Phytochemistry, 1966, Vol. 5, pp. 405 to 410. Pergamon Press Ltd. Printed in England

ACTIVATION OF SORGHUM URIDINE-5'-DIPHOSPHATE GLUCOSE PYROPHOSPHORYLASE BY OTHER RIBONUCLEOSIDE-5'-DIPHOSPHATE COMPOUNDS*

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(*Received* 29 *September* 1965)

Abstract—This investigation was conducted to determine the effect of ribonucleoside-5'-diphosphate derivatives of glucose other than UDPG upon the activity of UDPG pyrophosphorylase obtained from etiolated sorghum seedlings. UDPG pyrophosphorylase was isolated and partially purified on DEAE-cellulose. No ADPG, GDPG or TDPG pyrophosphorylase activities could be found in either the supernatant solution after centrifuging it at 30,000*g* or in the partially purified fraction. High concentrations of pyrophosphate was shown to inhibit the rate of formation of α-D-glucose-1-phosphate from UDPG. This inhibition was not observed when ADPG was added to the reaction medium. Furthermore, ADPG increased the apparent activity of the enzyme at low pyrophosphate concentrations. It was shown that this activation was not the result of activation of an ADPG pyrophosphorylase by UDPG. Other adenine nucleotides (ATP, ADP and 5'-AMP) had no effect on the partially purified pyrophosphorylase. Although GDPG increased the rate of α-D-glucose-1-phosphate formation in the crude extract, it had no effect on the partially purified enzyme preparation.

INTRODUCTION

THE enzymic synthesis of uridine diphosphate-5'-glucose (UDPG) from uridine triphosphate and α-D-glc-1-P was first reported by Munch-Peterson *et al.*[1] Since that time UDPG pyrophosphorylase (UTP: α-D-glucose-1-phosphate uridylyl-transferase, EC., 2.7.7.9) has been observed in extracts of tissues from many species in both the plant and animal kingdoms. In addition, similar reactions between α-D-glc-1-P and the appropriate ribonucleoside triphosphate have been shown to lead to the formation of the following ribonucleoside diphosphate glucose compounds: ADPG,[2] GDPG,[3] TDPG,[4, 5] CDPG[6] and IDPG.[7] Furthermore, some tissues contain more than one nucleoside diphosphate glucose pyrophosphorylase. For instance, it has been demonstrated that extracts of mammary tissue catalyze the formation of UDPG, GDPG,[8] CDPG, TDPG, ADPG[9] and IDPG.[7]

* This investigation was supported by Research Grant GB 1439 from the National Science Foundation and by a research grant from the Menshon Committee on Education in National Security at the Ohio State University, Columbus, Ohio.

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Abbreviations used: UDPG, UDP-glucose; ADPG, ADP-glucose; GDPG, GDP-glucose; TDPG TDP-glucose; CDPG, CDP-glucose; IDPG, IDP-glucose; GDPM, GDP-mannose; GTP, guanosine-5'-triphosphate; ATP, adenosine-5'-triphosphate; ADP, adenosine-5'-diphosphate; 5'-AMP, adenosine-5'-phosphate.

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There is evidence suggesting that the various nucleoside diphosphate glucosyl transferases show specificity toward the glucosyl donor. The labeling data of Kauss and Kandler[10] indicate that ADPG is involved in starch synthesis and that UDPG is concerned with the synthesis of sucrose. However Leloir[11] suggests that both UDPG and ADPG may serve as glucosyl donors in the synthesis of starch in corn even though ADPG appears to be a more effective donor than UDPG *in vitro*. This suggestion is based upon the fact that the UDPG concentration is much greater than ADPG therefore allowing the rate of transfer of glucose from UDPG to be about the same as the transfer from ADPG.

In a recent report by Elbein *et al.*,[12] GDPG was shown to be the glucosyl donor in the biosynthesis of cellulose in extracts of mung beans, while UDPG, TDPG, ADPG, CDPG, α-D-glc-1-P and D-glucose would not serve as glucosyl donors.

The control mechanisms underlying the biogenesis of the various glucose polymers have not been extensively studied. Murphy and Wyatt[13] have shown that in the fat body of the moth glycogen synthetase and trehalose-6-P synthetase (EC., 2.4.1.15) compete for UDPG until the latter enzyme is subject to feedback inhibition by accumulated trehalose. The observations[9] that GDPG pyrophosphorylase is inhibited by mannose-1-P and GDPM, and that IDPG pyrophosphorylase is inhibited by GTP and GDPM may be interpreted in terms of regulation of nucleoside diphosphate hexose pyrophosphorylase activity by intermediate metabolites. Bernstein and Robbins[14] have shown in *E. coli* that UDPG competitively inhibits dTDPG pyrophosphorylase and that UDPG pyrophosphorylase is strongly inhibited by both dTDPG and dTDP-L-rhamnose. Melo and Glaser[15] have obtained a dTDPG pyrophosphorylase from *Pseudomonas aeruginosa* and have shown that it is subject to feedback inhibition by dTDP-L-rhamnose.

