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Biosynthesis of Bacterial Glycogen: Activator Specificity of the Adenosine Diphosphate Glucose Pyrophosphorylases from the Genus *Rhodospirillum*

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The adenosine diphosphate (ADP) glucose pyrophosphorylases from *Rhodospirillum fulvum*, *Rhodospirillum molischianum*, and *Rhodospirillum tenue* were partially purified, and their kinetic properties were studied. The enzyme from the three organisms was found to be activated by pyruvate and thus was similar to the *Rhodospirillum rubrum* enzyme that had been previously studied (C. E. Furlong, and J. Preiss, J. Biol. Chem. **244**:2539-2548, 1979). The enzymes from *R. fulvum*, *R. molischianum*, and *R. tenue* were also activated by oxamate, an analog of pyruvate. Other α-keto acids, α-ketobutyrate and hydroxypyruvate, activated to a smaller extent. The presence of pyruvate increased the apparent affinity for adenosine 5'-triphosphate and $MgCl_2$ for all three enzymes. The *R. molischianum* enzyme has very little sensitivity to inhibition by adenosine 5'-monophosphate, ADP, or inorganic phosphate. However, *R. tenue* ADPglucose pyrophosphorylase is very sensitive to inhibition by adenosine 5'-monophosphate, and the *R. fulvum* enzyme is inhibited by ADP. Increasing pyruvate concentration reversed the inhibition caused by adenosine 5'-monophosphate or ADP. Since ADPglucose is the glycosyl donor for synthesis of glycogen, it is possible that in vivo glycogen synthesis is regulated by the concentration of pyruvate and, in the case of *R. fulvum* and *R. tenue*, by the ratio of pyruvate concentration to inhibitor concentration.

Biosynthesis of glycogen in bacteria occurs via these reactions: ADPglucose pyrophosphorylase (EC 2.7.7.27) catalyzes the synthesis of ADPglucose from ATP and glucose 1-phosphate (15-17). The glucosyl moiety of ADPglucose is then transferred in a reaction catalyzed by glycogen synthase to form additional α1,4-glucosidic linkages (6, 9, 15-17). Formation of the α-1,6-glucosyl branch parts is subsequently catalyzed by branching enzyme (1, 22).

Regulation of bacterial glycogen synthesis occurs at the level of ADPglucose synthesis. It has been found that glycolytic intermediates activate ADPglucose pyrophosphorylase activity, whereas compounds related to energy metabolism, e.g., AMP, ADP, or P_i, inhibit it. The specificity of activation varies with the groups of bacteria studied. It appears that the nature of the activator is related to the carbon assimilatory pathways prevalent in the organism (15-17).

Previous studies (7) have shown that the ADPglucose pyrophosphorylase of *Rhodospirillum rubrum* is specifically activated by pyruvate. Studies of other ADPglucose pyrophosphorylases from the species of the genus *Rhodospirillum* were therefore initiated to determine whether their activator specificities were the same. This paper describes the kinetic regulatory properties of the enzyme from *Rhodospirillum fulvum*, *Rhodospirillum molischianum*, and *Rhodospirillum tenue*. A preliminary report of these studies has been made (J. Preiss and E. Greenberg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, K35, p. 132).

MATERIALS AND METHODS

Bacteria *R. fulvum* SK10060, *R. molischianum* ATCC 7139, and *R. tenue* ATCC 25093 were obtained from Norbert Pfennig, Department of Bacteriology of the University of Göttingen, Göttingen, Federal Republic of Germany. The cultures were maintained at room temperature under illumination on agar slants containing 0.3% yeast extract (Difco) and 1.5% agar.

Growth conditions. The organisms were grown in 20-liter carboys at room temperature under illumination with a series of flood lamps in media containing, per liter, 2.5 g of DL-malic acid, 1.0 g of yeast extract, 1.25 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 53 mg of $CaCl_2$, 10 mg of ferric citrate, 20 mg of disodium EDTA, 0.6 g of KH_2PO_4, 0.9 g of K_2HPO_4, 1 ml of a trace element solution, and 7.5 ml of a vitamin solution. The pH of the medium before sterilization was

6.5. The trace element solution contained, in 100 ml, 0.3 g of ferric citrate, 2 mg of $MnSO_4 \cdot H_2O$, 1 mg of H_3BO_3, 1 mg of $CuSO_4 \cdot 5H_2O$, 2 mg of $(NH_4)_6Mo_7O_{24} \cdot 7H_2O$, 1 mg of $ZnSO_4$, 50 mg of disodium EDTA, and 20 mg of $CaCl_2 \cdot 2H_2O$. The vitamin solution contained, in 100 ml, 20 mg of nicotinic acid, 20 mg of nicotinamide, 40 mg of thiamine·hydrochloride, and 1 mg of biotin. After about 2 weeks of growth the bacteria were harvested with a Sharples centrifuge and stored as a paste at −12°C. About 40 to 50 g of bacteria was obtained from 20 liters of medium.

The DEAE-cellulose used was microgranular, preswollen DE52 (Whatman). Ultrafiltration was done with an Amicon ultrafiltration apparatus using PM-30 membranes.

ADPglucose was identified by descending paper chromatography on Whatman no. 1 paper by using the following solvent systems: solvent A, 95% ethanol–1 M ammonium acetate, pH 3.8 (5:2); solvent B, isobutyric acid–1 M ammonia–0.1 M EDTA, pH 7.0 (100:60:1.6).

ADP-[^{14}C]glucose was prepared from [^{14}C]glucose as previously described (19). Other reagents and compounds used were obtained at the highest purity available from commercial sources.

Assay of ADPglucose pyrophosphorylase: assay A (pyrophosphorolysis). Pyrophosphorolysis of ADPglucose was followed by the formation of [^{32}P]-ATP from ADPglucose and $^{32}PP_i$ (21). The reaction mixtures assaying the *R. tenue* and *R. molischianum* enzymes contained 20 μmol of Tris-chloride buffer (pH 8.0), 2 μmol of $MgCl_2$, 2.5 μmol of NaF, 100 μg of bovine plasma albumin, 0.5 μmol of $^{32}PP_i$ (5×10^5 to 50×10^5 cpm/μmol), 0.4 μmol of ADPglucose, 3 μmol of pyruvate, and enzyme in a volume of 0.25 ml. The reaction mixture for the assay of the *R. fulvum* enzyme was the same except that it contained only 0.2 μmol of ADPglucose.

