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Enzymes of Glucose Metabolism in *Frankia* sp.

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Enzymes of glucose metabolism were assayed in crude cell extracts of *Frankia* strains HFPArI3 and HFPCcI2 as well as in isolated vesicle clusters from *Alnus rubra* root nodules. Activities of the Embden-Meyerhof-Parnas pathway enzymes glucokinase, phosphofructokinase, and pyruvate kinase were found in *Frankia* strain HFPArI3 and glucokinase and pyruvate kinase were found in *Frankia* strain HFPCcI2 and in the vesicle clusters. An $NADP^+$-linked glucose 6-phosphate dehydrogenase and an NAD-linked 6-phosphogluconate dehydrogenase were found in all of the extracts, although the role of these enzymes is unclear. No $NADP^+$-linked 6-phosphogluconate dehydrogenase was found. Both dehydrogenases were inhibited by adenosine 5-triphosphate, and the apparent K_m's for glucose 6-phosphate and 6-phosphogluconate were 6.86×10^{-4} and 7.0×10^{-5} M, respectively. In addition to the enzymes mentioned above, an $NADP^+$-linked malic enzyme was detected in the pure cultures but not in the vesicle clusters. In contrast, however, the vesicle clusters had activity of an NAD-linked malic enzyme. The possibility that this enzyme resulted from contamination from plant mitochondria trapped in the vesicle clusters could not be discounted. None of the extracts showed activities of the Entner-Doudoroff enzymes or the gluconate metabolism enzymes gluconate dehydrogenase or gluconokinase. Propionate- versus trehalose-grown cultures of strain HFPArI3 showed similar activities of most enzymes except malic enzyme, which was higher in the cultures grown on the organic acid. Nitrogen-fixing cultures of strain HFPArI3 showed higher specific activities of glucose 6-phosphate and 6-phosphogluconate dehydrogenases and phosphofructokinase than ammonia-grown cultures. Activities of malic enzyme, glucokinase, and pyruvate kinase were similar in both treatments.

Since the isolation in 1978 of *Frankia*, the nitrogen-fixing symbiotic actinomycete, by Callaham et al. (14), efforts have been made to elucidate the carbon metabolic pathways in various *Frankia* isolates and in fractions of actinomycetous root nodules. Most of the reports have centered on the identification of enzymes from the tricarboxylic acid cycle and the glyoxylate cycle (1, 8, 17) in *Frankia* extracts. Blom and Harkink (8) assayed for enzymes of the Embden-Meyerhof-Parnas (EMP) pathway of glycolysis in pure cultures of *Frankia* strain AvCI2 and did not find activity of the irreversible enzymes, e.g., phosphofructokinase, pyruvate kinase, and hexokinase, but did find activity of the glycolytic enzymes involved in gluconeogenesis. Huss-Danell et al. (17) assayed vesicle clusters of *Frankia* isolated from *Alnus glutinosa* nodules for EMP enzymes and found a similar pattern. To date, these are the only published studies on the catabolism of glucose by *Frankia*. Even though many *Frankia* isolates will not grow readily on glucose or other sugars as sole carbon sources (33, 36), recently Lopez et al. (23, 24) showed that *Frankia* strain HFPArI3 synthesizes and metabolizes large amounts of trehalose and glycogen in pure culture. Since both of these carbon reserves must be catabolized by way of glucose, the existence of some glycolytic pathway(s) in *Frankia* was inferred. In this report, a survey of the activities of key enzymes of several pathways of glucose metabolism was made in two *Frankia* isolates from different plant hosts and in vesicle clusters isolated from N_2-fixing alder nodules.

MATERIALS AND METHODS

Chemicals. All enzymes, cofactors, and substrates used in the enzyme assays were the highest purity available from Sigma Chemical Co., St. Louis, Mo.

Bacterial strains. *Frankia* sp. strains HFPArI3 (ArI3) (7) and HFPCcI2 (CcI2) (38) were used.

Media. The BAP medium of Murry et al. (28) supplemented with 5 mM sodium propionate or 20 mM trehalose, or B medium (28) supplemented with 5 mM sodium propionate was used for growing all cells. The carbon sources were filter sterilized and added separately.

