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# SUGAR NUCLEOTIDE INHIBITION OF UDP-GLUCOSE PYROPHOSPHORYLASE FROM CALF LIVER

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## SUMMARY

UDPglucose pyrophosphorylase (UTP:α-D-glucose-1-phosphate uridylyltransferase, EC 2.7.7.9) was partially purified from calf liver. Kinetic properties and inhibition of the enzyme by UDP sugars were determined with an isotopic assay in which the product, UDP-D-[$^{14}$C]glucose, was collected and measured on DEAE-cellulose discs. The Michaelis–Menten constants for the substrates UTP and glucose-1-*P* were 0.1 mM and 0.03 mM, respectively. With glucose-1-*P* at 0.04 mM and UTP at 0.2 mM several UDP sugars inhibited the enzyme. The compounds and percent inhibitions at the concentrations indicated were 0.08 mM UDP-D-glucose (87%), 0.45 mM UDP-D-glucuronic acid (56%), 0.43 mM UDP-D-galacturonic acid (48%), 0.36 mM UDP-D-xylose (32%), 0.45 mM UDP-D-mannose (23%), and 0.35 mM UDP-D-galactose (14%). UDP-D-glucose gave the lowest $K_i$ value (0.005 mM), followed by UDP-D-glucuronic acid ($K_i$ = 0.15 mM), UDP-D-galacturonic acid ($K_i$ = 0.21 mM), and UDP-D-xylose ($K_i$ = 0.24 mM). These four compounds exhibited competitive inhibition with respect to UTP and noncompetitive inhibition with respect to glucose-1-*P*. One or more of these sugar nucleotides may regulate the UDPglucose pyrophosphorylase reaction *in vivo*.

## INTRODUCTION

The enzyme UDPglucose pyrophosphorylase (UTP:α-D-glucose-1-phosphate uridylyltransferase, EC 2.7.7.9) which catalyzes the formation of UDP-D-glucose from UTP and glucose-1-*P* is widespread in nature. Recent *in vitro* studies of the enzyme from pollen of *Lilium longiflorum* have demonstrated sugar nucleotide inhibition of enzyme activity when assayed isotopically in the direction of UDPglucose synthesis[1]. The most potent sugar nucleotide tested was the product itself, UDP-

Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2′-ethanesulfonic acid; Bicine, *N*,*N*′-bis-(2-hydroxyethyl)glycine.

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D-glucose ($K_i$ = 0.13 mM), followed by UDP-D-glucuronic acid ($K_i$ = 0.75), UDP-D-galacturonic acid ($K_i$ = 0.93), UDP-D-xylose ($K_i$ = 1.60), UDP-D-galactose ($K_i$ = 4.80), and UDP-D-mannose ($K_i$ = 9.60). All of these sugar nucleotides showed mixed competitive–noncompetitive inhibition with respect to the substrate UTP and purely noncompetitive inhibition with respect to the substrate glucose-1-*P*. Although UDP-D-glucose has been shown to be a potent inhibitor of UDPglucose pyrophosphorylase from animal tissues[2,3], to our knowledge there are no reports regarding possible inhibition of the animal enzyme by the other sugar nucleotides which inhibit the pollen enzyme.

The present work was undertaken in order to determine whether the enzyme from an animal source is also inhibited *in vitro* by the same sugar nucleotides showing inhibition of the pollen enzyme, and to characterize the mode of inhibition if inhibition is observed. The enzyme in animal tissues is of paramount importance since UDP-D-glucose-linked pathways are involved in the synthesis of glucuronides[4], glycogen[5], glycoproteins[6] and glycolipids[7]. UDP-D-glucose itself may be used for some syntheses such as glycogen, or it may be converted to other sugar nucleotides which then participate in synthetic reactions. It is conceivable, therefore, that the UDP-glucose pyrophosphorylase reaction in these tissues may be subject to regulation by product inhibition[2,3] or feedback inhibition *via* the other sugar nucleotides.

