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# URIDINE DIPHOSPHATE GLUCOSE DEHYDROGENASE OF CALF LIVER

## PROPERTIES AND INHIBITION CHARACTERISTICS WITH URIDINE DIPHOSPHATE XYLOSE ANALOGUES*

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**Abstract**—UDP-glucose dehydrogenase of calf liver dissociated in guanidine-HCl into six subunits. The number of reactive sulfhydrylgroups of native and guanidine-HCl-treated enzyme was found to be 20 ± 1 and 46 ± 1, respectively, per mole of native enzyme. A crude preparation of UDP-glucose pyrophosphorylase from yeast was used for the preparation of 5-hydroxyuridine diphosphate xylose and 5-aminouridine diphosphate xylose. 5,6-Dihydrouridine diphosphate xylose was prepared by catalytic reduction of UDP-xylose. The nature of the inhibition produced by UDP-xylose or its analogues was similar with respect to either UDP-glucose or $NAD^+$, the two substrates for the enzyme. Hydrogenation of the 5,6-double bond or substitution of a hydroxyl group at the C-5 position of the pyrimidine portion of UDP-xylose decreased its inhibitory activity. Substitution of an amino group at the C-5 position, however, did not alter the activity of the allosteric inhibitor.

UDP-GLUCOSE dehydrogenase[1–4] for pea seedling, calf liver, chick cartilage and *Cryptococcus laurentii* is strongly inhibited by UDP-xylose.[4,5] Kinetic studies which indicate an allosteric modification of this enzyme by UDP-xylose suggest that the nucleotide sugar serves as a regulator of UDP-glucuronate synthesis.[4,5] These findings and prior interest in the mechanism of action of the dehydrogenase[6] led to the preparation of the analogues of UDP-xylose, $H_2$UDP-xylose†, HO-UDP-xylose and $H_2$N-UDP-xylose.† The kinetics of inhibition of the dehydrogenase using these analogues were compared with those of UDP-xylose.

## MATERIALS AND METHODS

*Materials.* Xylose 1-phosphate (di-monocyclohexylammonium salt) was purchased from Sigma Chemical Company. Phosphoenol pyruvate, phosphoenol pyruvate kinase, ATP and 6-azauridine 5′-diphosphate (calcium salt) were obtained from Calbiochem; UTP, $NAD^+$, UDP-glucose and UDP-xylose were from P.L. Biochemicals. A 5% rhodium on alumina catalyst was procured from Engelhard Industries, Inc.

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† Abbreviations used: $H_2$UDP, 5,6-dihydrouridine 5′-diphosphate; HO-UDP, 5-hydroxyuridine 5′-diphosphate; HO-UTP, 5-hydroxyuridine 5′-triphosphate; $H_2$N-UDP, 5-aminouridine 5′-diphosphate; $H_2$N-UTP, 5-aminouridine 5′-triphosphate.

(Newark, N.J.). Carboxymethylcellulose and hydroxylapatite were products of Bio-Rad Laboratories. Other reagents were obtained from standard commercial sources.

*Enzyme assay.* UDP-glucose dehydrogenase activity was measured spectrophotometrically by following the rate of $NAD^+$ reduction carried out in a 1-ml quartz cuvette in a Gilford multiple sample absorbance recorder. The standard assay mixture in a final volume of 1 ml contained $NAD^+$, 2·0 mM; UDP-glucose, 0·5 mM; tris-acetate buffer, pH 8·7, 50 mM; and a rate-limiting amount of enzyme protein. One unit[7] of enzyme is defined as the amount of protein necessary to cause an increase in absorbancy of 0·001 at 340 mμ/min at 26° with the standard assay mixture. The specific activity is expressed as units per milligram of protein. The specific activity of the enzyme used is indicated under each figure and table.

*Protein determination.* Protein content of the enzyme preparation was determined by the method of Lowry *et al.*,[8] using crystalline bovine serum albumin as the standard, except that protein content of column fractions was estimated at 280 mμ.