Cultural conditions. Bacteria were subcultured continuously or at least through two subcultures on a particular carbon source. Cultures grown on medium supplemented with ammonia were grown in 1-liter air-sparged, stirred bottles maintained at 28°C in the light. Logarithmic-phase cells (approximately 5 to 7 days old) were harvested by centrifugation and washed in cold 10 mM potassium phosphate buffer (pH 6.7). Alternatively, for carbon source experiments, cells were grown in 250-ml Erlenmeyer flasks in 100 ml of medium maintained on a shaker at 28°C in the light. These cells were harvested as described above. Cultures depressed for nitrogen fixation were obtained by the method of Murry et al. (28). Inocula were grown in 1-liter bottles as described above, harvested by centrifugation, washed twice in B medium, and inoculated into 300 ml of B medium supplemented with 5 mM sodium propionate in a 1-liter Erlenmeyer flask with a cotton plug. Approximately 1 ml (packed cell volume) was used as inoculum per 300 ml of media. Nitrogenase activity was monitored by the acetylene reduction technique (28), and cells with maximum activity (approximately 5 to 7 days old) were harvested for enzyme assays as described above. The yield from each 1-liter flask was approximately 1.5 to 2.0 ml (packed cell volume).

Isolation of vesicle clusters. Nodule material was obtained by inoculating aeroponically grown (39) seedlings of *Alnus rubra* with ArI3. A modification of the methods of Akkermans et al. (1) and Benson (5) was used to isolate endophytic vesicle clusters from *A. rubra* nodules. Nodules were har-

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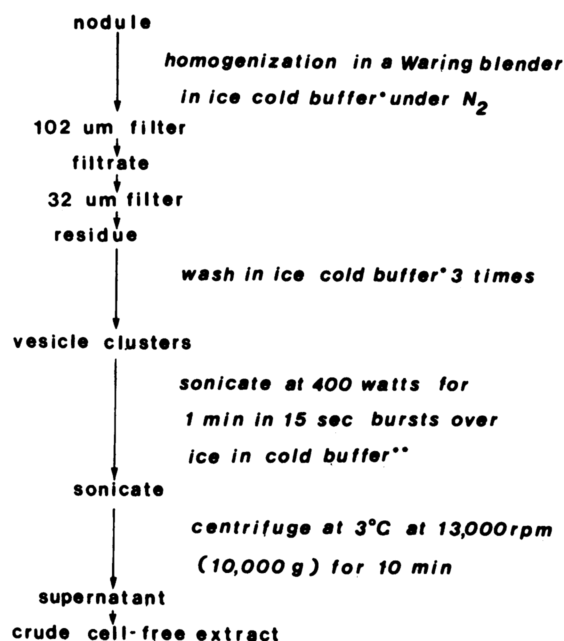

FIG. 1. Flow diagram of procedure used to isolate vesicle clusters and obtain cell extracts from *A. rubra* nodules.

vested, washed in distilled water, and placed in an appropriate volume (approximately 150 ml of buffer to 30 g [nodule wet weight]) of ice-cold 0.2 M glycylglycine buffer (pH 7.5)–4% soluble polyvinylpyrrolidone. The nodules were homogenized in a Waring blender inside a glove box flushed with nitrogen. Homogenization and filtration were performed anaerobically, as shown in the flow diagram (Fig. 1), to minimize damage to enzymes from oxidizable phenolic compounds which are present in abundance in alder nodules. Phenolic substances were left in the filtrate, which was discarded.

Preparation of cell extracts. Cells were harvested and washed as described above. The pellet was resuspended in the buffer to be used in a particular enzyme assay or in 10 mM phosphate buffer (pH 6.7), when the same extract was to be used to assay several different enzymes, and kept ice cold. Approximately 1 ml of packed cell paste to 2 ml of buffer was used. The probe from a Braun sonicator was precooled in ice, and the cell suspension was sonicated at 400 W over ice for a total of 1 min in 15-s bursts, between which the probe was cooled in ice. The sonicate was then centrifuged at 13,000 rpm (10,000 × *g*) in a Beckman refrigerated centrifuge at 3°C for 15 min. The pellet was discarded, and the supernatant was used for all enzyme assays. Cell extracts were always kept on ice. The protein concentration in the cell extracts was determined by the method of Bradford (10), with the reagents commercially prepared by Bio-Rad Laboratories, Richmond, Calif.