Liver tissue was chosen as the source of the UDPglucose pyrophosphorylase in this study since in this tissue UDP-D-glucose is the starting point for not only synthesis of glycogen, but for synthesis of many plasma glycoproteins (UDP-D-galactose is one substrate[8]) and glucuronides (liver detoxification pathways utilize UDP-D-glucuronic acid[4]) as well. Furthermore, much information is already available regarding the physical and chemical characteristics of the liver enzyme and its catalytic mechanism[9–12].

## MATERIALS AND METHODS

### *Reagents*

The enzymes, chemicals, and all other materials were obtained from commercial suppliers.

### *Analytical procedures*

All spectrophotometric measurements were made with a Zeiss PMQ II spectrophotometer equipped with a Sargent Model SRL recorder.

The concentrations of glucose-1-P, UTP, and UDP-D-glucose were determined by standard enzyme-linked spectrophotometric assays[13]. The concentrations of the sugar nucleotides other than UDPglucose tested as inhibitors were determined by absorbance at 262 nm.

Sugars and sugar acids on paper chromatograms and electrophoretograms were detected with Tollen's reagent spray (0.1 M $AgNO_3$–5 M $NH_4OH$–2 M NaOH; 1:1:2, by vol.) and *p*-anisidinephthalate spray (J. T. Baker Co.); sugar nucleotide standards were detected by fluorescence under ultraviolet light; and sugar phosphate standards were detected by ammonium molybdate reagent spray[14,15].

Protein was measured by the method of Lowry *et al.*[16] using crystalline bovine serum albumin as a standard.

The UDPglucose pyrophosphorylase activity was assayed in the direction of UDP-D-glucose synthesis using α-D-[U-$^{14}$C]glucose-1-*P* as the substrate. The details and validity of the assay have been described elsewhere[1]. Briefly, the reaction mixture contained HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2'-ethanesulfonic acid) buffer (pH 7.5), 100 mM; $MgCl_2$, 5 mM; crystalline bovine serum albumin, 20 μg; α-D-[$^{14}$C]glucose-1-*P*, spec. act. 2000 cpm/nmole; UTP; inorganic pyrophosphatase (Worthington Biochem. Co.), 0.8 μg (0.67 unit); and 2–10 μl of partially purified calf liver UDPglucose pyrophosphorylase in 0.1 ml total volume in 10 mm × 75 mm glass test tubes. The concentrations of UTP and glucose-1-*P* are given with the results of each experiment. The reaction was initiated by adding UDP-glucose pyrophosphorylase, incubated for 5 min at 30 °C in a shaking water bath, and terminated by placing in boiling water for 60 s. *Escherichia coli* alkaline phosphatase (Worthington Biochem. Co.), 10 μg (0.2 unit), was added to the cooled reaction mixtures which were then incubated 60 min at 37 °C for conversion of unreacted D-[$^{14}$C]glucose-1-*P* to D-[$^{14}$C]glucose. After alkaline phosphatase treatment a 25-μl aliquot was pipetted onto a 2.5-cm diameter DEAE-cellulose paper disc (Reeve Angel Co.). Each assay disc, together with 2 additional discs underneath, was placed over one of fifteen 2.3 cm diameter holes cut into a water-impervious plastic adapter fitted on top of the base of a 3000-ml fritted glass pyrex filter. Fifteen assay discs were washed simultaneously, each with 200 ml of pH 6.5 glass-distilled water to remove the D-[$^{14}$C]-glucose. A flow rate of 50 ml/min per disc was maintained with a partial vacuum. The discs were subsequently dried at 65 °C for 30 min in an air flow oven, placed in scintillation vials (the assay disc on top, the additional discs underneath) with 10 ml toluene containing 0.4% (w/v) PPO and 0.04% POPOP, and counted at 64% efficiency in a Packard Tri-Carb Model 3375 liquid scintillation counter, Retention of UDP-D-[$^{14}$C]glucose was 90–93%. Product formed was proportional to time over the 5-min incubation period and was proportional (within 15%) to volume of enzyme over the range used.