*Preparation of HO-UDP-xylose.* HO-UDP-xylose was prepared from HO-UTP[9] and xylose 1-phosphate using a crude enzyme preparation[10] from brewer's yeast. A 40-ml reaction mxiture contained $1 \times 10^{-3}$ M HO-UTP (sodium salt), $5 \times 10^{-3}$ M xylose 1-phosphate (di-monocyclohexylammonium salt), $3 \times 10^{-2}$ M tris-HCl (pH 7·0), $5 \times 10^{-3}$ M $MgCl_2$, $2{\cdot}5 \times 10^{-3}$ M cysteine, $5 \times 10^{-4}$ M ATP, $5 \times 10^{-3}$ M phosphoenol pyruvate, 300 units of phosphoenol pyruvate kinase, and an enzyme preparation from 2·5 g of dried yeast. After incubation at 37° for 60 min, the reactions were terminated by heating the incubation mixture at 100° for 2 min. The precipitates were removed by centrifugation and the supernatant solutions were placed on a column of DEAE-cellulose ($HCO_3^-$), 2 × 25 cm. Elution from the column was carried out at 4°, using a gradient of 0·15 M triethylammonium bicarbonate and 1 l. water in the mixer. The solution containing the first broad peak was lyophilized and separated from impurities by descending paper chromatography in ethanol–0·5 M ammonium acetate, pH 7·5 (5:2, v/v) on Whatman No. 3 MM filter paper. The product was located on the paper by its color reaction with $FeCl_3$.[11] The $R_f$ value was 0·76. After elution from the paper and lyophilization, the white amorphous powder was dissolved in a minimal volume of water and desalted on a column of Sephadex G-10. The solution containing HO-UDP-xylose was lyophilized and kept at −20° until used. The yield was 20 per cent (based on HO-UTP). An 85 per cent yield of UDP-xylose was obtained from UTP and xylose 1-phosphate under the same conditions, except that the pH of the reaction mixture was 8·0. Spectral characteristics of HO-UDP-xylose: $\lambda_{max}^{pH\,2}$, 283 mμ; $\lambda_{max}^{pH\,12}$, 304 mμ; $A_{280}:A_{260}$, 1·8 at pH 2 and 1·0 at pH 12. The molar ratio of total phosphorus to acid-stable phosphorus was 2. The xylose residue, released by hydrolysis for 20 min at 100° in 0·1 N HCl, was identified by paper chromatography in *n*-butanol–ethanol–water (2:1:1, v/v).

*Preparation of $H_2N$-UDP-xylose.* $H_2N$-UDP-xylose was prepared from $H_2N$-UTP[12] and xylose 1-phosphate using the crude enzyme preparation from yeast. The reaction conditions were the same as described for HO-UDP-xylose preparation, except that the product was eluted from a DEAE-cellulose column with 0·1 M triethylammonium bicarbonate, and located on paper by the color produced by *p*-(dimethylamino)-benzaldehyde.[13] The yield was about 1 per cent (based on $H_2N$-UTP). The molar ratio of total phosphorus to acid-stable phosphorus was 2, and the xylose residue was identified as described above.

*Preparation of $H_2$UDP-xylose.* $H_2$UDP-xylose was prepared by catalytic hydrogenation of UDP-xylose in aqueous hydrochloric acid (pH 3·8) at 0° in the presence of a 5% rhodium on alumina catalyst. The procedure is similar to that described for the preparation and purification of $H_2$UDP-glucose.[6,13] The molar ratio of total phosphorus to acid-stable phosphorus of the product was 2·0.

*Preparation of 6-azauridine 5'-triphosphate (6-aza-UTP).* A solution of 6-azauridine 5'-diphosphate (calcium salt) was converted to the sodium salt on a column of Dowex-50 ($Na^+$). The nucleoside diphosphate was phosphorylated to the triphosphate by nucleoside diphosphokinase from brewer's yeast.[14] The product was purified on a DEAE-cellulose ($HCO_3^-$) column using a triethylammonium bicarbonate gradient. The solution containing 6-aza-UTP was lyophilized and converted to its sodium salt on a Dowex-50 ($Na^+$) column. Attempts to convert 6-aza-UTP to azauridine diphosphate xylose were unsuccessful at various pH values (pH 6–8).

*Enzyme preparations.* Crude UDP-glucose pyrophosphorylase was prepared from dried brewer's yeast (Anhauser-Busch, Inc., Van Nuys, Calif.), as described by Rabinowitz and Goldberg.[10] The enzyme preparation showed UDP-xylose pyrophosphorylase activity and was used for the preparation of HO-UDP-xylose and $H_2$N-UDP-xylose. Nucleoside diphosphokinase was prepared from brewer's yeast.[14] UDP-glucose dehydrogenase was prepared according to the method of Wilson,[15] except that hydroxylapatite chromatography, as described below, was used in the final step of the purification instead of DEAE-cellulose chromatography.

