Carbohydrate Catabolism of *Mima polymorpha*

I. Supplemental Energy from Glucose Added to a Growth Medium

EMILY J. BELL AND ADRIENNE MARUS

Department of Biological Sciences and Graduate Division of Microbiology,
University of Cincinnati, Cincinnati, Ohio

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ABSTRACT

BELL, EMILY J. (University of Cincinnati, Cincinnati, Ohio), AND ADRIENNE MARUS. Carbohydrate catabolism of *Mima polymorpha*. I. Supplemental energy from glucose added to a growth medium. J. Bacteriol. 91:2223-2228. 1966.—*Mima polymorpha*, unable to grow in the presence of nonphosphorylated sugars as sole source of carbon and energy, grows rapidly and well in the presence of acetate, ethyl alcohol, short-chained fatty acids, and permeable intermediates of the tricarboxylic acid cycle. Chemical evidence indicates, however, a limited uptake of glucose. Further, glucose, although incapable of supporting growth as the sole source of carbon and energy, does increase both the rate of growth and the total cell crop when added as an ancillary nutrient to cells growing in a mineral salts medium which contains 0.03 M acetate as the carbon and energy source. A yield of energy from an abortive catabolism of glucose is hypothesized. In addition to the enhancement of growth rate and total cell crop, this hypothesis is supported by the facts that: (i) transport systems for the slightly permeable phosphorylated hexoses appear to be induced when glucose is incorporated into a medium capable of supporting growth and (ii) the rate of induction and the total activity of an inducible enzyme, isocitrate lyase E.C. 4.1.3.1., are markedly increased in the presence of supplemental glucose.

Mima polymorpha, a gram-negative coccobacillus originally isolated by DeBord (1), will not grow in the presence of glucose and other nonphosphorylated carbohydrates as sole sources of carbon and energy. It does grow well, however, in the presence of acetate, ethyl alcohol, short-chained fatty acids, and permeable intermediates of the tricarboxylic acid cycle (J. S. Daniels, M.S. Thesis, University of Cincinnati, Cincinnati, Ohio, 1963). A high level of isocitrate lyase E.C. 4.1.3.1. (isocitritase) was demonstrated (10), as well as an active malate synthetase E.C. 4.1.3.2. (1), indicating a functional glyoxylate cycle operant in the physiology of the organism. Daniels and Bell (Bacteriol. Proc. p. 104, 1963) also reported an apparent repression of isocitrate lyase activity when the organism was grown for 24 hr in an acetate medium supplemented with glucose. This observation necessitated the present investigation of the fate of glucose in a growing culture, so that further metabolic control studies could be performed. The evidence to be presented clearly indicates a need for control studies, but does not confirm the reported glucose repression

effect on the synthesis of isocitritase. Rather, time studies show that supplemental energy obtained from an abortive catabolism of glucose allows a more rapid biosynthesis of the enzyme and an initially higher level of specific activity. The data indicate, further, that energy derived from partial glucose metabolism is made available to the cell for the synthesis of certain transport systems.

MATERIALS AND METHODS

M. polymorpha ATCC 9957 was maintained on both Trypticase Soy Agar slants and in a liquid mineral salts, acetate medium of the following composition: 0.2% $\text{NH}_4\text{H}_2\text{PO}_4$, 0.3% KH_2PO_4 , 0.7% K_2HPO_4 , 0.01% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.03 M carbon source (acetate). This same basal medium with various sources added was used in all experiments, and was prepared to an initial pH of 7.0 ± 0.1 . Carbon sources were autoclaved separately at two times concentration unless, as in the case of phosphorylated intermediates, filtered sterile through a Swinney filter adapter fitted with a Seitz filter disc. Experimental inocula were prepared from either maintenance medium, as necessary, by twice washing the cells in chilled physiological saline, and were standardized to

contain 10^6 cells per milliliter. A sample of the resuspended washed cells was diluted in a Klett-Sumerson photoelectric colorimeter to a standard turbidity which corresponded to a known level of viable cells and to a determined dry weight. A calculated volume of saline then was added aseptically to the cell suspension to achieve the desired cell number. All cultures were incubated for various periods by shaking in a New Brunswick incubator shaker, model G 26, at 28 C and 250 rev/min.