Contaminating enzyme activities in the partially purified UDPglucose pyrophosphorylase were measured as follows. Breakdown of UTP and conversion of α-D-glucose-1-*P* to D-glucose-6-*P* were tested spectrophotometrically[13]. Breakdown products of labeled compounds possibly resulting from phosphatase or phosphodiesterase activity were also assayed by descending ion exchange chromatography on 45-mm strips of DEAE-cellulose paper (DE81) with water or 0.05 M or 0.1 M LiCl. Epimerization or hydrolysis of UDP sugars and UDP sugar acids was tested for using standard paper chromatographic and electrophoretic procedures. Conversion of UDP-D-[$^{14}$C]-glucose to UDP-D-[$^{14}$C]galactose or UDP-D-[$^{14}$C]glucuronate, conversion of UDP-D-[$^{14}$C]glucuronate to UDP-D-[$^{14}$C]xylose or UDP-D-[$^{14}$C]galacturonate, conversion of UDP-D-[$^{14}$C]xylose to UDP-L-[$^{14}$C]arabinose were tested for by incubating the labeled compounds with 10-fold excess (over that used in kinetic experiments) of UDPglucose pyrophosphorylase preparation for 30 min at 30 °C in simulated reaction mixtures lacking D-[$^{14}$C]glucose-1-*P*. The UDP-bound sugars and sugar acids were hydrolyzed to free sugars or sugar acids with phosphodiesterase (Sigma) and alkaline phosphatase[17], and the digests were subjected to paper chromatography or paper electrophoresis. Sugars were readily separated from sugar acids by ascending chromatography on 21-cm DEAE-cellulose strips developed with water. Descending chromatography on 55 cm Whatman No. 1 strips in ethyl acetate–pyridine–water (8:2:1, by

vol.) gave good separations among xylose, glucose ($R_{xylose}$ = 0.59), galactose ($R_{xylose}$ = 0.49), arabinose ($R_{xylose}$ = 0.81), and mannose ($R_{xylose}$ = 0.71). 8 h horizontal electrophoresis (7 V/cm) on Whatman 3MM paper in 20 mM sodium borate–boric acid buffer (pH 8.6) at 4 °C separated glucuronic acid from galacturonic acid[18]. For the detection of new compounds and breakdown products, all paper strips were scanned with a Packard Model 7201 radiochromatogram scanner, and mobilities of radioactive compounds were compared to mobilities of standards. The paper strips were then cut into sections corresponding to the location of radioactive peaks. These sections were placed in 10 ml of the PPO–POPOP–toluene scintillant, and the radioactivity was determined.

## RESULTS

### *Extraction and purification of UDPglucose pyrophosphorylase*

The procedure outlined below was based in part on the procedure published by Albrecht *et al.*[9]. All steps were carried out at 2–4 °C.

*Step 1: Extraction.* Male calf liver was the source of the enzyme. It was placed on ice immediately after slaughter, frozen at −20 °C 30 min later, and stored in the freezer for 4 weeks. A portion of the frozen calf liver (130 g) was chopped into pieces about 1 cm thick and homogenized in 150 ml of 0.03 M KOH containing 0.005 M EDTA and 0.005 M mercaptoethanol. Homogenization was done intermittently during six 20-s intervals in a Waring blender. Another 150 ml of the extraction medium was added to the homogenate and the total homogenate was stirred for 5 min. The homogenate was then strained through glass wool and centrifuged for 30 min at 25 000 × *g*. The resulting solution constituted Fraction I.

*Step 2: Protamine sulfate precipitation.* To Fraction I, 100 ml of extraction medium containing 400 mg of protamine sulfate (Elanco) was added slowly with stirring. After 20 min the suspension was centrifuged at 25 000 × *g* for 30 min. The supernatant fluid constituted Fraction II.

