 4). As the cells grew and accumulated polysaccharide, the $\Delta\psi$ declined, and the intracellular potassium level paralleled the decrease in $\Delta\psi$. The cessation of growth was associated with an abrupt increase in intracellular ATP level and a rapid decline in high-affinity glucose transport (500 μM [^{14}C]glucose).

Growth kinetics of the wild type in continuous culture. When wild-type *P. ruminicola* $B_1$4-M was grown in continuous culture (0.1 h^{-1}, pH 6.7) in basal medium containing 5 mM glucose, the optical density at 600 nm (1-cm cuvette) reached a steady value of 0.6 in 2 days (Fig. 5). The glucose-limited cells had 3.0 mM ATP, an intracellular potassium concentration of 191 mM, and a $\Delta\psi$ of 130 mV (Table 1). The ratio of cellular polysaccharide to protein was 0.53, and these glucose-limited cells had 3.3-fold-greater high-affinity glucose transport activity than exponentially growing cells (102 versus 30 nmol/mg of protein/min).

When the concentration of glucose in the medium reservoir was increased to 50 mM, the ammonia was completely utilized, glucose accumulated in the culture vessel, and the ratio of polysaccharide to protein increased at least 2.5-fold (Table 1). The glucose-excess continuous cultures, however, never reached a steady state, and the density of cells in the culture vessel fluctuated dramatically (Fig. 5). When the optical density was increasing, the cells had ATP, potassium, and $\Delta\psi$ values which were comparable to those of the glucose-limited continuous cultures, but high-affinity glucose transport activity was fivefold lower. Once the optical density started to decrease, the ATP level remained more or less constant, but there was a decrease in $\Delta\psi$ and in the intracellular potassium level. High-affinity glucose transport was virtually eliminated.

Methylglyoxal accumulation. Batch cultures which were limited by glucose never produced methylglyoxal, but methylglyoxal accumulated (approximately 3 mM) when glucose was in excess (Fig. 6a). Increasing the phosphate concentration of the medium from 3.5 to 40 mM had no effect on methylglyoxal accumulation (data not shown). Methylglyoxal accumulation was correlated with a decrease in viability, and as little as 1.0 mM methylglyoxal inhibited the growth of the wild type (Fig. 6b). The glucose-resistant mutants produced threefold less methylglyoxal than the wild type after 24 h of incubation with 50 mM glucose (data not shown), but the mutants and the wild type showed similar sensitivity to methylglyoxal (Fig. 6b). Glucose-limited continuous cultures did not produce methylglyoxal, but methyl-

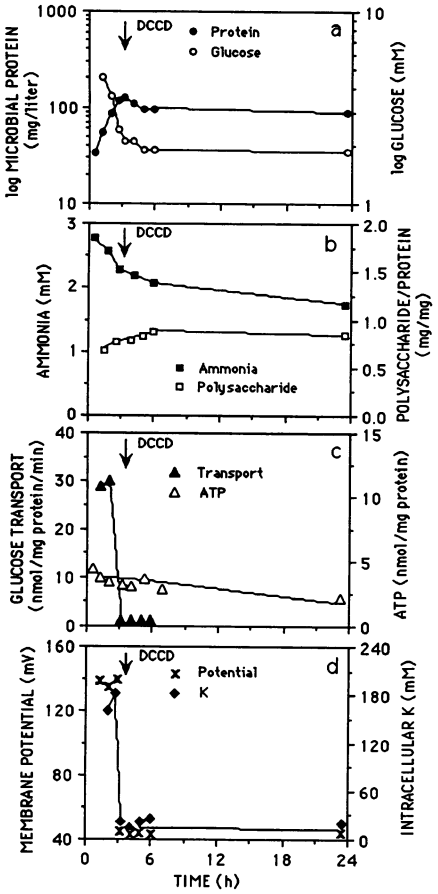

FIG. 3. Effect of DCCD (150 μM, added at the time marked by the arrow) on the growth and glucose utilization (a), ammonia utilization and polysaccharide production (b), high-affinity glucose transport and intracellular ATP level (c), and membrane potential ($\Delta\psi$) and intracellular potassium level (d) of wild-type *P. ruminicola* B_14-M in medium which contained 5 mM glucose.