Enzyme assays. Enzyme assays were performed at room temperature in a Beckman DB double beam spectrophotometer equipped with a Perkin-Elmer chart recorder. Phosphofructokinase (EC 2.7.1.11) was assayed by using a modification of the procedure of Kemerer et al. (20). The reaction mixture contained, in a final volume of 3 ml, 240 μmol of glycylglycine buffer (pH 8.2), 3 μmol of $MgCl_2 \cdot 6H_2O$, 20 μmol of fructose 6-phosphate, 6 U of commercial aldolase, 20 U of commercial glycerophosphate dehydrogenase-triose phosphate isomerase mixture, 0.45 μmol of NADH (in 0.2 M glycylglycine buffer [pH 8.2]), 3 μmol of ATP, and cell extract.

Gluconokinase (EC 2.7.1.12) was assayed by the method of Keele et al. (19). The reaction mixture contained, in a final volume of 3 ml, 75 μmol of Tris-hydrochloride buffer (pH 7.65), 3.0 μmol of $NADP^+$, 10 μmol of $MgCl_2$, 10 μmol of ATP, 2 U of commercial 6-phosphogluconate dehydrogenase, 20 μmol of sodium gluconate, and cell extract. Glucokinase (EC 2.7.1.2) was assayed by the method of Anderson and Kamel (3). The reaction mixture contained, in a final volume of 3 ml, 200 μmol of glycylglycine buffer (pH 7.5), 20 μmol of $MgCl_2$, 10 μmol of ATP, 4 μmol of $NADP^+$, 2 U of commercial glucose 6-phosphate dehydrogenase, 20 to 200 μmol of D-glucose, and cell extract. Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (EC 1.1.1.49 and EC 1.1.1.44, respectively) were both assayed by a modification of the method of DeMoss (15). The reaction mixtures contained, in a final volume of 3 ml, 300 μmol of glycylglycine buffer (pH 7.5), 0.6 μmol of NAD or $NADP^+$, 10 μmol of $MgCl_2$, 5 μmol of glucose 6-phosphate or 6-phosphogluconate, and cell extract. Gluconate dehydrogenase (EC 1.1.99.3) was assayed by the method of Keele et al. (19). The reaction mixture contained, in a final volume of 3 ml, 60 μmol of glycylglycine buffer (pH 10.0), 5 μmol of $MgCl_2$, 3 μmol of NAD or $NADP^+$, 50 μmol of sodium gluconate, and cell extract.

The "ED enzymes", 6-phosphogluconate dehydratase (EC 4.2.1.12) and phospho-2-keto-3-deoxy-gluconate aldolase (EC 4.1.2.14) were determined by the method of Keele et al. (18), measuring the production of pyruvate from 6-phosphogluconate. The reaction mixture contained, in a final volume of 1 ml, 100 μmol of glycylglycine buffer (pH 7.65), 5 μmol of 6-phosphogluconate, 6 μmol of $FeSO_4$, 3 μmol of reduced glutathione, and cell extract. The reaction was stopped by the addition of 1 ml of 0.1% 2,4-dinitrophenylhydrazine in 2 N HCl. The determination of pyruvate was made as follows. 1 ml of the sample was extracted with 3 ml of ethyl ether. The ether phase was extracted with 6.0 ml of 10% $NaCO_3$, and 5 ml of the lower phase was then removed and mixed with 5.0 ml of 1.5 N NaOH. The samples were then read at 420 nm in a spectrophotometer after incubation at room temperature for 10 min. Pyruvate kinase (EC 2.7.1.40) was assayed by the method of Bucher and Pfleiderer (12). The reaction mixture contained, in a final volume of 3 ml, 0.45 μmol of NADH, 0.69 μmol of ADP, 150 μmol of Tris-hydrochloride buffer (pH 7.5), 24 μmol of $MgSO_4$, 225 μmol of KCl, 10 U of commercial lactic dehydrogenase, 3 μmol of phosphoenolpyruvate, and cell extract. Malic enzyme(s) (EC 1.1.1.38, 1.1.1.39, and 1.1.1.40) were assayed by the method of Ochoa (29). The reaction mixture contained, in a final volume of 3 ml, 75 μmol of glycylglycine buffer (pH 7.4), 3 μmol of $MnCl_2$, 1.5 μmol of NAD or $NADP^+$, 1.5 μmol of malate (pH 7.4), and cell extract. All assays based on the reduction of NAD or $NADP^+$ or oxidation of NADH followed the change in optical density at 340 nm, and a value of 6.2×10^3 was used as the molar extinction coefficient of $NADH_2$ and $NADPH_2$ (6). In most cases, correction for the activities of endogenous activity of nonspecific $NADH_2$ or $NADPH_2$ oxidases was not necessary, since the levels were extremely low or nonexistent.