*Step 3: First $(NH_4)_2SO_4$ fractionation.* Fraction II was brought to 40% saturation with the addition of a saturated solution of $(NH_4)_2SO_4$ at pH 7.2. The solution was stirred for 20 min and then centrifuged at 25 000 × *g* for 30 min. The supernatant fluid was brought to 60% saturation with $(NH_4)_2SO_4$, stirred for 20 min, and centrifuged as before. The precipitate was dissolved in a total volume of 22 ml of 0.01 M Bicine [*N*,*N*′-bis-(2-hydroxyethyl)glycine] buffer, pH 8.5, 0.005 M mercaptoethanol, and 0.001 M EDTA and dialyzed for 6 h against 2 × 4 l of the same buffer. The dialyzed fluid was Fraction III.

*Step 4: First DEAE-cellulose column chromatography.* A portion, 5 ml, of Fraction III was applied to a 0.9 cm × 28 cm DEAE-cellulose (Whatman DE 52) column previously equilibrated with 0.01 M Bicine buffer, pH 8.5. After sample application the column was washed overnight with the same buffer at a flow rate of 18 ml/h. Elution of the enzyme from the column was accomplished with a linear gradient of NaCl generated at the same flow rate. Fractions of 5 ml were collected. The mixing chamber contained 100 ml of 0.01 M Bicine buffer, pH 8.5, and the reservoir chamber contained 100 ml of 0.01 M Bicine buffer, pH 8.5, 0.2 M in NaCl. Protein was monitored by its absorbance at 280 nm. Those fractions having the highest specific activity were pooled (Fraction IV).

*Step 5: Second $(NH_4)_2SO_4$ fractionation.* The protein in Fraction IV precipitating between 40 and 60% saturation was recovered as described in Step 3 except that a saturated solution of $(NH_4)_2SO_4$ adjusted to pH 8.5 was used. The recovered protein precipitate (Fraction V) was dissolved in 3.0 ml of 0.01 M HEPES buffer, pH 7.5, 0.001 M EDTA and 0.005 M mercaptoethanol and dialyzed for 6 h against 2 × 4 l of the same buffer.

*Step 6: Second DEAE-cellulose column chromatography.* The dialyzed Fraction V was applied to a 0.9 cm × 25 cm DEAE-cellulose (Whatman DE 52) column previously equilibrated with 0.01 M HEPES buffer, pH 7.5. After sample application the column was washed overnight with the same buffer at a flow rate of 30 ml/h. Elution of the enzyme was accomplished with a 200-ml linear gradient of from 0.01 to 0.2 M NaCl in 0.01 M HEPES buffer, pH 7.5. The flow rate during elution was 30 ml/h, with 5-ml fractions being collected. Protein was monitored by its absorbance at 280 nm. Again, those fractions having the highest specific activity were pooled (Fraction VI).

*Step 7: Third $(NH_4)_2SO_4$ fractionation.* Fraction VI was brought to 60% saturation with the addition of a saturated solution of $(NH_4)_2SO_4$ at pH 7.5. The solution was kept at 2–4 °C for 3 h prior to centrifugation. The 15 000 × *g* pellet was dissolved in 8 ml of 0.01 M HEPES buffer, pH 7.5, 0.01 M merceptoethanol. The enzyme was purified 25-fold overall, and the yield was 6% (Table I).