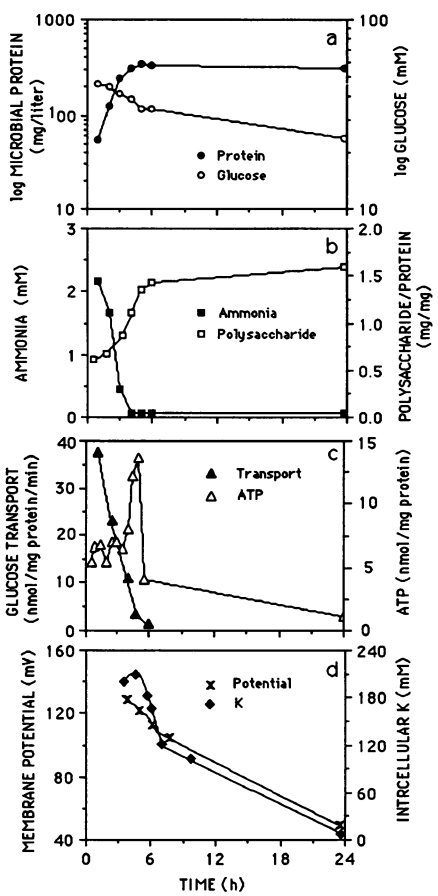

FIG. 4. Growth and glucose utilization (a), ammonia utilization and polysaccharide production (b), high-affinity glucose transport and intracellular ATP level (c), and membrane potential ($\Delta\psi$) and intracellular potassium level (d) of wild-type *P. ruminicola* B_14-M in medium which contained 50 mM glucose.

glyoxal was produced by the glucose-excess continuous cultures. The methylglyoxal concentration was twofold greater when the optical density was decreasing (Table 1). The effect of methylglyoxal was similar to glucose toxicity. Glucose-limited batch cultures which were treated with 4 mM methylglyoxal showed a transient increase in the intracellular ATP level and a decrease in $\Delta\psi$, intracellular potassium level, and high-affinity glucose transport (Fig. 7).

Other strains of *P. ruminicola* also produced methylglyoxal when they were incubated in basal medium with an excess of glucose, but the results were variable (Table 2). Strains 20-63, M384, and 20-78 produced nearly as much methylglyoxal as B_14, but methylglyoxal production could not be detected with strain 23, 118B, or GA33. GA33 did not grow well in nitrogen-limited medium, and growth was not observed for several days.

DISCUSSION

In *Escherichia coli*, methylglyoxal accumulates when there is an elevated metabolic flux through the glycolytic scheme of metabolism (6). Methylglyoxal production increases when cyclic AMP overcomes the catabolite repression of xylose or glucose 6-phosphate and the rate of sugar transport into the cell is elevated (1). Recent work showed that a multicopy plasmid carrying the *uhpT* gene caused an increased rate of sugar phosphate transport and methylglyoxal production in *E. coli* (9). *E. coli* was inhibited by as little as 0.25 mM methylglyoxal, but the minimal inhibitory dose is proportional to the cell concentration (17).

When *P. ruminicola* B_14-M was grown under nitrogen limitation with an excess of glucose, the number of viable cells decreased rapidly, but there was initially little evidence

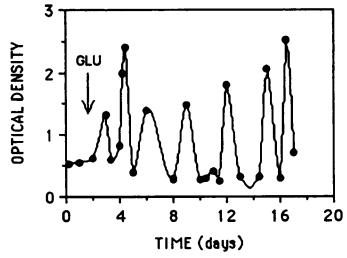

FIG. 5. Growth of *P. ruminicola* B_14-M in continuous culture ($0.1\ h^{-1}$, pH 6.7) with 5.0 mM glucose (before the arrow) and 50 mM glucose (after the arrow).

TABLE 1. Effect of glucose concentration on growth kinetics of *P. ruminicola* $B_1$4-M in continuous culture (0.1 h^{-1}, pH 6.7)

Glucose concn (mM)	Optical density[a]	Residual concn (mM)		Ratio of cellular polysaccharide to protein (mg/mg)	Intracellular concn (mM)		Membrane potential (mV)	Glucose consumption[b] (nmol/mg of protein/min)	Glucose transport[c] (nmol/mg of protein/min)	Methylglyoxal concn (mM)
		Glucose	Ammonia		ATP	Potassium				
5.5	0.60	0.0	2.37	0.53	3.0	191	130	96	102	0
55	1.7↑	40.3	0.0	1.37	2.6	233	130	NSS	19	0.7
55	1.3↓	48.0	0.8	2.24	3.3	10	88	NSS	2	1.4