Assay B (synthesis). The synthesis of ADPglucose was measured by determination of the ADP-[^{14}C]glucose formed from ATP and [^{14}C]glucose-1-P (8). All reaction mixtures contained, in 0.2 ml, 20 μmol of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.0), 0.1 μmol of [^{14}C]glucose-1-P (5×10^5 to 10×10^5 cpm/μmol), 50 μg of bovine plasma albumin, 0.30 μg of yeast inorganic pyrophosphatase (Sigma, 600 U/mg), and enzyme. Reaction mixtures measuring *R. fulvum* activity contained 0.75 μmol of $MgCl_2$, 0.2 μmol of ATP, and 0.2 μmol of pyruvate. In reaction mixtures containing no pyruvate the amount of $MgCl_2$ added was increased to 4 μmol.

The reaction mixtures measuring ADPglucose synthesis catalyzed by the *R. molischianum* enzyme contained 0.1 μmol of ATP, 1.0 μmol of $MgCl_2$, and 0.1 μmol of pyruvate. When no pyruvate was added, $MgCl_2$ was increased to 4 μmol. Reaction mixtures assaying ADPglucose synthesis catalyzed by the *R. tenue* enzyme contained 0.2 μmol of ATP, 0.6 μmol of $MgCl_2$, and 0.2 μmol of pyruvate. When the assay was done in the absence of pyruvate, $MgCl_2$ was increased to 1.5 μmol and ATP was used at 0.4 μmol. The reaction mixture was incubated for 10 min at 37°C. The reaction was terminated by heating for 1 min in a boiling-water bath and assayed as described previously (8). All assays were done in the linear range of enzyme activity except where noted.

Assay of glycogen synthase. Glycogen synthase activity was measured by following the transfer of [^{14}C]glucose from ADP-[^{14}C]glucose to glycogen (18). The reaction mixture contained, in 0.2 ml, 10 μmol of Tris-hydrochloride (pH 8.0), 2 μmol of reduced glutathione, 5 μmol of KCl, 0.1 μmol of $MgCl_2$, 100 μg of bovine plasma albumin, 0.5 mg of rabbit liver glycogen, 0.14 μmol of ADP-[^{14}C]glucose (3×10^5 to 5×10^5 cpm/μmol), and enzyme. Incorporation of glucose into glyocgen was assayed as described previously (18).

Assay of branching enzyme. Branching enzyme was measured as described previously (10). The assay contained 25 μg of crystalline phosphorylase *a* (Sigma), 5 μmol of [^{14}C]glucose-1-P (5×10^4 cpm/μmol), 10 μmol of sodium citrate buffer (pH 7.0), 0.1 μmol of AMP, and enzyme in a volume of 0.1 ml.

Protein determination. Proteins were assayed by the method of Lowry et al. (12).

Ultracentrifugation in sucrose density gradients. Sucrose density gradient centrifugation was carried out according to the procedure of Martin and Ames (13). Linear sucrose gradients (4.3 ml) were prepared by mixing 5% (wt/vol) sucrose and 25% (wt/vol) sucrose solutions, both containing 50 mM Tris-chloride buffer (pH 7.5) and 5 mM dithioerythritol (DTE). The gradients were layered with 100 μl of the above buffer solution containing 50 μg of rabbit heart lactate dehydrogenase, 100 μg of rabbit muscle pyruvate kinase, and about 30 μl of ADPglucose pyrophosphorylase. Centrifugation was carried out for 16 to 20 h, and 7-drop fractions were collected. Lactate dehydrogenase activity (11) and pyruvate kinase (2) were assayed as described previously. ADPglucose pyrophosphorylase activity was measured by assay A.

Purification of ADPglucose pyrophosphorylases: sonic oscillation and heat treatment. Bacterial cell paste (10 g of *R. fulvum* or *R. molischianum* or 5 g of *R. tenue*) was suspended in 50 ml of ice-cold 0.05 M glycylglycine buffer (pH 7.0) containing 5 mM DTE and exposed to sonic oscillation for 4 to 4.5 min. The extract was made 0.03 M in phosphate by the addition of 1.74 ml of M potassium phosphate buffer (pH 7.0) and then incubated for 5 min at 60°C. The denatured protein was centrifuged at 30,000 × *g* for 15 min at 4°C. The precipitate obtained from the *R. fulvum* cell paste was washed with 25 ml of the above glycylglycine-DTE buffer and pooled with the 30,000 × *g* supernatant fluid after centrifugation.

***R. fulvum* ADPglucose pyrophosphorylase.** The heat-treated extract was diluted with an equal volume of water and adsorbed on a DEAE-cellulose column (1 by 8 cm) equilibrated with 15 mM potassium phosphate buffer (pH 7.5) containing 0.5 mM DTE and 1 mM EDTA. The column was washed with 12 ml of the equilibrating buffer, and then the enzyme was eluted with a linear gradient containing 60 ml of the equilibrating buffer in the mixing chamber and 60 ml of 0.2 M potassium phosphate buffer (pH 7.0) containing 0.5 mM DTE, 1 mM EDTA, and 0.3 M KCl in the reservoir chamber. Fractions (2 ml) were collected, and the enzyme appeared after 28 ml of the gradient had passed through the column. Those fractions con-

taining high amounts of activity were pooled, and the protein was precipitated with solid ammonium sulfate added to 0.7 saturation. After centrifugation, the protein was dissolved in 30 mM potassium phosphate buffer (pH 7.0) containing 0.5 mM DTE and dialyzed against this buffer (200 ml) overnight.