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Transmission electron microscopy. Isolated vesicle clusters were fixed for 2 h at room temperature in 5% glutaraldehyde in 75 mM sodium phosphate buffer (pH 7.1). They were post-fixed for 1 h in buffered 1% OsO_4, dehydrated in a graded acetone series, and infiltrated and embedded in Spurr low-viscosity resin. Sections were stained for 10 min in 2% aqueous uranyl acetate and for 5 min in Reynold lead citrate before examination on a JEOL 100 CX electron microscope.

RESULTS

Comparison of glucose metabolism in two *Frankia* strains. The specific activities of key enzymes of several pathways of glucose metabolism were surveyed in propionate-grown ArI3 and CcI2 from *A. rubra* and *Casuarina cunninghamiana*, respectively. The results of this survey are shown in Table 1. Three irreversible enzymes in the Embden-Meyerhof-Parnas (EMP) pathway, phosphofructokinase, pyruvate kinase, and glucokinase were detected in ArI3 and pyruvate kinase and glucokinase were detected in CcI2. This suggests that in contrast to previous reports (8, 17), the classical glycolysis probably does operate in at least one and possibly two *Frankia* strains, although specific activities of phosphofructokinase in ArI3 were low. The discrepancy in these findings may be due to differences in assay techniques, or perhaps all *Frankia* strains derived from alder do not have the same metabolic pathways.

Both isolates also had activity of NADP-specific glucose 6-phosphate dehydrogenase, one of the principal enzymes in the pentose phosphate pathway (PPP) and Entner-Doudoroff pathway. No NAD-linked activity was found in cell extracts with this enzyme. In contrast, 6-phosphogluconate dehydrogenase was detected in both isolates but was exclusively NAD-linked. The specific activities of this enzyme were comparable to those of glucose 6-phosphate dehydrogenase. There is some controversy whether the presence of NAD-linked 6-phosphogluconate dehydrogenase indicates operation of the PPP; a similar enzyme has been found in *Rhizobium* (25–27), but its function there is unclear. Since there was no detectable activity of the "Entner-Doudoroff enzymes" in any of the preparations, the activity of the glucose 6-phosphate dehydrogenase is not associated with the Entner-Doudoroff pathway.

Of the enzymes for gluconate metabolism, gluconate dehydrogenase, the key enzyme in the ketogluconate pathway, was not detected in any of the extracts, suggesting that this catabolic route is not available to *Frankia*, in contrast to some rhizobia (35). Gluconokinase was also absent from both extracts. An $NADP^+$-linked malic enzyme was found in both isolates, but no NAD-linked malic enzyme was detected. Malic enzyme functions in the synthesis of pyruvate for gluconeogenesis.

Effect of carbon source on specific activities of enzymes. The effect of the carbon source for growth on the specific activities of the glucose metabolic enzymes was investigated (Table 1). An organic acid, propionate, was compared with a sugar, trehalose, as carbon source. Enzyme activities from cultures grown on the two carbon sources were similar, except for malic enzyme. The propionate-grown cells had higher enzyme activities (approximately 10 times higher) than did the trehalose-grown cells. Malic enzyme can function in the production of pyruvate from malate for gluconeogenesis.

Glucose metabolism in nitrogen-fixing cultures. As has been described by others (13, 28, 37), when NH_4Cl is omitted from the growth medium, ArI3 cells are induced to form vesicles, (the purported site of nitrogenase) and to fix

TABLE 1. Specific activities of some enzymes of glucose metabolism in cultured *Frankia* strains ArI3 and CcI2

Enzyme	Cofactor	Sp act[a] of enzyme in strain[b]:			
		ArI3			CcI2
		Propionate + NH_4Cl	Propionate − NH_4Cl	Trehalose	Propionate + NH_4Cl
Glucose 6-phosphate	$NADP^+$	25.4	71.3	22.2	86.1
dehydrogenase	NAD	<1	<1	<1	<1
6-Phosphogluconate	NAD	24.1	63.6	26.2	41.5
dehydrogenase	$NADP^+$	<1	<1	<1	<1
Glucokinase		150.6	137.8	27.2	54.0
Phosphofructokinase		9.6	18.7	2.0	ND
Pyruvate kinase		23.7	15.5	16.0	31.5
Malic enzyme	$NADP^+$	77.7	69.7	5.3	18.3
	NAD				
"ED enzymes"		<1	<1	<1	<1
Gluconate dehydrogenase		<1	<1	<1	<1
Gluconokinase		<1	ND[c]	ND	<1

[a] Expressed in nmoles of NAD or $NADP^+$ reduced or NADH oxidized per milligram of protein per minute, except for "ED enzymes", which are expressed in nanomoles of pyruvate produced per milligram of protein per minute.