TABLE I

PURIFICATION OF UDP-GLUCOSE PYROPHOSPHORYLASE FROM CALF LIVER

| *Purification step* | *Volume (ml)* | *Total protein (mg)* | *Total activity (units)** | *Specific activity (units/mg)* |
|---|---|---|---|---|
| 1. Crude extract | 195 | 10 140 | 3 699.5 | 0.36 |
| 2. Protamine sulfate | 225** | 9 000 | 3 413.8 | 0.38 |
| 3. First $(NH_4)_2SO_4$, 40–60% | 22 | 4 452.8 | 3 122.7 | 0.70 |
| 4. First DEAE-cellulose, pH 8.5 | 16.6 | 982.7 | 2 091.0 | 2.13 |
| 5. Second $(NH_4)_2SO_4$, 40–60% | 13.2 | 844.8 | 1 605.6 | 1.90 |
| 6. Second DEAE-cellulose, pH 7.5 | 52.9 | 67.4 | 324.7 | 4.82 |
| 7. Third $(NH_4)_2SO_4$, 60% | 8 | 24.1 | 215.9 | 8.95 |

* A unit of enzyme activity specifies that amount catalyzing the formation of 1 μmole of UDPglucose per min at 30 °C.

** This and all subsequent values have been normalized to the total volume in each previous step of which only a portion was actually taken for subsequent steps.

*Properties of partially purified UDPglucose pyrophosphorylase*

The partially purified UDPglucose pyrophosphorylase was essentially free of enzymes catalyzing the transformation of UTP, α-D-[$^{14}$C]glucose-1-*P*, UDP-D-[$^{14}$C]-glucose, UDP-D-[$^{14}$C]xylose, or UDP-D-[$^{14}$C]glucuronate (see Materials and Methods). These activities would include UDPglucose 4'-epimerase, UDParabinose 4'-epimerase, UDPglucose dehydrogenase, UDPglururonic acid decarboxylase, UDPglucuronic acid 4'-epimerase, phosphatase, and phosphodiesterase.

*Product identification.* The $^{14}$C-labeled product synthesized by the enzyme was identified as UDPglucose in the following ways. A reaction mixture containing α-D-

[$^{14}$C]glucose-1-*P* and UTP was incubated with the 25-fold purified enzyme for 10 min at 30 °C, heat treated, and digested with alkaline phosphatase. A reaction mixture containing no UTP was run as a control. A portion (0.025 ml) of each of the digested reaction mixtures was spotted on 55-cm Whatman 3MM strips. Some strips were equilibrated for 5 h with ethanol–water (5:2, v/v)[19] and developed for 32 h with 95% ethanol–1.0 M ammonium acetate (5:2, v/v), pH 7.5, (Solvent A) in the descending direction[20]. Other strips were developed for 55 h with 1,2-dimethoxyethane–methyl ketone–0.5 M morpholinium tetraborate pH 8.6, in 0.01 M EDTA (7:2:3, by vol.), (Solvent B). The dried chromatograms were scanned with the Packard Model 7201 scanner and with ultraviolet light for location of $^{14}$C-labeled compounds and for uridine-containing compounds, respectively. The $^{14}$C-labeled product synthesized in the complete reaction mixture cochromatographed with authentic UDPglucose with both Solvents A and B. With Solvent B the $R_{\text{UDPglucose}}$ values of various UDP-sugars were UDP-D-glucuronic acid, 0.67; UDP-D-galacturonic acid, 0.40; UDP-D-galactose, 0.63; UDP-D-mannose, 0.70; and UDP-D-xylose, 1.1. Similarly, when aliquots of the complete and control reaction mixtures were spotted onto DEAE-cellulose strips and developed, descending, with 50 mM LiCl solution, the newly appearing alkaline phosphatase-resistant $^{14}$C-labeled compound in the complete reaction mixture cochromatographed with authentic UDP-D-[$^{14}$C]glucose.