[a] Arrows indicate whether the optical density was increasing (↑) or decreasing (↓).
[b] Decrease in glucose concentration per milligram of cell protein times the dilution rate. NSS indicates that the culture was not operating under steady-state conditions and this parameter was not determined.
[c] Transport of [^{14}C]glucose (external concentration, 500 μM).

that methylglyoxal was involved (15). All peaks on the high-pressure liquid chromatogram could be accounted for as glucose, succinate, acetate, or lactate, and an inoculum grew in the cell-free culture supernatant if ammonia was added (15). These earlier assumptions are, however, contradicted by the present data: (i) the succinate peak split into two peaks when the column temperature was increased from 50 to 65°C, (ii) the latter peak had the same retention time as methylglyoxal, (iii) the concentration of methylglyoxal in the culture supernatant was actually 3 mM, (iv) a small inoculum (<0.1 optical density unit) was unable to grow in the culture supernatant even if ammonia was added, (v) methylglyoxal concentrations as low as 1.0 mM inhibited growth, and (vi) glucose-resistant mutants produced methylglyoxal at a threefold slower rate than the wild type.

Kadner et al. (9) noted that 0.5% casein hydrolysate provided "considerable protection" from methylglyoxal toxicity, but the role of the nitrogen source in methylglyoxal production was largely ignored. The present experiments indicated that methylglyoxal production is strongly influenced by the balance of catabolic and anabolic rates. *P. ruminicola* $B_1$4-M only produced methylglyoxal when it was grown in nitrogen-limited medium with an excess of glucose, and under these conditions, the cells had a very high ratio of polysaccharide to protein. The glucose-resistant mutants took up glucose at a slower rate and produced methylglyoxal at a threefold slower rate.

The regulation of methylglyoxal synthase is not clear. Kadner et al. (9) suggested that the excessive transport of sugar phosphate into *E. coli* might deplete "the intracellular P_i pool to a growth-limiting level" and "favor methylglyoxal production as methylglyoxal synthase is inhibited by P_i."

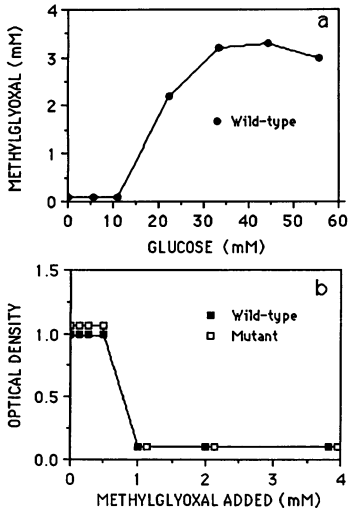

FIG. 6. (a) Production of methylglyoxal by wild-type *P. ruminicola* $B_1$4-M cultures which were incubated with increasing amounts of glucose, and its effect on viability. (b) Effect of methylgloxyal addition (4 mM) on the growth of wild-type and mutant *P. ruminicola* strains in batch culture (10 mM glucose).

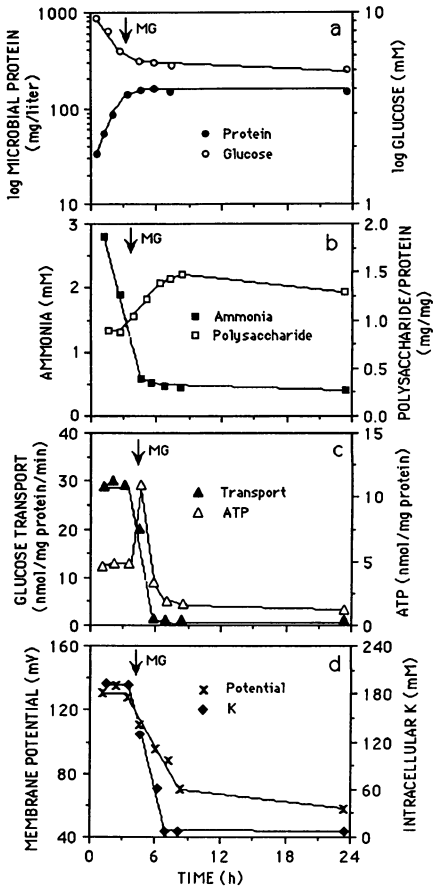

FIG. 7. Effect of methylglyoxal (MG; 4 mM added at the time marked by the arrow) on the growth and glucose utilization (a), ammonia utilization and polysaccharide production (b), high-affinity glucose transport and ATP level (c), and membrane potential ($\Delta\psi$) and potassium level (d) of wild-type *P. ruminicola* $B_1$4-M (10 mM glucose).