A slurry of hydroxylapatite, which had been washed twice with an equal volume of 0·05 M EDTA, pH 6·0, was used to prepare the column (1·5 × 40 cm). The column was washed with 500 ml of 0·05 M potassium phosphate buffer, pH 7·0. Usually two or three enzyme preparations from the preceding purification procedure were combined (total $A_{280}$, about 80) and applied on the hydroxylapatite column. Unadsorbed protein was eluted with 60 ml of the potassium phosphate buffer. A linear gradient of potassium phosphate, pH 7·0, was passed through the column using 400 ml of 0·05 M phosphate buffer in the mixing chamber and 400 ml of 0·65 M phosphate buffer in the reservoir. Fractions (about 5 ml) with ratios (units to $A_{280}$) which were greater than 15,000 were combined and concentrated by ultrafiltration. Thc concentrated fraction was dialyzed against 1000 ml of 0·05 M potassium phosphate buffer, pH 7·0, for 3 hr with one change of buffer, and clarified by centrifugation.

*Disc electrophoresis.* Polyacrylamide disc electrophoresis was carried out by the method of Davis[16] as modified by Dietz and Lubrano.[17] The concentration of running gel was 5·5%, and both the spacer gel and sample gel were omitted. The protein samples (30–100 μg) in 10% sucrose were placed on top of the running gel just prior to electrophoresis. Electrophoresis was carried out at 2·5 mA per tube and at room temperature. When enzyme assays were desired, the electrophoresis was carried out at 4°. After electrophoresis, the gels were stained for protein with either Coomassie brilliant blue or Amido Black.[16,18] UDP-glucose dehydrogenase activity was located by[18] the phenazine methosulfate and nitro blue tetrazolium staining technique. The gel was covered with 3 ml of a mixture containing 1 mM UDP-glucose, 2 mM $NAD^+$, 10 mM NaCl, 0·5 mM $MgCl_2$, 0·025% nitro blue tetrazolium, 0·0025% phenazine methosulfate, and 0·125 M potassium phosphate buffer, pH 7·6, and was incubated at 37° for 1 hr.

*Determination of sulfhydryl group.* Sulfhydryl groups of UDP-glucose dehydrogenase were titrated with 5,5'-dithiobis(2-nitrobenzoic acid) as described by Wassarman and

Major.[19] The reaction was carried out in a cuvette, at a final volume of 1 ml, containing: potassium phosphate buffer, pH 8·0, 0·1 M; 5,5′-dithiobis(2-nitrobenzoic acid), 0·1 mM; and the UDP-glucose dehydrogenase (0·45 or 0·225 mg). The reaction was initiated by the addition of enzyme. The course of the reaction was followed at 412 mμ on a Gilford multiple sample absorbance recorder at room temperature. A cuvette containing the same phosphate buffer and 5,5′-dithiobis(2-nitrobenzoic acid) (plus guanidine hydrochloride when appropriate) was always included to serve as a blank. A molar extinction coefficient of 13,600 $M^{-1}$ $cm^{-1}$ for the reduction product of 5,5′-dithiobis(2-nitrobenzoic acid) was used.[20]

## RESULTS

*Physical properties of UDP-glucose dehydrogenase.* The preparation from hydroxylapatite chromatography had a specific activity of 20,300–23,000 units/mg and showed a single protein band in acrylamide gel electrophoresis at pH 8·3. Enzymatic activity in this band was shown by reaction with phenazine methosulfate and nitro blue tetrazolium.

The best preparation gave straight line slopes from the plot of log fringe displacement vs. (radius)$^2$ from sedimentation equilibrium experiments at two different protein concentrations. From the slopes of the plot, the molecular weight was determined to be 300,000 $\pm$ 15,000, a value comparable to those reported by Wilson[15] and Zalitis and Feingold.[21]

The same enzyme was dialyzed for 2 days against 0·05 M potassium phosphate buffer, pH 7·0, containing 4 M guanidine hydrochloride and 0·1 M β-mercaptoethanol, and then subjected to sedimentation equilibrium analysis as for the native enzyme. The molecular weight calculated from the slope of the plot of log fringe vs. (radius)$^2$ was 51,000 $\pm$ 2500. This treatment resulted in the loss of enzymatic activity and reactivation was not achieved by removal of guanidine hydrochloride.