*Inhibition of the enzyme-catalyzed synthesis of UDPglucose by UDP sugars and UDP sugar acids*

The following UDPsugars and their corresponding sugar-1-phosphates were tested at the indicated concentrations for inhibition of the partially purified calf liver UDPglucose pyrophosphorylase: UDP-D-glucose (0.085 mM), UDP-D-glucuronic acid (0.45 mM), UDP-D-galacturonic acid (0.43 mM), UDP-D-xylose (0.36 mM), UDP-D-mannose (0.45 mM), UDP-D-galactose (0.35 mM), α-D-galactose-1-*P*, α-D-mannose-1-*P*, α-D-xylose-1-*P*, and α-D-glucuronic acid-1-*P* (all sugar phosphates at 0.4 mM). With the substrates glucose-1-*P* at 0.037 mM and UTP at 0.195 mM the following per cent inhibitions were observed: UDP-D-glucose (87%), UDP-D-glucuronic acid (56%), UDP-D-galacturonic acid (48%), UDP-D-xylose (32%), UDP-D-mannose (23%), UDP-D-galactose (14%). The sugar 1-phosphates showed little or no inhibition.

*Kinetic parameters and inhibitor studies*

The four most effective inhibitors from the above survey were studied further to determine the type of inhibition with respect to each substrate. All the kinetic studies were conducted under conditions which gave a linear relationship between enzyme concentration and amount of UDP-D-glucose formed.

*The effect of UTP concentration.* Fig. 1 illustrates the dependence of rate of UDP-D-glucose synthesis on the concentration of UTP in the absence and the presence of various levels of three of the four most effective sugar nucleotide inhibitors. Results for UDP-D-galacturonic acid (not shown) closely resembled the data presented for UDP-D-glucuronic acid. In each case Lineweaver–Burk plots gave straight lines, and the average $K_m$ for UTP in the absence of inhibitor was 0.12 mM. Each of the four sugar nucleotides caused an increase in the $K_m$ for UTP without any effect on $V$, indicating competitive inhibition with respect to UTP. Dixon[21] plots of

$1/v$ *vs* inhibitor concentration with three different levels of each inhibitor at each of eight different levels of UTP (glucose-1-*P* at 0.19 mM) were used to estimate inhibitor constants ($K_i$). A representative example of the data is given for the inhibitor UDP-D-glucose (Fig. 2). The $K_i$ value graphically estimated for each inhibitor was: UDP-D-glucose, 0.005 mM; UDP-D-glucuronic acid, 0.15 mM; UDP-D-galacturonic acid, 0.21 mM; UDP-D-xylose, 0.24 mM.

*The effect of glucose-1-P concentration.* Fig. 3 illustrates the dependence of rate of UDPglucose synthesis on the concentration of glucose-1-*P* in the absence and