The sources of the major reagents employed in the nutritional studies were as follows: glucose-1-phosphate (G1P), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1,6-diphosphate (F1,6P), 6-phosphogluconic acid (6PG), and glucono- δ -lactone from Mann Research Laboratories, New York, N.Y.; glyceraldehyde-6-phosphate, ribose-5-phosphate, gluconic acid, and 2-ketogluconic acid from Nutritional Biochemicals Corp., Cleveland, Ohio; 3-phosphoglyceric acid, 2-phosphoglyceric acid, and phospho-enolpyruvic acid from Sigma Chemical Co., St. Louis, Mo. Sodium salts of the sugar phosphate esters were prepared by treating an acidic aqueous suspension of the barium salts with a slight excess of sodium sulfate. The precipitated barium sulfate was removed by centrifugation, and the resultant solution was adjusted to the desired pH.

Subsequent to the initial growth curve experiment in which optical density was correlated with dry-weight determinations and a viable-cell count, all growth was measured turbidimetrically. Enzyme assays were performed in a Gilford model 2000 recording spectrophotometer. Cell-free extracts for the isocitritase assays were prepared by disruption of twice-washed cells in a Raytheon 9-kc, 50-w sonic oscillator at 150 v for 15 min. Cell debris was removed by centrifugation for 10 min at $10,000 \times g$ at 0 C in a Servall refrigerated centrifuge. Isocitrate lyase activity was determined by the method of Dixon and Kornberg (2). Specific activity is expressed as micromoles of glyoxylate formed per milligram of protein per hour. Protein was measured by the method of Lowry et al. (5). Glucose uptake was measured with Glucostat reagent (Worthington Biochemical Corp., Freehold, N.J.).

RESULTS

Since a late, repressive effect by glucose on isocitrate lyase induction had been noted previously (Daniels and Bell, *Bacteriol. Proc.*, p. 104, 1963), the effect of glucose as an ancillary nutrient on the growth of the organism was investigated. Basal salts medium containing 0.03 M acetate and the same medium supplemented with 0.03 M glucose were inoculated with a twice-washed suspension of acetate-grown cells. Preliminary experiments, in which rate of growth and total cell crop were measured as a function of acetate concentration showed that a concentration of 0.03 M was optimal for growth. Growth curves showing optical density and dry weight changes are given

in Fig. 1. The results show that, although the differences are not great, there is slightly less lag in growth initiation, a greater rate of growth, and a larger cell crop. These indicate that glucose is taken up by the growing cells and does affect the metabolism of the growing organism.

To substantiate an uptake of glucose by growing cells, the per cent uptake was calculated from the residual glucose remaining after 24 hr of incubation of cells in basal-glucose medium, and in the basal medium containing several concentrations of acetate and glucose. The results (Table 1) show that glucose uptake does occur and does increase as growth increases in the presence of acetate. The increased glucose uptake observed in the presence of 0.03 M acetate and increasing concentrations of glucose does not result in increased growth, and, possibly, is a reflection of more active uptake by cells growing in the presence of optimal levels of acetate and in the presence of increasing concentrations of a permeable substrate. This, however, has not been subjected to test. These results do suggest, in conjunction with the effect on the rate and level of growth, that glucose is catabolized to some extent.

Table 2 shows the growth response of the organism in the presence of various other utilizable carbohydrates, all of which were incorporated into the mineral salts medium to a concentration of 0.03 M. Good growth was obtained

FIG. 1. Comparative growth, as measured by optical density changes and dry-weight determinations, of *Mima polymorpha* growing in the presence of acetate alone and acetate plus glucose.