***R. molischianum* ADPglucose pyrophosphorylase.** A saturated ammonium sulfate solution (48 ml) was added to 32 ml of the heat-treated supernatant, and after 15 min the precipitate was centrifuged at 30,000 × *g* for 10 min, suspended in 50 mM HEPES (pH 7.0) containing 2 mM DTE, and dialyzed overnight against 1 liter of the same buffer. The dialyzed suspension was centrifuged for 1 h at 105,000 × *g*, and the clear supernatant obtained was adsorbed on a DEAE-cellulose column (1 by 11 cm) equilibrated as indicated above. The column was washed with 100 ml of the equilibration buffer, and then the enzyme was eluted with a linear gradient composed of 90 ml of the equilibration buffer in the mixing chamber and 90 ml of 150 mM potassium phosphate buffer (pH 7.0), containing 0.5 mM DTE, 1 mM EDTA, and 0.3 M KCl, in the reservoir chamber. Fractions of 3 ml were collected, and the enzyme appeared after 39 ml of the gradient had passed through the column. Fractions containing activity were pooled, and the enzyme was precipitated with solid ammonium sulfate added to 0.7 saturation. After centrifugation the enzyme was dissolved in the 50 mM HEPES (pH 7.0)–2 mM DTE buffer and dialyzed against 300 ml of the same buffer.

***R. tenue* ADPglucose pyrophosphorylase.** The heat-treated fraction was adsorbed on a DEAE-cellulose column (1.5 by 11 cm) equilibrated as indicated above. The column was washed with 200 ml of the equilibration buffer, and the enzyme was eluted with a linear gradient containing 200 ml of the equilibration buffer in the mixing chamber and 200 ml of 0.15 M potassium phosphate buffer (pH 7.0), containing 0.5 mM DTE, 1 mM EDTA, and 0.2 M KCl, in the reservoir chamber. Fractions (8 ml) were collected, and the enzyme appeared in the eluate after 120 ml of gradient had passed through the column. The active fractions were pooled (70 ml), diluted to 140 ml with 50 mM HEPES (pH 7.0) containing 0.5 mM DTE, and concentrated to 20 ml with an Amicon ultrafiltrator using a PM-30 membrane filter. The enzyme was diluted to 50 ml with the HEPES-DTE buffer and concentrated to about 5 ml.

RESULTS

Levels of glycogen biosynthetic enzymes in *R. fulvum*, *R. molischianum*, and *R. tenue*. The levels of ADPglucose pyrophosphorylase, glycogen synthase, and branching enzyme in the crude extracts of the three photosynthetic organisms are seen in Table 1. The glycogen synthase of the three organisms was highly specific for ADPglucose as no incorporation of glucose was noted with UDPglucose, CDPglucose, or TDPglucose (unpublished data). Small activity was observed with GDPglucose as the glucosyl donor, ranging from about 3 to 10% of that observed with ADPglucose. A slight amount of glycogen phosphorylase activity was also noted in *R. molischianum* and *R. tenue*, i.e., 28 and 5% of the glycogen synthase activity observed in those extracts, respectively. The glycogen synthase and phosphorylase activities were not observed when 1.7 U of hog pancreatic α-amylase was added to the reaction mixture. Glycogen synthase activity was not affected by the presence of either 1 mM glucose-6-P, fructose-6-P, fructose-1,6-P_2, NADPH, pyruvate, 3-phosphoglycerate, or phosphoenolpyruvate in the crude extract of the three organisms (results not shown).

TABLE 1. *ADPglucose pyrophosphorylase, glycogen synthase, and branching enzyme levels in crude extracts of R. fulvum, R. molischianum, and R. tenue*[a]

Organism	ADPglucose pyrophosphorylase (nmol min^{-1} mg^{-1})	Glycogen synthase (nmol min^{-1} mg^{-1})	Branching enzyme (nmol min^{-1} mg^{-1})
R. fulvum	88	15.3	167
R. molischianum	51	37.8	85
R. tenue	52	22.6	109

[a] Extracts were prepared by sonic oscillation of 1 g of cells suspended in 10 ml of cold 0.05 M glycylglycine (pH 7.0) buffer plus 5 mM DTE for 2 to 3 min at 15-s intervals, with frequent cooling to maintain the temperature below 15°C. ADPglucose pyrophosphorylase was measured via assay A. Other assays are described in the text.

Partial purification of ADPglucose pyrophosphorylase. The results of the purification procedures described in Materials and Methods gave enrichments of 13- to 40-fold of the enzyme activities. The DEAE-cellulose fractions were essentially free of ATP-, glucose-1-P-, and ADPglucose-degrading activities. The specific activities of the enzyme from *R. fulvum*, *R. molischianum*, and *R. tenue* were 17.1, 11.2, and 16.0 μmol of ATP formed per 10 min per mg of protein, respectively.

pH optima and requirements for ADPglucose synthesis. The presence of ATP, glucose-1-P, and Mg^{2+} were absolutely required for ADPglucose synthesis for the reactions catalyzed by the enzyme from the three organisms. Inorganic pyrophosphatase increased the extent of linearity of the reaction with respect to time. In the presence of the activator, pyruvate, the pH optima were fairly broad for the three enzymes and fairly independent of the buffers used in the range of pH 7.0 through 8.0. The *R. tenue*, *R. fulvum*, and *R. molischianum* enzymes showed optimum activity at pH 7.0 in HEPES buffer and at pH 8.0 in bicine buffer. There was

no change in the pH optima in the absence of pyruvate for the *R. fulvum* and *R. molischianum* enzymes. However, in the absence of the activator pyruvate, the pH optimum was shifted to 6.0 to 6.6 for the *R. tenue* ADPglucose pyrophosphorylase activity. Very little activation by pyruvate was noted in that range.