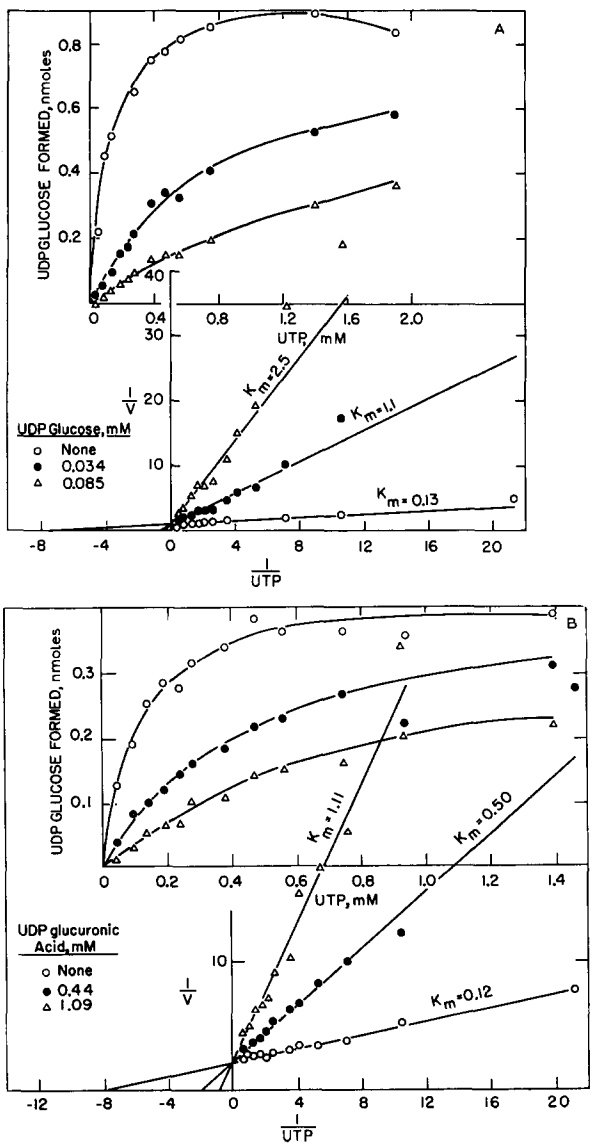


Fig. 1.

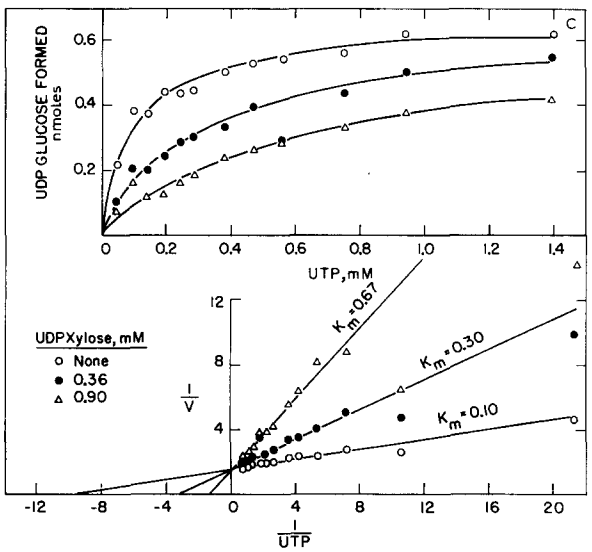


Fig. 1. Dependence of velocity of UDPglucose synthesis on UTP concentration in the absence and presence of various levels of several sugar nucleotide inhibitors. A. UDPglucose. B. UDPglucuronic acid. C. UDPxylose. The concentration of glucose-1-*P* was held constant at 0.19 mM. Assay conditions are given in Materials and Methods.

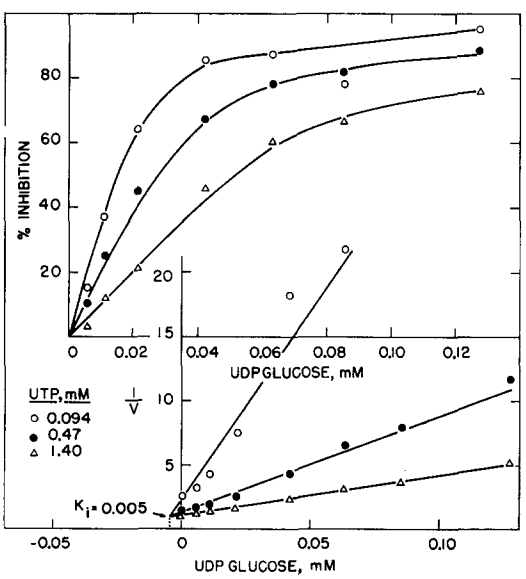


Fig. 2. Inhibitor saturation curves (above) and Dixon plot (below) for the inhibitor UDPglucose with UTP as the varied substrate. The concentration of glucose-1-*P* was held constant at 0.19 mM.

presence of various levels of the four UDP sugars and UDP sugar acids. Again, the data indicate a good fit to Michaelis–Menten kinetics. Lineweaver–Burk plots yielded estimates of $K_m$ equal to 0.029 mM glucose-1-*P* and $V$ equal to 0.67 with no inhibitor present. Each of the inhibitors caused a marked decrease in the $V$ without any