The present communication describes the influence of ADPG, GDPG and TDPG upon the activity of UDPG pyrophosphorylase obtained from *Sorghum vulgare* seedlings.

RESULTS AND DISCUSSION

Table 1 shows the influence of ADPG, GDPG and TDPG upon the velocity of the UDPG pyrophosphorylase reaction. This table shows that neither the crude nor partially purified enzyme preparation caused the release of glc-1-P in the presence of ADPG, GDPG or TDPG unless UDPG was also present. Furthermore, the rate of change in absorbance increased two- to three-fold when ADPG was added to the reaction medium along with UDPG when either the crude or partially purified enzyme preparations were used. However, the increased in rate of the reaction caused by the addition of GDPG to the system was observed only with the crude enzyme preparation. The small increase in rate in the presence of TDPG was only observed with the partially purified enzyme. These data do not establish whether the increased rate of reaction was due to α-D-glc-1-P derived from only UDPG, from ADPG as well as UDPG or less likely, but nevertheless, possible, activation of a NADP linked dehydrogenase in which UDPG or ADPG acted as a substrate. Phosphoglucomutase was required in order to obtain reaction which eliminated an ADPG activated UDPG dehydrogenase.

Experiments were designed to determine the specificity of the partially purified enzyme for

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ADPG and other adenine nucleotides. Table 2 shows that ATP, ADP and 5′-AMP had no influence upon the velocity of the reaction whereas ADPG increased the velocity more than three-fold with this preparation. Other nucleotide mono, di- and triphosphates tested had no effect upon the velocity of the reaction.

TABLE 1. INFLUENCE OF ADPG, GDPG AND TDPG UPON THE VELOCITY OF THE UDPG PYROPHOSPHORYLASE REACTION

Nucleoside diphosphate glucose	Δ Absorbance/min*	
	Crude enzyme	Partially purified enzyme
UDPG	0·25	0·25
ADPG	0·00	0·00
GDPG	0·00	0·00
TDPG	0·00	0·00
UDPG and ADPG	0·76	0·65
UDPG and GDPG	0·47	0·26
UDPG and TDPG	0·21	0·35

* The enzyme preparations were incubated at room temperature (25°) with the following components: nucleoside-5′-diphosphate glucose, 5 μmoles; inorganic pyrophosphate, 1 μmole; $MgSO_4$, 20 μmoles; 2,6-dichlorophenol indophenol, 0·1 μmole; phenazine methosulfate, 0·3 μmole; NADP, 0·5 μmole; tris-HCl buffer, pH 7·5, 100 μmoles; glucose-6-phosphate dehydrogenase, 1 international unit, phosphoglucomutase, 1 international unit; all in a total volume of 3·0 ml.

The velocity of the reaction was estimated by measuring the linear decrease in absorbance at 600 mμ.

TABLE 2. INFLUENCE OF ADENINE NUCLEOTIDES UPON THE VELOCITY OF THE UDPG PYROPHOSPHORYLASE CATALYZED REACTION

Adenine nucleotide added	Δ Absorbance/min*
None	0·14
ADPG	0·56
ADP	0·16
ATP	0·14
AMP	0·15

* Each reaction mixture contained 5 μmoles of UDPG and 5 μmoles of the indicated adenine nucleotide. Other reaction conditions were the same as those described in Table 1.

Further investigations of this activating phenomenon by ADPG have revealed that it is dependent upon the concentration of inorganic pyrophosphate present in the reaction medium. Figure 1 shows that inorganic pyrophosphate acted as a substrate inhibitor at relatively high concentrations. The addition of ADPG to the reaction medium appeared to remove the pyrophosphate inhibition and in addition also increased the velocity of the reaction at very low concentrations of pyrophosphate. Little activation by ADPG was observed over a

range of pyrophosphate concentrations of 0·2 to 0·7 μmole per 3·0 ml. Thus it appears that ADPG extends the range of zero order kinetics with respect to pyrophosphate. Similar investigations with GDPG and the partially purified enzyme failed to show any effect by this nucleotide at any concentration of inorganic pyrophosphate.