Nucleoside triphosphate specificity of the ADPglucose pyrophosphorylases. Significant GDP mannose pyrophosphorylase activity was also noted in the *R. molischianum* and *R. tenue* crude extracts, whereas the *R. fulvum* extracts contained CDPglucose pyrophosphorylase activity. The activity of the partially purified enzymes towards nucleoside triphosphates other than ATP is shown in Table 2. For the *R. fulvum* enzyme less than 1% activity for synthesis of most other sugar nucleotides was observed. About 12% activity was noted with XTP. When 100-fold more enzyme was used, significant but relatively low activity was noted with dATP, UTP, CTP, and XTP. The *R. molischianum* enzyme showed about 16, 9, 6, and 5% of the activity observed with ADPglucose with the sugar nucleotides UTP, XTP, dATP, and CTP, respectively. Higher concentrations of enzyme gave significant synthesis of the sugar nucleotides from those nucleoside triphosphates as well as from TTP and GTP; thus the enzyme appears to be relatively nonspecific. The *R. tenue* enzyme showed appreciable activity only with CTP, XTP, and dATP, and only at very high protein concentrations. All nucleotide triphosphates except UTP required the presence of the activator pyruvate for optimal activity. The dependency on activator presence was usually much greater than that required for ADPglucose formation from ATP. However, UDPglucose synthesis from UTP was not activated by pyruvate. It is quite possible that in those cases a UDPglucose pyrophosphorylase was contaminating the ADP glucose pyrophosphorylase. Because of the activation by pyruvate, synthesis of the other sugar nucleotides is considered to be an innate property of the ADPglucose pyrophosphorylase.

TABLE 2. *Nucleoside triphosphate specificity of the ADPglucose pyrophosphorylases*[a]

Nucleoside triphosphate	ADPglucose formed (nmol per 10 min) with protein added:					
	R. fulvum (μg)		*R. molischianum* (μg)		*R. tenue* (μg)	
	1.3	127	1.36	68	1.5	150
ATP	17	62	13.7	69	12.1	68
dATP	<0.1	6.2	0.81	15.1	0.2	2.1
CTP	<0.1	1.2	0.7	22.1	1.0	1.8
GTP	<0.1	<0.1	0.13	1.6	<0.1	0.4
TTP	<0.1	<0.1	0.14	2.5	<0.1	<0.1
UTP	0.1	6.7	2.2	51	<0.1	0.3
XTP	0.27	14.3	1.2	—	0.15	1.4
ITP	—	—	<0.1	<0.1	<0.1	<0.1

[a] The conditions of assay B were used with 1 mM pyruvate as the activator. The DEAE-cellulose fractions were the enzymes used in the experiment. The reaction rate was linear when the lower protein concentration was used for each enzyme. The use of the higher protein concentration gave nonlinear rates in the 10-min incubation time but permitted the isolation of the sugar nucleotides other than ADPglucose for identification.

The radioactive products synthesized from dATP, CTP, GTP, TTP, and UTP cochromatographed in solvents A and B with the respective sugar nucleotide standards. The product obtained with XTP could not be compared with an authentic sample of XDP glucose because it was not available.

Activation of ADPglucose synthesis. The three photosynthetic bacterial ADPglucose pyrophosphorylases were activated by pyruvate and by the pyruvate analog oxamate. Glycolytic intermediates tested and found not to give any activation at 1 mM were glucose-6-P, fructose-6-P, fructose-1,6-P_2, 3-phosphoglyceraldehyde, dihydroxyacetone-P, 2-phosphoglycerate, 3-phosphoglycerate, phosphoenolpyruvate, and DL-lactate. Other intermediates also inactive were NADH, NADPH, citrate, succinate, fumarate, ribose-5-P, and 2-keto-3-deoxy-6-phosphogluconate. α-Ketobutyrate and hydroxypyruvate at 1 mM concentration gave six- and fourfold stimulation, respectively, of ADPglucose synthesis catalyzed by the *R. fulvum* enzyme.

Figure 1 shows the pyruvate activation curve for the *R. fulvum* enzyme at two $MgCl_2$ concentrations, 2.5 and 3.75 mM. At 3.75 mM, $MgCl_2$ was saturating the enzyme in the presence of pyruvate, and the $A_{0.5}$ value (concentration of activator giving half-maximal activation) was 18.5 μM. At 2.5 mM $MgCl_2$ the $A_{0.5}$ value for pyruvate was increased to 42 μM. The curves were slightly sigmoidal since Hill plots (3) of the data (Fig. 1B) gave slope values of 1.3.

Table 3 summarizes the data obtained for the pyruvate activation curves for the *R. tenue*, *R. molischianum*, and *R. fulvum* ADPglucose pyrophosphorylases. $MgCl_2$ or ATP concentrations or both were varied, and these compounds affected the $A_{0.5}$ value or maximal velocity. Lowering of the ATP concentrations from 1 mM to a subsaturation level, 0.5 mM, increased the pyruvate $A_{0.5}$ value for the *R. tenue* enzyme. A decrease of ATP to subsaturating levels decreased only V_{max} for the *R. molischianum* enzyme and had little effect on the pyruvate $A_{0.5}$ value. In contrast, a lowering of the $MgCl_2$ concentration decreased the pyruvate $A_{0.5}$ value al-

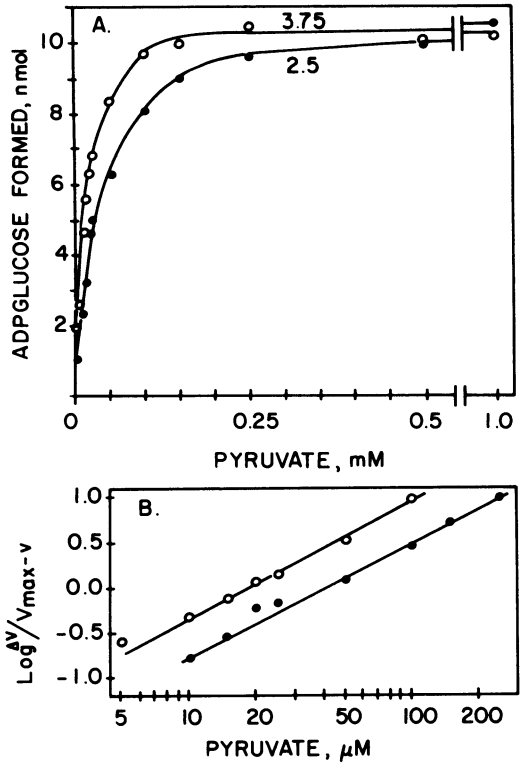

FIG. 1. *Effect of pyruvate concentration on the rate of ADPglucose synthesis catalyzed by the R. fulvum enzyme. The conditions are those of assay B except for the $MgCl_2$ conditions that are indicated in the graph. (B) Hill plot of the data. ΔV is the increase of velocity due to the addition of activator, i.e., the velocity obtained upon addition of a certain amount of activator to the reaction mixture minus the velocity observed for the reaction mixture containing no activator. V_{max} was obtained from reciprocal plots of V against activator concentration.*

most twofold but essentially had no effect on V_{max}.