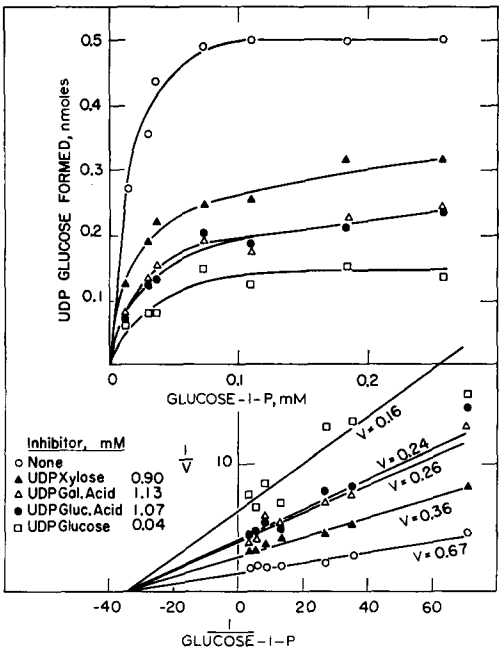


Fig. 3. Dependence of velocity of UDPglucose synthesis on glucose-1-*P* concentration in the absence and presence of the sugar nucleotides found to be inhibitory. The concentration of UTP was held constant at 0.59 mM.

apparent effect on the $K_m$, indicating noncompetitive inhibition with respect to the substrate glucose-1-*P*.

## DISCUSSION

The inhibition of the calf liver UDPglucose pyrophosphorylase by sugar nucleotides shows both similarities and differences when compared with the same enzyme isolated from lily pollen[1]. The order of effectiveness of the sugar nucleotides as inhibitors is identical for the liver and pollen enzymes: UDPglucose > UDPglucuronic acid > UDPgalacturonic acid > UDPxylose. However, for each of these sugar nucleotides a comparison of the $K_i$ values reveals a markedly greater sensitivity to inhibition by the liver enzyme[1]. This difference is most marked for UDPglucose which exhibits a $K_i$ 20-fold lower for the calf liver enzyme than for the pollen enzyme.

Another difference lies in the type of inhibition observed. The sugar nucleotides all gave competitive inhibition with respect to the substrate UTP when the liver enzyme was assayed, but mixed competitive–noncompetitive inhibition was observed with the pollen enzyme. In this respect the calf liver enzyme resembles the enzymes from rat liver[2], erythrocyte, heart, and mung bean[3].However, UDPglucosewas the only sugar nucleotide inhibitor tested with the enzyme from the latter four tissues. The sugar nucleotides all exhibited noncompetitive inhibition with respect to the substrate glucose-1-*P* (Fig. 3), and these results are the same as reported for the pollen enzyme[1]. An explanation for the competitive inhibition with respect to UTP is that the various UDP sugars bind to that part of the substrate site which is normally occupied by UTP.

The *in vivo* significance of the *in vitro* inhibitions depends on intracellular concentrations of the sugar nucleotides involved. The calf liver enzyme is inhibited by such a low UDPglucose concentration that product inhibition by UDPglucose seems a likely regulatory mechanism as suggested earlier for rat liver[2]. The liver UDPglucose content has been reported for a variety of animals[22,23] but apparently not for cow. The level of bovine liver UDPglucose may be relatively low because Pieck *et al.*[24] reported that total UDPhexose per cell in cow and calf liver was only 1/8 that found in rat liver. However, the $K_i$ for UDP glucose inhibition of the calf liver UDPglucose pyrophosphorylase is considerably lower than the $K_i$ for the rat liver enzyme[2] since the former is only 1/8 as large (Fig. 2, this communication). Thus, considerably different levels of UDPglucose in livers of the two species may cause similar degrees of inhibition to the UDP-glucose pyrophosphorylases involved. The physiological significance of inhibition by UDPglucuronic acid, UDPxylose, and UDPgalactose is questionable because these compounds were considerably less effective inhibitors than was UDPglucose, and the content of UDPglucuronate in liver is less than that of UDPglucose[22,25]. However, it is possible that several sugar nucleotides act additively to cause significant inhibition of UDPglucose pyrophosphorylase *in vivo*.

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