[b] All cultures grown on BAP (+NH_4Cl) medium supplemented with 5mM sodium propionate or 20 mM trehalose, or on B(-NH_4Cl) medium supplemented with 5 mM propionate. Cells on B medium had nitrogenase activity estimated by acetylene reduction of at least 100 nmol of ethylene formed/mg of protein/h. Values shown are the average of two to five separate assays.

[c] ND, Not determined.

dinitrogen. The specific activities of several enzymes in ArI3 were compared in cells grown with and without ammonium chloride to determine whether nitrogen-fixing cells differed in the pathway of glucose metabolism. These results are shown in Table 1. The activities of pyruvate kinase, malic enzyme, and glucokinase were similar in both treatments, but the specific activities of the glucose 6-phosphate and 6-phosphogluconate dehydrogenases and phosphofructokinase were about 2.5 times higher in the N_2-fixing cells than in the nonfixing cells grown with added nitrogen. A comparison of the specific activities of the enzymes in the N_2-fixing cells alone shows that those of the two dehydrogenases were at least 3 times higher than that of phosphofructokinase, suggesting that these enzymes play an important role in the dissimilation of glucose in these cells and that the role of the EMP pathway may be a minor one.

Survey of enzymes in vesicle clusters. With the use of a filtration technique similar to that of Akkermans et al. (1) and Benson (5), vesicle clusters from *A. rubra* nodules infected with ArI3 were isolated and assayed for enzyme activity. Any conclusions to be drawn from these data must be tentative, since the isolation procedure does not result in pure preparations of *Frankia*. As was previously observed by Akkermans et al. (2), plant mitochondria are intermingled frequently with the vesicles (Fig. 2), and therefore enzymes commonly encountered in mitochondria cannot necessarily be assumed to be present in the *Frankia*. Of the soluble enzymes not normally found in plant mitochondria (9), glucokinase, pyruvate kinase, glucose 6-phosphate dehydrogenase ($NADP^+$), and 6-phosphogluconate dehydrogenase (NAD) were all found in the cell extracts from vesicle clusters (Table 2). The pyridine nucleotide specificities of the two dehydrogenases were identical to those found in pure cultures of ArI3 and unlike what would be commonly seen in plant tissues. This is evidence that these enzymes indeed originated from the *Frankia* endophyte. In addition,

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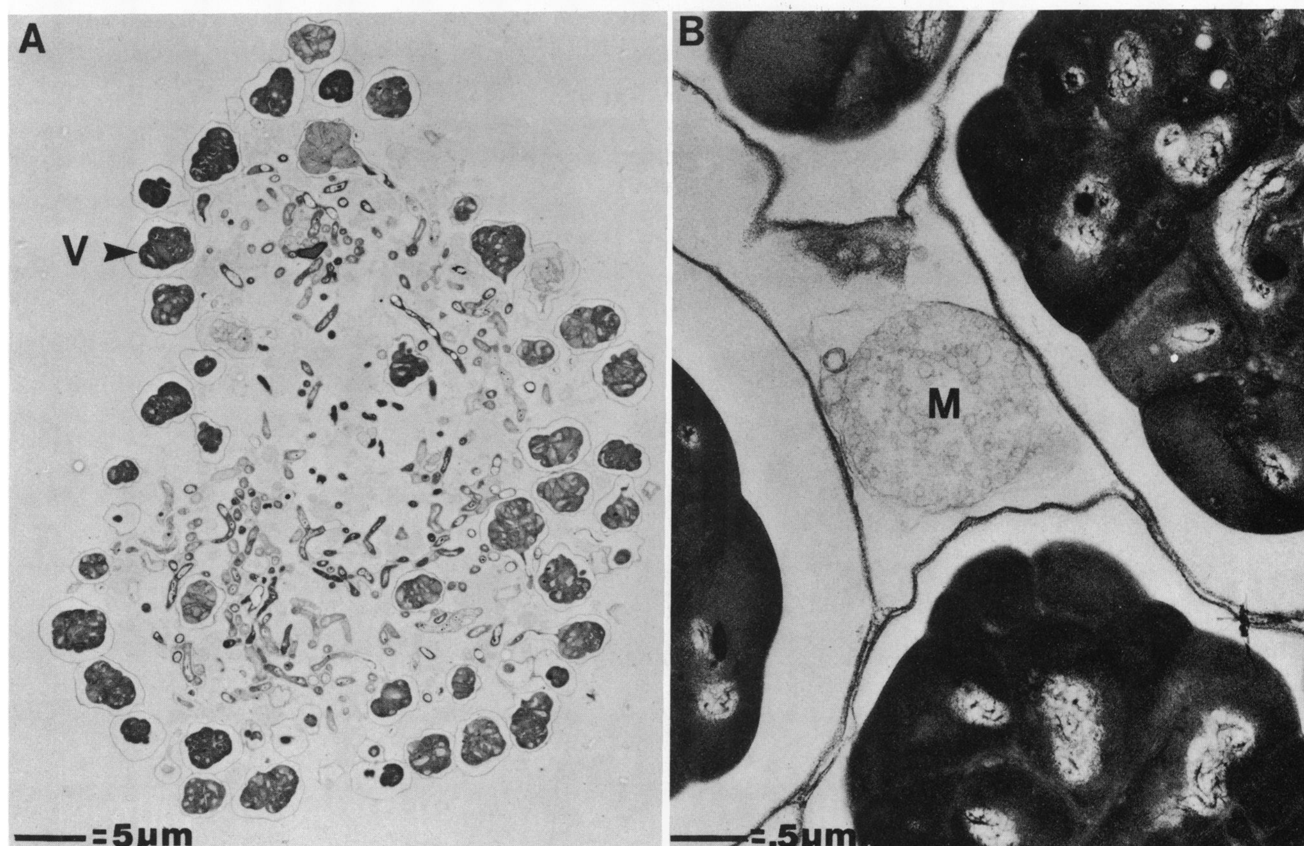