Figure 2 shows the oxamate activation curves for the three enzymes. The curves were hyperbolic in shape with Hill plot slope values, $\bar{n}$, ranging from 0.96 to 1.06. The $A_{0.5}$ values for oxamate were 6.8, 13, and 31 μM for the *R. tenue*, *R. molischianum*, and *R. fulvum* enzymes, respectively.

Effect of pyruvate on the kinetic parameters of the *R. fulvum*, *R. molischianum*, and *R. tenue* ADPglucose pyrophosphorylases. Figure 3 shows the effect of pyruvate on the ATP saturation curve for the *R. tenue* enzyme. The presence of 1 mM pyruvate decreased the concentration of ATP required for half-maximal activity, $S_{0.5}$, from 1.1 to 0.39 mM. The curves in the presence or absence of pyruvate were sigmoidal with Hill plot slope values, $\bar{n}$, being 2.4 to 2.6. Pyruvate also decreased to a small extent the $S_{0.5}$ values for ATP for the *R. molischianum* and *R. fulvum* enzymes (Table 4). However, the ATP saturation curves for these enzymes were hyperbolic. Pyruvate did not appreciably change the $S_{0.5}$ value for glucose-1-P. Table 4 shows that pyruvate decreased the $S_{0.5}$ value for glucose-1-P for the *R. molischianum* enzyme from 78 to 50 mM but did not affect the glucose-1-P $S_{0.5}$ values for either the *R. fulvum* or *R. tenue* enzymes. The $S_{0.5}$ values for $MgCl_2$ for the three enzymes were decreased about 2.25- to 5.75-fold (Table 4). The $MgCl_2$ curves were highly sigmoidal either in the presence or absence of pyruvate, with the Hill plot slope values being about 3.0. The activator pyruvate had only a small effect on the maximal velocity for ADPglucose synthesis for either the *R. fulvum* or *R. molischianum* enzymes but increased the maximal velocity about threefold for the *R. tenue* ADPglucose pyrophosphorylase.

Inhibition of the *R. fulvum* and *R. tenue* ADPglucose pyrophosphorylases. The *R. tenue* enzyme was sensitive to inhibition at zero or low concentrations of pyruvate. Figure 4 shows inhibition of the *R. tenue* enzyme by either ADP, P_i, or AMP at 50 μM pyruvate, a concentration giving 60% of the maximal velocity, and at 15 μM pyruvate, a concentration of activation resulting in 36% of the maximal velocity (Table 5). AMP was the most effective inhibitor, giving 50% inhibition at 62 μM in the absence of pyruvate and at 0.26 mM in the presence of 50 mM pyruvate. The ADP and P_i inhibitor curves were sigmoidal, with Hill slope values of 1.6, whereas the AMP inhibition curves were hyperbolic. No inhibition of the *R. tenue* enzyme was observed with either 2.5 mM AMP, ADP, or P_i in the presence of 1 mM pyruvate.

The inhibitor, AMP, also affected the *R. tenue* enzyme pyruvate activation curve. Figure 5 shows that in the presence of 0.2 and 0.5 mM AMP, the $A_{0.5}$ value for pyruvate was increased from 28 to 78 and 140 μM, respectively. Furthermore, the hyperbolic activation curve was changed into sigmoidal shape, with the Hill slope value increasing from 1.0 to 1.4 and 1.8, respectively.

ADP was found to be the most effective inhibitor of the *R. fulvum* ADPglucose pyrophosphorylase. In the presence of 0.025 mM pyruvate about 50% inhibition was observed with 2.5 mM AMP or with 1 mM ADP. No inhibition was observed with 2.5 mM P_i. Figure 6 shows the inhibition by ADP in the presence of various concentrations of pyruvate. The inhibition

TABLE 3. *Pyruvate activator constants for the ADPglucose pyrophosphorylases of R. fulvum, R. molischianum, and R. tenue*[a]

Enzyme from:	$MgCl_2$ concn (mM)	ATP concn (mM)	Pyruvate $A_{0.5}$ (μM)	$\bar{n}$[b]	V_{max}[c] (μmol/ml)	V_0[d] (μmol/ml)
R. fulvum	2.5	1.0	42	1.3	134	13.7
	3.75	1.0	18.5	1.3	138	26
R. tenue	3	1.0	25	1.0	20.5	2.4
	3	0.5	56	1.0	17.0	1.05
R. molischianum	10	1.0	11.5	1.1	90.4	47.5
	5	1.0	18	1.1	86	9.8
	10	0.1	10.5	1.05	40	3.9

[a] Assay B was used and is described in the text.
[b] $\bar{n}$ is the slope obtained from the Hill plot.
[c] V_{max} was determined as indicated in Fig. 1.
[d] V_0 is the activity obtained in the absence of pyruvate and at the concentrations of ATP and $MgCl_2$ listed in the table.