TABLE 1. *Growth of Mima polymorpha and per cent glucose uptake in the presence of varying concentrations of acetate and supplemental glucose**

Concn of substrate added		Optical density, 24 hr	Glucose uptake
Acetate	Glucose		
M	M		%
—	0.03	0.00	4.5
0.01	—	0.19	—
0.01	0.03	0.16	5.4
0.03	—	0.32	—
0.03	0.005	0.38	20.0
0.03	0.01	0.38	17.0
0.03	0.05	0.40	17.0

* No growth occurred in any control flask containing various concentrations of glucose as sole carbon source. Glucose recovery from uninoculated medium was 100%.

TABLE 2. *Growth of Mima polymorpha in the presence of various carbon and energy sources*

Substrate, 0.03 M	Optical density, 48 hr
Acetate.....	0.30
Ethyl alcohol.....	0.40
Propionate.....	0.30*
Butyrate.....	0.42
Pyruvate.....	0.28
Lactate.....	0.51
α -Ketoglutarate.....	0.12†
Succinate.....	0.32
Malate.....	0.47
Oxaloacetate.....	0.57
Glucose-1-phosphate.....	0.01
Glucose-6-phosphate.....	0.03†
Fructose-6-phosphate.....	0.11
Fructose-1,6-diphosphate.....	0.40
Glyceraldehyde-3-phosphate.....	0.28
6-Phosphogluconate.....	0.08

* After 72 hr of incubation.

† After 96 hr of incubation.

in the presence of acetate, ethyl alcohol, propionate, butyrate, pyruvate, lactate, succinate, malate, oxalacetate, fructose-1,6-diphosphate, and glyceraldehyde-3-phosphate. No growth, even after prolonged incubation of up to 14 days, occurred in the presence, as sole carbon and energy source, of glucose, fructose, arabinose, ribose, xylose, erythrose, sucrose, maltose, lactose, 3-phosphoglyceric acid, 2-phosphoglyceric acid, phospho-enolpyruvic acid, glycerol, mannitol, inositol, sorbitol, glucono- δ -lactone, gluconic acid, 2-ketogluconic acid, citric acid,

cis-aconitic acid, methanol, and formate. No growth occurred in the presence of some of these substrates (e.g., citrate) even when the initial pH was varied greatly. In some instances, at least, lack of an active permeation system may have prevented uptake of an otherwise utilizable substrate.

The next experiment was designed to test this possibility with glucose. The data (Tables 1 and 3), however, suggest that an inducible transport system will not be formed for glucose in the presence of sparker amounts of an energy source. Growth (Table 1) in the presence of a suboptimal concentration of (0.01 M) acetate is not enhanced when 0.03 M glucose is added as a supplemental nutrient. A similar result is seen in Table 3 where the addition of 0.03 M glucose to 0.005 M acetate does not stimulate growth over that occurring in acetate alone, even after 48 hr. Nor can an inducible transport system be formed in the presence of other readily utilizable substrates such as ethyl alcohol, lactate, malate, and oxaloacetate.

Growth in the presence of G1P, G6P, and F6P was slight (Table 2), owing, probably, to the limited permeability of this class of compounds. If, however, glucose is taken up and furnishes supplemental energy to the growing cells (as suggested by the growth-curve data), then this energy might be used for the induction of transport mechanisms for these relatively impermeable substrates, and increased growth should result. Further, ancillary glucose should have no growth stimulation effect on freely permeable substrates when the latter are supplied in concentrations insufficient for optimal growth. The results of the experiment designed to test this hypothesis are presented in Table 4.