FIG. 2. Electron micrographs of vesicle clusters isolated from *A. rubra* root nodule infected with ArI3. (A) low-magnification view of entire cluster. (B) higher magnification showing a plant mitochondrion embedded in the cluster. V, Vesicle, M, mitochondrion.

the specific activities of these enzymes were high, in particular that of the NAD-specific 6-phosphogluconate dehydrogenase, again suggesting that these two enzymes are important in *Frankia* glucose catabolism both in pure culture and in the symbiotic state.

An NAD-linked malic enzyme with very high specific activity was also detected in the vesicle cluster preparation. The fact that this enzyme was NAD specific, in contrast to the $NADP^+$-specific malic enzyme of *Frankia* cultures, may indicate that it arose from the plant mitochondrial contamination. No NADP-specific malic enzyme activity was detected in the vesicle clusters, and no NAD-specific malic enzyme was detected in pure cultures of *Frankia*. Huss-Danell et al. (17) reported low specific activities (10 nmol of NADH or NADPH mg of protein^{-1} min^{-1}) of malic enzyme in *A. glutinosa* vesicle clusters, but the pyridine nucleotide specificity of the vesicle cluster fraction was not shown.

Some kinetic properties of the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. To learn more about the glucose 6-phosphate and 6-phosphogluconate dehydrogenases in crude cell extracts of ArI3, a preliminary investigation of some of the kinetic properties of these enzymes was undertaken. Both enzymes showed Michaelis-Menten kinetics when velocity was plotted as a function of

TABLE 2. Specific activities of some enzymes of glucose metabolism in vesicle clusters from *A. rubra* nodules inoculated with ArI3

Enzyme	Cofactor	Sp act[a]
Glucose 6-phosphate dehydrogenase	$NADP^+$	110.04
	NAD	<1
6-Phosphogluconate dehydrogenase	NAD	614.76
	$NADP^+$	<1
Malic enzyme	$NADP^+$	<1
	NAD	879.0
Glucokinase		24.7
Phosphofructokinase		ND[b]
Pyruvate kinase		90.95
"ED enzymes"		<1

[a] Expressed in nanomoles of NAD or $NADP^+$ reduced or NADH oxidized per milligram of protein per minute except "ED enzymes", which are expressed as nanomoles of pyruvate per milligram of protein per minute. Values shown are the average of two to five separate assays.
[b] ND, Not determined.