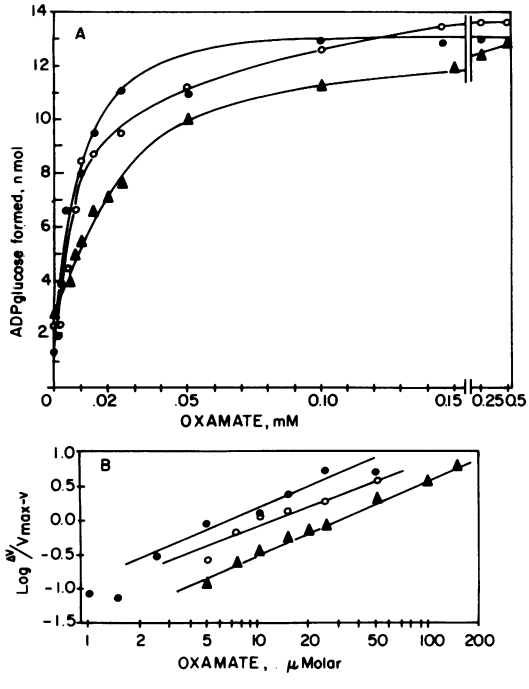

FIG. 2. *Activation of ADPglucose pyrophosphorylase by oxamate.* (●) *R. tenue enzyme;* (○) *R. molischianum enzyme;* (▲) *R. fulvum enzyme.* ΔV *is the same as indicated in Fig. 1. (B) Hill plot of the data.*

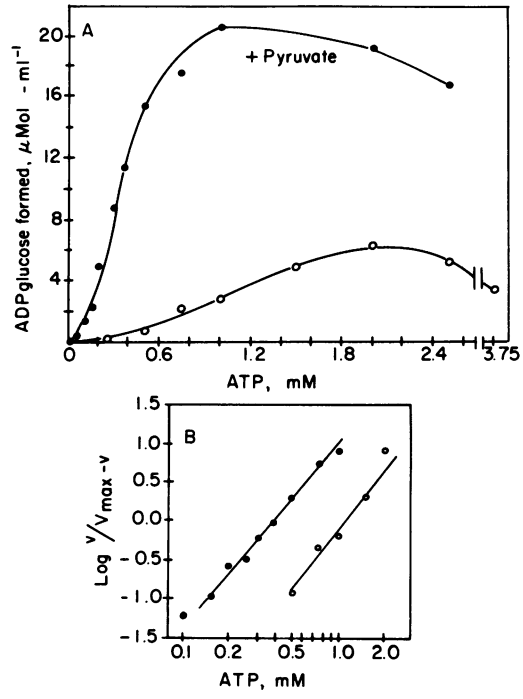

FIG. 3. *Effect of pyruvate on the R. tenue ATP saturation curve. The conditions are those of assay B with the pyruvate concentration, when added, being 1 mM. (B) Hill plot of the data.*

curves were sigmoidal with Hill plot slopes, $\bar{n}$, of 2.0. Increasing concentrations of pyruvate decreased the sensitivity of the enzyme towards inhibition. The concentrations of ADP required for 50% inhibition ($I_{0.5}$) were 0.93, 1.25, and 3.25 mM when the concentrations of pyruvate in the reaction mixture were 5 μM, 20 μM, and 1 mM, respectively (Fig. 6).

The *R. molischianum* enzyme was relatively insensitive to inhibition. No inhibition by either 2.5 mM P_i, ADP, or AMP was observed in the presence of 1 mM pyruvate. When no activator was present in the reaction mixture in unactivated conditions the inhibition by 2.5 mM AMP and 2.5 mM ADP was about 50%. No inhibition was observed with 2.5 mM P_i.

Estimation of the molecular weight of the ADPglucose pyrophosphorylase. In sucrose density gradient ultracentrifugation experiments

TABLE 4. *Substrate kinetic parameters of the R. fulvum R. molischianum, and R. tenue ADPglucose pyrophosphorylases*[a]

Enzyme	Substrate	Activato.	$S_{0.5}^{b}$ (mM)	$\bar{n}^{c}$	V_{max} (μmol mg^{-1} 10 min^{-1})
R. fulvum	ATP	None	0.18	0.99	
		Pyruvate (1 mM)	0.12	0.95	
	αGlucose 1-P	None	0.096	0.94	12.6
		Pyruvate (1 mM)	0.089	0.91	15.0
	$MgCl_2$	None	6.4	2.9	
		Pyruvate (1 mM)	1.4	3.0	
R. molischianum	ATP	None	0.11	0.97	
		Pyruvate (0.5 mM)	0.05	1.0	
	αGlucose 1-P	None	0.05	1.0	10.6
		Pyruvate (0.5 mM)	0.078	0.98	13.0
	$MgCl_2$	None	9.2	3.0	
		Pyruvate (0.5 mM)	1.6	3.2	
R. tenue	ATP	None	1.1	2.6	
		Pyruvate (1 mM)	0.39	2.4	
	αGlucose 1-P	None	0.04	1.0	5.8
		Pyruvate (1 mM)	0.04	1.0	15.9
	$MgCl_2$	None	4.5	3.9	
		Pyruvate (1 mM)	2.0	4.5	

[a] Assay B was used and is described in the text.
[b] $S_{0.5}$ is the concentration required for half-maximal activity.
[c] $\bar{n}$ is the slope obtained from the Hill plot.

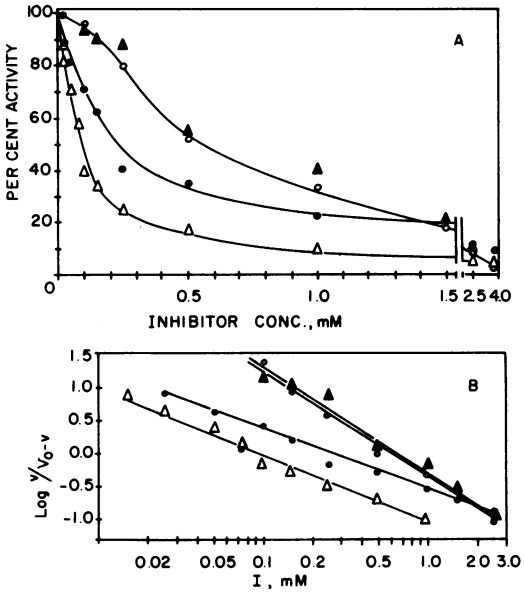

FIG. 4. *Inhibition of R. tenue ADPglucose pyrophosphorylase by AMP, ADP, and P_i.* (○) *ADP,* (▲) *P_i, and* (●) *AMP in the presence of 50 μM puruvate.* (△) *AMP in the presence of 15 μM pyruvate. (B) Hill plot of the data. The conditions are those of assay B.*

the ADPglucose pyrophosphorylases of *R. fulvum*, *R. molischianum*, and *R. tenue* migrated as one peak between the rabbit muscle pyruvate kinase and lactate dehydrogenase activity peaks.