TABLE 2. DNA homology of and methylglyoxal production by various strains of *P. ruminicola* in nitrogen-limited medium with an excess of glucose for 48 h

Strain	Methylglyoxal concn (mM)	% DNA homology[a]
B_14	3.6	100.0
20-63	2.8	16.8
M384	3.4	16.6
20-78	1.3	16.6
GA33	0.0	20.8
118B	0.0	12.6
23	0.0	20.9

[a] Based on the DNA homology of Mannarreli et al. (12).

Because a 10-fold increase in the phosphate concentration of the medium had little effect on methylglyoxal production, it did not appear that phosphate was a key regulator of methylglyoxal production by *P. ruminicola* B_14-M. Since significant amounts of D- or L-lactate were never detected, *P. ruminicola* B_14-M did not appear to have glyoxalase, an enzyme which converts methylglyoxal to D-lactate.

Kadner et al. (9) speculated that methylglyoxal might be only indirectly involved in the toxicity of sugar phosphate for *E. coli*, but there was little support for the hypothesis that "acidification of the cytoplasm as a result of elevated proton entry coupled directly or indirectly to the uptake" was responsible for the toxicity: "The initial pH of the medium had only a slight effect on the susceptibility of the strains to growth inhibition." Fravel and McBrien (5) indicated that methylglyoxal inhibited protein and DNA synthesis, but the effect on other cell components was not considered.

Previous workers had indicated that methylglyoxal inhibits DNA replication and protein synthesis (17), but the mechanism of these long-term effects was not delineated. In *P. ruminicola*, methylglyoxal addition (or nitrogen deprivation) caused a rapid decline in $\Delta\psi$, intracellular potassium levels, and high-affinity glucose transport, but intracellular ATP levels increased. From the increase in ATP levels, it is conceivable that methylglyoxal could be having a direct effect on the F_1F_0 ATPase. If the F_1F_0 were not affected and the decrease $\Delta\psi$ was being caused by a nonspecific leak of ions across the cell membrane, the ATP level should have decreased rather than increased. DCCD, an inhibitor of the F_1F_0 ATPase, had a similar effect on $\Delta\psi$, potassium level, and high-affinity glucose transport. DCCD did not cause an increase in ATP levels, but this inhibitor has no direct effect on protein synthesis.

Previous work indicated that *P. ruminicola* did not have a phosphotransferase system for glucose (13) and that glucose uptake was not stimulated by sodium (15). Since glucose transport could be inhibited by DCCD even though intracellular ATP concentrations were high, the high-affinity glucose transport system of *P. ruminicola* B_14-M is probably a $\Delta\psi$-driven proton symport mechanism. The kinetics of glucose transport by *P. ruminicola* B_14-M did not show simple saturation kinetics, and it appeared that the carrier had more than one binding site for glucose ($n_{app} > 1.0$). Because the glucose-resistant mutant had a similar n_{app} and relationship between V/S and V, it appeared that the glucose-resistant mutant simply had less glucose carrier in the membrane.

Recent work by Mannarelli et al. (12) indicated that various strains of bacteria designated as *P. ruminicola* sometimes had very low DNA homology. B_14, one of the best-studied strains, had less than 21% homology with the DNA of any of the other strains examined. Strains 23 and 118B had greater than 80% DNA homology (12), and neither of these strains produced methylglyoxal (Table 2). Strains 20-63, M384, 20-78, and B_14 all produced methylglyoxal even though the DNA homology was less than 30%. From these comparisons, it appears that even genetically distinct strains can produce methylglyoxal.

The impact of methylglyoxal on ruminal fermentation is as yet not known. Since the diversity of the ruminal microflora is very great (8), it is unlikely that methylglyoxal production by a single species would cause a significant increase in methylglyoxal levels in vivo. However, preliminary experiments indicated that the intracellular methylglyoxal level was at least threefold greater than the extracellular concentration (data not shown). Given the observation that the decrease in $\Delta\psi$ was rapid, it is possible that methylglyoxal could become toxic even before the extracellular concentration increases. Because methylglyoxal is a toxic substance in mammals (17), ruminal methylglyoxal production would almost surely be detrimental.

ACKNOWLEDGMENTS

This research was supported by the U.S. Dairy Forage Research Center, Madison, Wis.

The technical assistance of Irina Kudryavtseva is greatly appreciated.

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