TABLE 3. Influence of ATP on affinities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase for their respective substrates

Enzyme	Substrate	Apparent K_m[a] (M) with:	
		no ATP	2×10^{-3} M ATP
Glucose 6-phosphate dehydrogenase	Glucose 6-phosphate	6.86×10^{-4}	3.0×10^{-3}
	$NADP^+$	2.1×10^{-5}	2.1×10^{-5}
6-Phospho-gluconate dehydrogenase	6-Phospho-gluconate	7.0×10^{-5}	1.8×10^{-4}
	NAD	1.6×10^{-5}	1.6×10^{-3}

[a] For each assay, n = 2 to 4, r values ranged from 0.92 to 0.99. Specific activities ranged from 14 to 41 nmol of NAD or $NADP^+$ reduced per mg of protein per min. All cells of *Frankia* strain ArI3 were grown in BAP medium (28) supplemented with 5 mM propionate.

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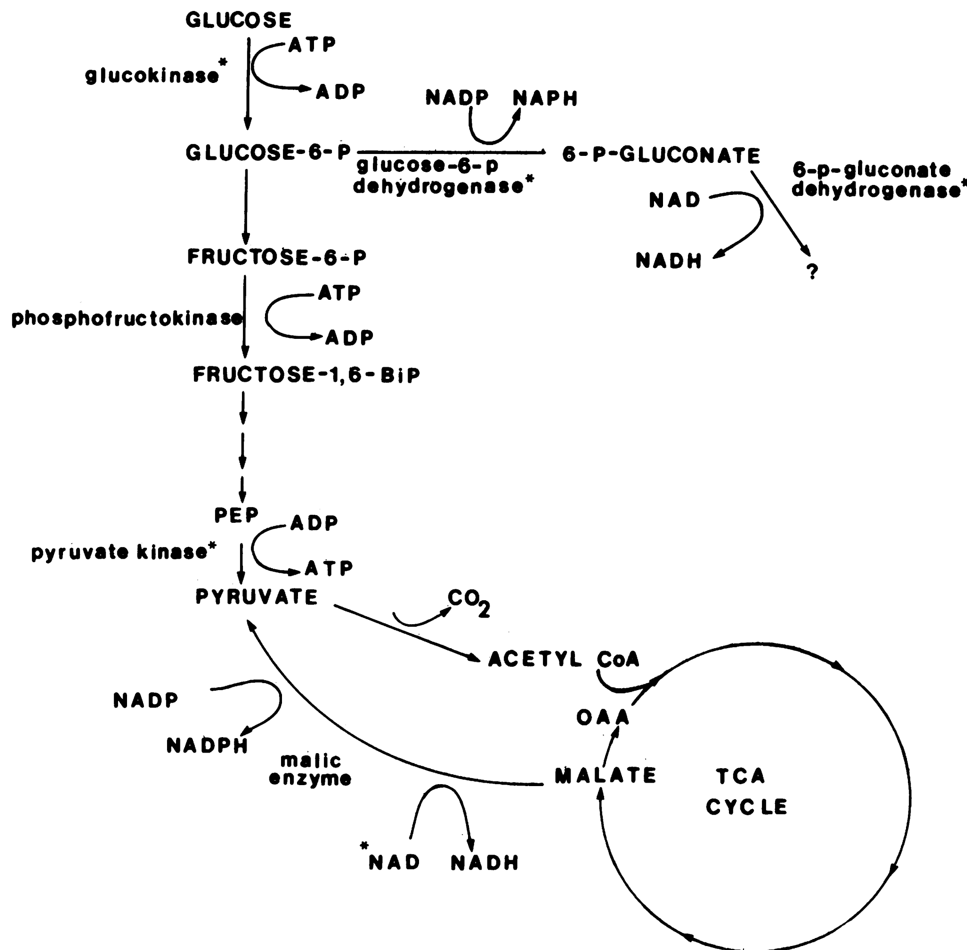

FIG. 3. Summary of the pathways of glucose metabolism that may be operating in *Frankia*. All enzymes shown have been identified in cell extracts of ArI3 cultures, enzymes with an asterisk have been identified in cell extracts of ArI3 vesicle-clusters isolated from *A. rubra* nodules.

substrate concentration. The apparent K_m values for glucose 6-phosphate, $NADP^+$, 6-phosphogluconate, and NAD are given in Table 3. Both enzymes showed an inhibitory effect of added ATP at physiological levels. The apparent affinity of the enzymes for glucose 6-phosphate and 6-phosphogluconate, respectively, was decreased by ATP at a concentration of 2 mM by 2.5 to 4 times (Table 3). The inhibition by ATP affected the apparent K_m of both enzymes for glucose 6-phosphate and 6-phosphogluconate, but not the V_{max}, and was therefore of the competitive type for these substrates. ATP did not affect the apparent K_m of the two dehydrogenases for $NADP^+$ and NAD (Table 3); however, the catalytic activity (V_{max}) of the enzymes was decreased by 4.5 times for glucose 6-phosphate dehydrogenase and by 1.3 times for 6-phosphogluconate dehydrogenase in the presence of 2 mM ATP.