TABLE 5. *Inhibition of R. tenue ADPglucose pryophosphorylase*[a]

Activator (concn)	Inhibitor	$I_{0.5}$ (mM)	$\bar{n}$
None	AMP	0.062	1.0
	ADP	0.55	1.5
	P_i	0.42	1.6
Pyruvate (15 μM)	AMP	0.095	1.0
Pyruvate (50 μM)	AMP	0.26	1.0
	ADP	0.62	1.6
	P_i	0.66	1.6

[a] The conditions of the experiment were those of assay B. In the absence of activator the concentrations of ATP and $MgCl_2$ were 2.0 and 7.5 mM, respectively.

The apparent molecular weights of the ADPglucose pyrophosphorylases were estimated to be 205,000 ± 15,000, 202,400 ± 3,430, and 186,000 ± 9,800 for the *R. fulvum*, *R. molischianum*, and *R. tenue* enzymes, respectively. Thus the native molecular weights of the enzymes appear to be similar to what has been observed for other bacterial ADPglucose pyrophosphorylases (17).

DISCUSSION

The glycogen synthases from the above three organisms are highly specific for ADPglucose. The ADPglucose pyrophosphorylases of the above three organisms are activated by pyruvate, suggesting that regulation of glycogen syn-

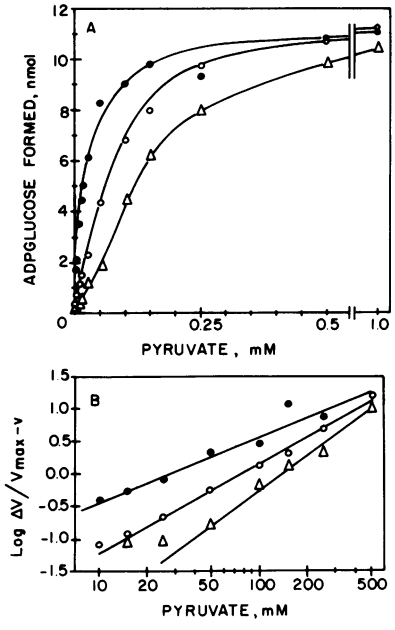

FIG. 5. *Effect of AMP on activation of the R. tenue ADPglucose pyrophosphorylase by pyruvate.* (●) *No AMP;* (○) *0.2 mM AMP; and* (△) *0.5 mM AMP. (B) Hill plot of the data.*

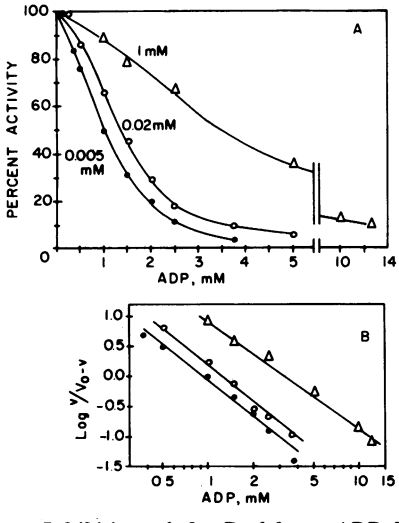

FIG. 6. *Inhibition of the R. fulvum ADPglucose pyrophosphorylase by ADP. The conditions of the assay were those of assay B. The concentration of pyruvate present in the reaction mixtures is indicated in (A). (B) Hill plot of the data.*

thesis in *R. fulvum*, *R. molischianum*, and *R. tenue* may occur at the level of ADPglucose synthesis. No other naturally occurring glycolytic intermediates were found to be activators. Oxamate, an analog of pyruvate, was shown to be an effective activator, and the α-keto acids α-ketobutyrate and hydroxypyruvate activated to a smaller extent.

The biggest effect of pyruvate was to increase the apparent affinity for both ATP and $MgCl_2$ for all three enzymes (Table 4). Maximal velocity was only slightly increased by pyruvate for the *R. fulvum* and *R. molischianum* enzymes but was increased almost threefold in the *R. tenue* system. Another major effect of pyruvate was to desensitize the *R. tenue* and *R. fulvum* ADPglucose pyrophosphorylases to inhibition (Fig. 4 to 6 and Table 5). Higher concentrations of pyruvate increased the $I_{0.5}$ values for the inhibitors. Alternatively, the inhibitor could increase the $A_{0.5}$ for the activator (Fig. 5). Thus it is highly possible that in vivo, ADPglucose pyrophosphorylase activity and glycogen synthesis is regulated by the ratio of pyruvate to inhibitor. Since ATP is a substrate and ADP or AMP or both are inhibitors of the enzyme, one may postulate that glycogen synthesis is regulated by the energy charge of the cell and that the levels of pyruvate, the activator, can potentiate the high energy charge state towards higher rates of ADPglucose-synthesizing activity (4, 20). However, the ADPglucose pyrophosphorylases of *R. molischianum* and *R. rubrum* (7) are not effectively inhibited by either P_i, AMP, or ADP. Energy charge levels may not be very effective in playing a role in the regulation of these enzymes.

Thus the ADPglucose pyrophosphorylases from organisms of the genus *Rhodospirillum* have the same activator specificity. Crude extracts of *Rhodospirillum photometricum* have also been assayed for ADPglucose pyrophosphorylase activity, and only pyruvate has been found to activate the pyrophosphorolysis of ADPglucose (unpublished data). Although the enzymes appear to have the same activator specificity, their regulatory properties are slightly different. As indicated before, the *R. rubrum* and *R. molischianum* enzymes have very little sensitivity to inhibition by AMP, ADP, or P_i. The enzyme most sensitive to inhibition is the *R. tenue* enzyme, which is most effectively inhibited by AMP. The most effective inhibitor for the *R. fulvum* enzyme is ADP. The ATP saturation curve for the *R. fulvum* and *R. molischianum* enzymes is hyperbolic, whereas it is sigmoidal for the *R. tenue* and *R. rubrum* enzymes (7). Similarly, the pyruvate activation curve is hyperbolic for the *R. tenue* and *R. molischianum* enzymes and sigmoidal for the *R. rubrum* (7) and *R. fulvum* enzymes.