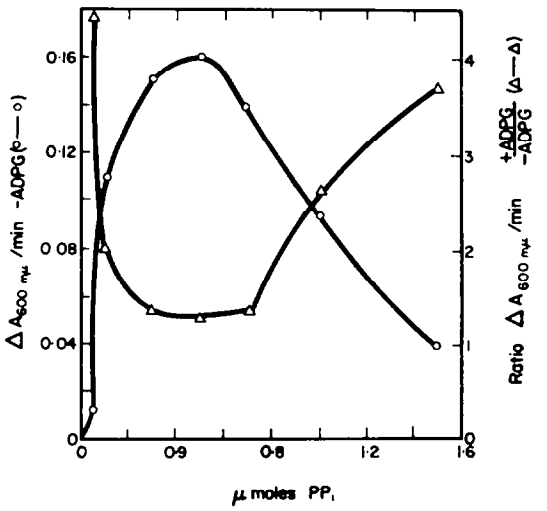

FIG. 1. INFLUENCE OF ADPG UPON PYROPHOSPHATE INHIBITION OF UDPG PYROSPHOSPHORYLASE

The reaction conditions are as described in Tables 1 and 2 except the concentration of inorganic pyrophosphate is varied as shown on the abscissa. The influence of inorganic pyrophosphate concentration upon the velocity of the reaction in the absence of ADPG.

Δ—Δ The influence of pyrophosphate concentration upon ADPG activation of UDPG pyrophosphorylase and is expressed as the ratio of velocities (+ADPG/−ADPG).

TABLE 3. INFLUENCE OF ORTHO PHOSPHATE UPON ADP-GLUCOSE ACTIVATION OF UDP-GLUCOSE PYROPHOSPHORYLASE

PPi μmoles/3 ml	Ratio of UDPG-Pyrophosphorylase activity*			
	(+ADPG/−ADPG)		(+$HOPO_3^-$/−$HOPO_3^-$)	
	−$HOPO_3^-$	+$HOPO_3^-$	−ADPG	+ADPG
1·5	3·7	1·0	2·4	0·65
1·0	2·6	1·6	1·0	0·64
0·7	1·3	1·5	0·70	0·79
0·5	1·7	1·3	0·92	0·73
0·3	1·4	1·3	0·40	0·50
0·1	2·0	1·7	0·60	0·55
0·05	11·0	1·6	0·60	0·50

* Reactions were carried out with various inorganic pyrophosphate concentrations as indicated. When ADPG and/or ortho phosphate were present in the reaction medium 5 μmoles of each was added.

The values obtained show the influence of orthophosphate upon ADPG activation (columns 1 and 2) and the influence of ADPG upon the orthophosphate effect (columns 3 and 4).

It was also found that inorganic phosphate would remove a large portion of the ADPG activating effect. Table 3 (columns 1 and 2) shows the results of experiments in which the effect of inorganic phosphate upon the ratios of rates of reduction of the indicator dye in the presence and absence of ADPG was measured. Column 3 shows that the ratio of activities obtained in the presence of phosphate divided by that in the absence of phosphate was dependent upon pyrophosphate concentration. At high concentrations of pyrophosphate, inorganic phosphate activated the reaction while at low concentrations it inhibited the reaction. Column 4 shows that when ADPG was in the reaction mixture inorganic phosphate inhibited the reaction. The data presented in Fig. 1 and Table 3 favor a hypothesis in which ADPG, inorganic phosphate and inorganic pyrophosphate are modifiers of the reaction by binding at a common non-catalytic site on the enzyme.

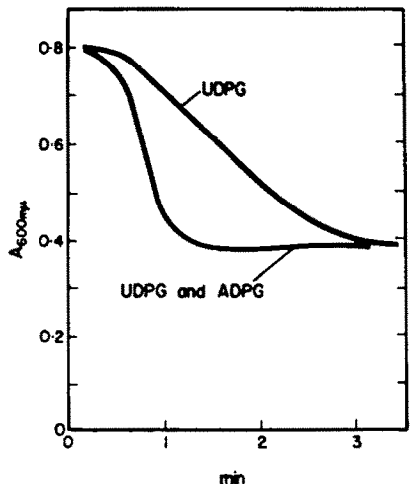

FIG. 2. THE LACK OF INFLUENCE OF ADPG UPON THE EXTENT OF THE UDPG PYROPHOSPHORYLASE REACTION.

The reaction conditions are the same as those described for Tables 1 and 2 except the reaction mixture contains 0·05 μmole of UDPG. The lower line shows the change in absorbance with time when the reaction mixture contains ADPG as compared (upper line) to the rate of change in absorbance in the absence of ADPG.