It may be seen that the addition of glucose does indeed stimulate growth in the presence of G1P, G6P, and F6P, suggesting that the "energy-yield" hypothesis might be true. Although glucose itself cannot be utilized as such for growth, the possibility that a phosphate ester exchange was occurring was considered. The data show, however, that 0.005 M acetate, added as a "sparker," also allows increased growth in the presence of F6P and especially in the presence of G1P where supplemental growth due to the presence of 0.005 M acetate (see Table 3) can be essentially ignored. Further, the addition of 0.03 M glucose to a medium containing a minimal concentration (0.005 M) of the freely permeable fructose-1,6-diphosphate, did not cause an increased growth response. This, too, suggests that a transphosphorylation reaction involving glucose is not responsible for the enhanced growth.

As a further test of the supplemental yield of energy from an abortive catabolism of glucose,

TABLE 3. Growth of *Mima polymorpha* in the presence of various substrates with and without supplemental glucose

Substrate	Concn	Glucose, 0.03 M	Optical density, 48 hr
Acetate	M		
	0.03	—	0.30
	0.005	—	0.12
Ethyl alcohol	0.03	—	0.38
	0.005	—	0.07
	0.005	+	0.09
Lactate	0.03	—	0.51
	0.005	—	0.12
	0.005	+	0.12
Malate	0.03	—	0.47
	0.005	—	0.06
	0.005	+	0.07
Oxaloacetate	0.03	—	0.57
	0.005	—	0.13
	0.005	+	0.13

TABLE 4. Growth of *Mima polymorpha* in the presence of phosphorylated intermediates with and without supplemental glucose

Substrate	Concn	Glucose, 0.03M	Optical density, 48 hr
Fructose-1,6-di-phosphate	M		
	0.03	—	0.40
	0.005	—	0.05
Glucose-6-phosphate	0.03	—	0.03*
	0.03	+	0.52*
	0.03	Plus acetate, 0.005 M	0.64
Glucose-1-phosphate	0.03	—	0.01
	0.03	+	0.70
	0.03	Plus acetate, 0.005 M	0.25
Fructose-6-phosphate	0.03	—	0.11
	0.03	+	0.22
	0.03	Plus acetate, 0.005 M	0.25

* After 96 hr of incubation.

the effect of this compound on the induction of a catabolic enzyme, isocitrate lyase, was studied. If there is an energy yield without accumulation of repressive metabolite intermediates then an actual stimulation of enzyme induction might occur. Accordingly, a time study was performed in which the specific activities of the enzyme syn-

thesized by cells growing in acetate (as inducer) were compared to those activities produced by cells growing in acetate plus supplemental glucose. To have sufficient cell numbers for the early analyses, Roux bottles containing the mineral salts supplemented with 0.3% casein hydrolysate were inoculated. After 18 hr of growth on this noninducing medium, the cells were washed thoroughly, and the experimental flasks were heavily inoculated with twice-washed noninduced cells. The glucose plus acetate flasks were inoculated 1 hr after the acetate flasks, so that the experimental time lag would be minimized. Isocitrate lyase activity was determined at time-zero and at approximately 3-hr intervals for 12 hr; 22-hr determinations were also performed. Growth was followed by optical-density measurements, by dry weight, and by increasing protein per milliliter, as determined in standardized cell-free sonic extracts.

Figure 2 shows the specific activities and growth

FIG. 2. Time study of isocitritase induction by *Mima polymorpha* growing in acetate alone and in acetate plus glucose. Growth curves are included for comparison. Cuvettes for the isocitritase assays contained: 50 μ moles of tris(hydroxymethyl)aminomethane buffer, pH 7.7; 10 μ moles of $MgCl_2$; 10 μ moles of phenyl hydrazine-HCl; 15 μ moles of cysteine-HCl; 30 μ moles of NaOH; 10 μ moles of sodium DL-isocitrate; and 0.01 ml of bacterial extract. Water was added to 3.0 ml; temperature was 28 C. Specific activity is expressed as micromoles of glyoxylate formed per hour per milligram of protein.

response in the two media. Not only is increased growth seen (also Fig. 1) when supplemental glucose is added to the growth medium, but the synthesis of isocitrate lyase is initiated more rapidly than when glucose is omitted. Further, the maximal specific activity is higher (23 vs. 17.5) when glucose is present. After 22 hr, however, the enzyme activity induced by acetate alone is higher than the glucose-stimulated activity. The rapid drop in activity in the presence of glucose after 4 to 10 hr may be due to the more rapid utilization of inducer. This has not been determined as yet.