DISCUSSION

An examination of the possible pathways of glucose catabolism by enzymatic analysis has shown that the EMP pathway of classical glycolysis is probably operating in at least one *Frankia* strain ArI3 (Fig. 3) and possibly in CcI2. This result is in contrast to earlier reports by Huss-Danell et al. (17) and Blom et al. (8), who did not find activity of reversible enzymes of glycolysis in *Frankia* vesicle clusters from *A. glutinosa*, or in pure cultures of *Frankia* strain AvCI1. The discrepancies may be due to the use of different techniques in enzyme detection or differences in strain metabolism as is seen in species of *Rhizobium* (16, 30, 31).

In addition to the EMP pathway, two enzymes usually associated with the PPP were also detected. However, the specificity of the 6-phosphogluconate dehydrogenase for NAD rather than $NADP^+$ places its role in the PPP in doubt. Most species of *Rhizobium*, both slow and fast growers, assayed to date have also been found to contain an NAD-linked 6-phosphogluconate dehydrogenase (25); however, in the fast growers the $NADP^+$-specific form is also present. From radiorespirometric analyses, it was concluded that the NAD-specific enzyme was not operating in the PPP in the slow-growing rhizobia (26, 27, 34). In other bacteria (11, 22), NAD-linked 6-phosphogluconate dehydrogenases operating in the PPP have been identified. It appears that the elucidation of the role of this enzyme in *Frankia* will have to await radiorespirometric investigation.

Regardless of the pathway(s) in which they operate, the glucose 6-phosphate and 6-phosphogluconate dehydrogenases appear to be important in glucolysis in *Frankia* as evidenced by their high specific activities in vesicle clusters and pure cultures with nitrogenase activity. The inhibition of these enzymes by ATP may indicate a regulatory function. ATP regulation of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase has been seen in a

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number of instances (4, 21, 22, 32). Lessie and Neidhardt (21) suggested that under certain conditions of growth, ATP inhibition of glucose 6-phosphate dehydrogenase in *Pseudomonas aeruginosa* can prevent wasteful degradation of hexose phosphate. The reasons for ATP regulation of 6-phosphogluconate dehydrogenase are not so clear. The end products of the reaction catalyzed by NAD-specific 6-phosphogluconate dehydrogenase in *Frankia* must be identified to understand its regulation by ATP.

The comparison of the specific activities of malic enzyme in ArI3 cultures grown on trehalose and propionate gives an indication of the possible importance of malic enzyme in gluconeogenesis in cells grown on organic acids (other than pyruvate) as a sole carbon source. It has been shown that ArI3 grown on propionate synthesizes large quantities of trehalose and glycogen (23). Presumably, cells growing on trehalose would have much lower rates of gluconeogenic enzymes since they would not have to synthesize glucose. It is interesting that no $NADP^+$-specific malic enzyme corresponding to that in pure cultures was found in the vesicle clusters of ArI3 isolated from alder nodules. If the NAD-specific malic enzyme found in the vesicle clusters was due to plant mitochondrial contamination, then the lack of a malic enzyme in the symbiotic endophyte would indicate that pyruvate for gluconeogenesis is generated by another metabolic route, or perhaps pyruvate is translocated directly to the endosymbiont from the plant cytosol. Benson (5) has shown that pyruvate is an excellent carbon source for culturing *Frankia* during isolation from *Alnus* nodules. As reported previously by Akkermans et al. (2), it is clear that plant mitochondria are closely intermingled with the endophyte vesicle clusters isolated from alder nodules (Fig. 2b) and that therefore any conclusions about the localization of enzymes in vesicle clusters must be quite tentative. Nevertheless, from published surveys of the tricarboxylic acid cycle (1, 17) and glyoxylate cycle (8) enzymes in both pure cultures and vesicle clusters of *Frankia* and from the survey in this report, a better understanding of the carbon metabolism in *Frankia* is now available. It appears that in symbiosis as well as in pure culture, glucose catabolism plays an important part in energy generation for *Frankia* sp.

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