Figure 2 shows that the extent of 2,6-dichlorophenol indophenol reduction was identical when the reaction medium contained either 0·05 μmole of UDPG and 1 μmole of ADPG or only 0·05 μmole of UDPG. The presence of ADPG increased the velocity about three-fold. Thus UDPG appears to be the source of α-D-glc-1-P.

The observation that GDPG has an activating effect only upon the crude enzyme preparation (Table 1) is of interest in considering the possibility of the existence of more than one UDPG pyrophosphorylase. Furthermore, aging of the crude enzyme preparation resulted in a rapid loss in UDPG pyrophosphorylase over a five hour period. However, the ratio of activity in the presence and absence of ADPG or GDPG remained essentially constant under defined concentrations of the nucleoside-5′-diphosphate sugars and inorganic pyrophosphate. Some evidence was obtained that indicated the extent of activation by ADPG varies with the age of the tissue.

At present one can only speculate as to the physiological importance of the modification by ADPG. It will be important to determine whether the velocity of formation of UDPG

from UTP and α-D-glc-1-P is modified in a similar manner by ADPG or other nucleotides. The hypothesis that an increase in ADPG concentration increases the UDPG pyrophosphorylase activity thus diverting a relatively larger portion of the α-D-glc-1-P pool toward UDPG is an attractive one and is in general agreement with observations in other systems in which metabolites have been shown to be feed-back modifiers.

The data presented in this paper may be interpreted as providing evidence for regulation of UDPG pyrophosphorylase by other nucleoside-5′-diphosphate glucose compounds.

EXPERIMENTAL

Investigations were carried out with a crude enzyme preparation and a partially purified enzyme which was obtained by extracting the shoots from 3-day-old etiolated sorghum seedlings with 0·02 M, pH 7·5 tris(hydroxymethylamino)methane–HCl buffer (3 ml/g). In typical experiments, 30 g of tissue were macerated with buffer in a mortar with washed sand followed by subjecting the macerate to sonication for 5 min in a 20 kc/s sonic oscillator. The temperature was held below 5° during preparation of the enzyme. The extracts were filtered through cheese cloth and were centrifuged at 30,000*g* for 10 min. The supernatant solution was removed and used as the crude enzyme preparation. The partially purified enzyme was obtained by concentrating the supernatant solution (crude enzyme preparation) to about one-third its volume with Sephadex G-75 and the gel was sedimented by centrifugation. The supernatant solution was passed onto a 1 × 15 cm DEAE-cellulose column and the column was eluted with 100 ml of 0·02 M tris–HCl–0·05 M KCl pH 7·5 buffer and the eluate discarded. The column was then eluted with 0·02 M tris–HCl–0·10 M KCl pH 7·5 buffer and the eluate was collected in a series of 2 ml fractions. The activity was obtained in tubes 9 through 13. These fractions were pooled and used as the partially purified enzyme. No estimate of the homogeneity of the enzyme has been obtained due to interference by phenolic compounds with the Folin-Ciocalteau reagent.

In experiments reported here, unless otherwise indicated enzyme assays were carried out as follows: 5 μmoles of UDPG were incubated with 1 μmole of inorganic pyrophosphate, 20 μmoles of $MgSO_4$, 0·1 μmole of 2,6-dichlorophenol indophenol, 0·3 μmole of phenazine methosulfate, 0·5 μmole of NADP, 100 μmoles of tris–HCl pH 7·5 buffer and 1 international unit of phosphoglucomutase and glucose-6-phosphate dehydrogenase; all in a volume of 3·0 ml. Sufficient enzyme was added to give a change of absorbance per min at 600 mμ of between 0·2 to 0·8. Phosphoglucomutase and glucose-6-P dehydrogenase activities were such as to cause complete reduction of the 2,6-dichlorophenol indophenol in about 10 sec in the presence of 0·1 μmole of a α-D-glc-1-P. This assay was used because of interference at 340 mμ by phenolic substances present in sorghum extracts and because of the increased sensitivity of the assay.

When the influence of ADPG, GDPG, TDPG or other nucleotides was measured, the apparent velocity of the UDPG pyrophosphorylase reaction was first measured for 1 to 2 min before addition of 1 μmole of the second nucleotide to the reaction mixture. Control experiments established that the sequence of addition had little effect upon the velocity of the reaction and that the velocity was uniform over approximately 80 per cent of the range of absorbancies recorded.

UDPG, ADPG, GDPG and TDPG were obtained commercially from California Corporation for Biochemical Research.

Acknowledgement—The excellent technical assistance of Miss Sharon Walters is gratefully acknowledged.