DISCUSSION

Absence of growth on glucose may be explained by the absence of a functional hexokinase, glucokinase, and 2-ketogluconokinase (6). The apparent lack of these and, possibly, other hexose kinases may explain the absence of growth on all nonphosphorylated sugars, since the metabolic lesion would prevent the formation of biosynthetic building blocks. Glucose is catabolized partially via the direct oxidative pathway (6), and the energy obtained from a cytochrome-linked dehydrogenase is available to the cell for supplemental energy. These studies have shown that this energy may be utilized by the cell for the following: (i) increased rate of growth and total cell crop, (ii) induction of transport systems for the slightly permeable phosphorylated intermediates, and (iii) more rapid induction to a higher level of activity of an inducible catabolic enzyme. It should be noted, however, that the evidence for (ii) is indirect, and that direct confirmation of the induction of transport systems is necessary before such a conclusion is entirely warranted.

Of interest is the ability of *M. polymorpha* to take up and to assimilate the phosphate esters of glucose and fructose. Although often assumed to be impermeable to the cell membrane, several phosphate esters may be taken up and assimilated, without primary hydrolysis by alkaline phosphatase. This was shown with *Escherichia coli* by Roberts and Wolffe (9) for glucose-6-phosphate and fructose-6-phosphate, by Lin et al. (4) for L- α -glycerophosphate, and by Fraenkel and Horecker (3) for glucose-6-phosphate with a glucokinaseless mutant of the same organism. The latter authors list the phosphate esters known to enter the cell without hydrolysis. These include glycerophosphate, G6P, and "probably G1P and F6P." The evidence presented in the present investigation (in conjunction with the absence of functional hexose kinases) suggests indirectly that F1,6P and 6PG may be added to

this list. Radioactive phosphate uptake studies are necessary, however, for confirmation. As pointed out by Lin et al. (4), it is probable that uptake of phosphorylated intermediates is by means of an energy-requiring transport mechanism. These data would tend to support this suggestion in that supplemental energy, available from the abortive catabolism of glucose, apparently allows the induction of a permeation system.

The acetate induction of isocitrate lyase synthesis by *M. polymorpha* follows a time course similar to that demonstrated for *E. coli* by Reeves and Ajl (8). The data presented in Fig. 2 do not show the usual lag in growth (during enzyme synthesis in the presence of acetate as sole carbon and energy source), since a massive inoculum was employed. An approximate 8-hr lag in growth does appear (Fig. 1) when the standard inoculum is employed. Although glucose was reported to repress the synthesis of isocitrate lyase, (Daniels and Bell, *Bacteriol. Proc.*, p. 104, 1963), the time-course study indicates that a lowered activity in the presence of glucose occurs only after some 10 hr of growth. Initially, glucose stimulates the synthesis of the enzyme, and higher levels of activity are reached. The rapid later decrease may be due to the complete utilization of the inducer, acetate. This has not been subjected to test. The absence of the "glucose effect" is of interest. It has been stated that this effect is on the formation of enzymes producing metabolites derived from glucose (7), and glucose repression of isocitrate lyase formation has been reported (11). The absence of glucose repression in *M. polymorpha* can be explained by the statement of Neidhardt (7) that "any general interference with glucose metabolism should result in a general reversal of the glucose effect." *M. polymorpha*, lacking glucokinase and gluconokinase (6), suffers a metabolic lesion which will not allow glucose catabolism to continue sufficiently for the accumulation of metabolic intermediates, building blocks, and possible catabolite repressors. Rather, supplemental energy released by the direct oxidation of this compound allows more rapid induction of the enzyme.

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