The physiological significance of pyruvate in metabolism in *R. rubrum* and as an activator of the *R. rubrum* ADPglucose pyrophosphorylase

has been previously discussed (7, 15–17). Experiments by Stanier et al. (23) shows that there is a correlation between the ability of *R. rubrum* to accumulate glycogen and the ability to convert the carbon present in the growth medium to pyruvate. The other organisms in the genus *Rhodospirillum*, with the exception of *R. photometricum*, cannot metabolize glucose, preferentially use tricarboxylic acid cycle intermediates for growth, and thus are similar to *R. rubrum* (14). However, the precise nature and flux of the carbon metabolic pathways are not known in sufficient detail in these organisms. It would be of interest to determine or correlate the levels of pyruvate in these organisms and their ability to accumulate glycogen. Such a study has been done for fructose-P_2 levels and glycogen accumulation rates in *Escherichia coli* (5). Fructose-P_2 has been shown to be the principal activator for the *E. coli* ADPglucose pyrophosphorylase.

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LITERATURE CITED

1. **Boyer, C., and J. Preiss.** 1977. Biosynthesis of bacterial glycogen: purification and properties of *Escherichia coli* B α-1,4-glucan: α-1,4-glucan 6-glycosyltransferase. Biochemistry **16**:3693–3696.
2. **Bücher, T., and G. Pfleiderer.** 1955. Pyruvate kinase from muscle. Methods Enzymol. **1**:435–440.
3. **Changeux, J. P.** 1963. Allosteric interactions on biosynthetic L-threonine deaminase from *E. coli* K 12. Cold Spring Harbor Symp. Quant. Biol. **28**:497–504.
4. **Chapman, A. G., L. Fall, and D. E. Atkinson.** 1971. The adenylate energy charge in *Escherichia coli* during growth and starvation. J. Bacteriol. **108**:1072–1086.
5. **Dietzler, D. N., M. P. Leckie, C. J. Lais, and J. Magnani.** 1975. Evidence for the regulation of bacterial glycogen synthesis *in vivo*. J. Biol. Chem. **250**:2383–2387.
6. **Fox, J., K. Kawaguchi, E. Greenberg, and J. Preiss.** 1976. Biosynthesis of bacterial glycogen: purification and properties of the *Escherichia coli* B ADP glucose: 1,4-α-D-glucan 4α glucosyltransferase. Biochemistry **15**:849–857.
7. **Furlong, C. E., and J. Preiss.** 1969. Biosynthesis of bacterial glycogen. VII. Purification and properties of adenosine diphosphoglucose pyrophosphorylase of *Rhodospirillum rubrum*. J. Biol. Chem. **244**:2539–2548.
8. **Ghosh, H. P., and J. Preiss.** 1966. Adenosine diphosphate glucose pyrophosphorylase—a regulatory enzyme in the biosynthesis of starch in spinach leaf chloroplasts. J. Biol. Chem. **241**:4491–4504.
9. **Greenberg, E., and J. Preiss.** 1964. The occurrence of adenosine diphosphate glucose: glycogen transglucosylase in bacteria. J. Biol. Chem. **239**:4314–4315.
10. **Hawker, J. S., J. L. Ozbun, H. Ozaki, E. Greenberg, and J. Preiss.** 1974. Interaction of spinach leaf adenosine diphosphate glucose α-1,4-glucan α-4-glucosyl transferase in synthesis of branched α-glucan. Arch. Biochem. Biophys. **160**:530–551.
11. **Kornberg, A.** 1955. Lactic dehydrogenase of muscle. Methods Enzymol. **1**:441–443.
12. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. **Martin, R. G., and B. N. Ames.** 1961. A method for determining the sedimentation behavior of enzymes: application to protein mixtures. J. Biol. Chem. **236**: 1372–1379.
14. **Pfennig, N., and H. G. Trüper.** 1972. The Rhodospirales (phototrophic or photosynthetic bacteria), p. 17–27. *In* A. I. Laskin and H. A. Lechevalier (ed.), Handbook of microbiology, vol. 1: Organismic microbiology. CRC Press, Cleveland.
15. **Preiss, J.** 1969. The regulation of the biosynthesis of α1,4-glucans in bacteria and plants, p. 125–161. *In* E. R. Stadtman and B. L. Horecker (ed.), Topics in cellular regulation, vol. 1. Academic Press, Inc., New York.
16. **Preiss, J.** 1973. ADPglucose pyrophosphorylase, p. 73–119. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 8. Academic Press, Inc., New York.
17. **Preiss, J.** 1978. Regulation of adenosine diphosphate glucose pyrophosphorylase. Adv. Enzymol. Relat. Areas Mol. Biol. **46**:317–381.
18. **Preiss, J., and E. Greenberg.** 1965. Biosynthesis of bacterial glycogen. III. The adenosine diphosphate glucose: α-4-glycosyl transferase of *Eshcerichia coli* B. Biochemistry. **4**:2328–2334.
19. **Preiss, J., and E. Greenberg.** 1972. ADP-[^{14}C]-glucose. Methods Enzymol. **28**:279–281.
20. **Shen, L., and D. E. Atkinson.** 1970. Regulation of adenosine diphosphate glucose synthase from *Escherichia coli*. Interactions of adenylate energy charge and modifier concentrations. J. Biol. Chem. **245**:3996–4000.
21. **Shen, L., and J. Preiss.** 1974. The activation and inhibition of bacterial adenosine diphosphoglucose pyrophosphorylase. Biochem. Biophys. Res. Commun. **17**: 424–429.
22. **Sigal, N., J. Cattaneo, J. P. Chambost, and A. Favard.** 1965. Characterization and partial purification of a branching enzyme from *Escherichia coli*. Biochem. Biophys. Res. Commun. **20**:616–620.
23. **Stanier, R. Y., M. Doudoroff, R. Kunisawa, and R. Contopoulou.** 1959. The role of organic substrates in bacterial photosynthesis. Proc. Natl. Acad. Sci. U.S.A. **45**:1246–1260.