*Titration of UDP-glucose dehydrogenase with 5,5′-dithiobis(2-nitrobenzoic acid).* The reactivity of the sulfhydryl groups of UDP-glucose dehydrogenase was examined at two protein concentrations (225 μg; specific activity, 20,300 units/mg) in the presence of a 70- to 140-fold molar excess of 5,5′-dithiobis(2-nitrobenzoic acid). Reactions were initiated by the addition of enzyme. Absorbance at 412 mμ was recorded continuously. As indicated in Fig. 1, the reaction rate was slow for the native enzyme as compared to the reaction rate in the presence of guanidine hydrochloride. When the enzyme was preincubated with 1·6 M guanidine hydrochloride at room temperature, about 39 sulfyhdryl (SH) groups per 300,000 g of protein were reacted after 1 min, whereas only 2·6 SH groups per 300,000 g of native enzyme were reacted during this time (Table 1). The reaction with the guanidine-treated enzyme was essentially completed in 10 min, when about 46 SH groups were reacted per mole of the protein; whereas only about 20 SH groups were reacted per mole of the native protein after 1·5 hr. No further reaction was observed after these time periods. These results suggest that guanidine hydrochloride exposes SH groups presumably by unfolding or dissociation of protein aggregates.

*Nature of inhibition.* The results of kinetic studies of the inhibition by nucleotide-xylose compounds of UDP-glucose dehydrogenase activity are presented as conventional double reciprocal plots in Figs. 2–8. Inhibition by UDP-xylose of the enzyme activity at pH 8·7 and pH 7·7 with respect to UDP-glucose (Fig. 2) or with respect to

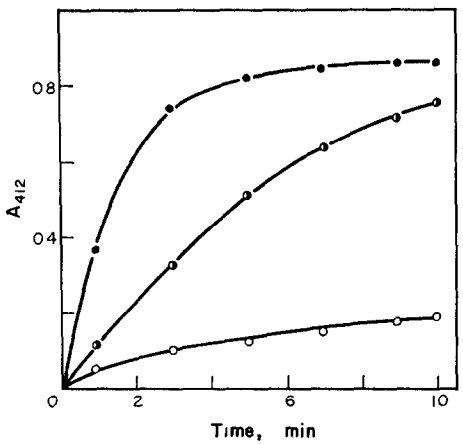


FIG. 1. Reaction of UDP-glucose dehydrogenase with 5,5′-dithiobis-(2-nitrobenzoic acid) in the presence of guanidine hydrochloride. UDP-glucose dehydrogenase was added into the reaction mixture containing: 0 M (○), 0·8 M (◐) and 1·6 M (●) guanidine hydrochloride.

TABLE 1. EFFECT OF GUANIDINE HYDROCHLORIDE ON THE REACTION OF UDP-GLUCOSE DEHYDROGENASE WITH 5,5′-DITHIOBIS(2-NITROBENZOIC ACID) (DTNB)*

| Time (min) | Moles of DTNB reacted/300,000 g of protein | |
|---|---|---|
| | Native enzyme | 1·6 M guanidine-HCl |
| 1 | 2·65 | 38·5 |
| 5 | 4·38 | 41·0 |
| 10 | 6·75 | 45·0 |
| 20 | 10·5 | 46·0 |
| 30 | 13·6 | 45·5 |
| 60 | 18·1 | |
| 90 | 20·2 | |

* UDP-glucose dehydrogenase (456 μg) was incubated with 0·1 m-mole potassium phosphate, pH 8·0, and 1·6 m-mole guanidine hydrochloride in 0·95 ml at room temperature for 30 min before addition of DTNB. Calculation was carried out as described in the text.

$NAD^+$ (Fig. 3) showed competitive kinetics at low inhibitor concentrations. The reciprocal of velocity plots showed an upward inflection with increasing UDP-xylose and decreasing substrate concentrations. This behavior of the enzyme is consistent with the report of Neufeld and Hall.[5]

Inhibition produced by $H_2$UDP-xylose with respect to UDP-glucose (Fig. 4) or with respect to $NAD^+$ (Fig. 5) also showed competitive kinetics. The inhibition kinetics were not competitive with HO-UDP-xylose or $H_2$UDP-xylose. Plots of these data with respect to UDP-glucose (Figs. 6 and 8a) or with respect to $NAD^+$ (Figs. 7 and 8b) did not intersect at either axis, but intersected at points which were closer to

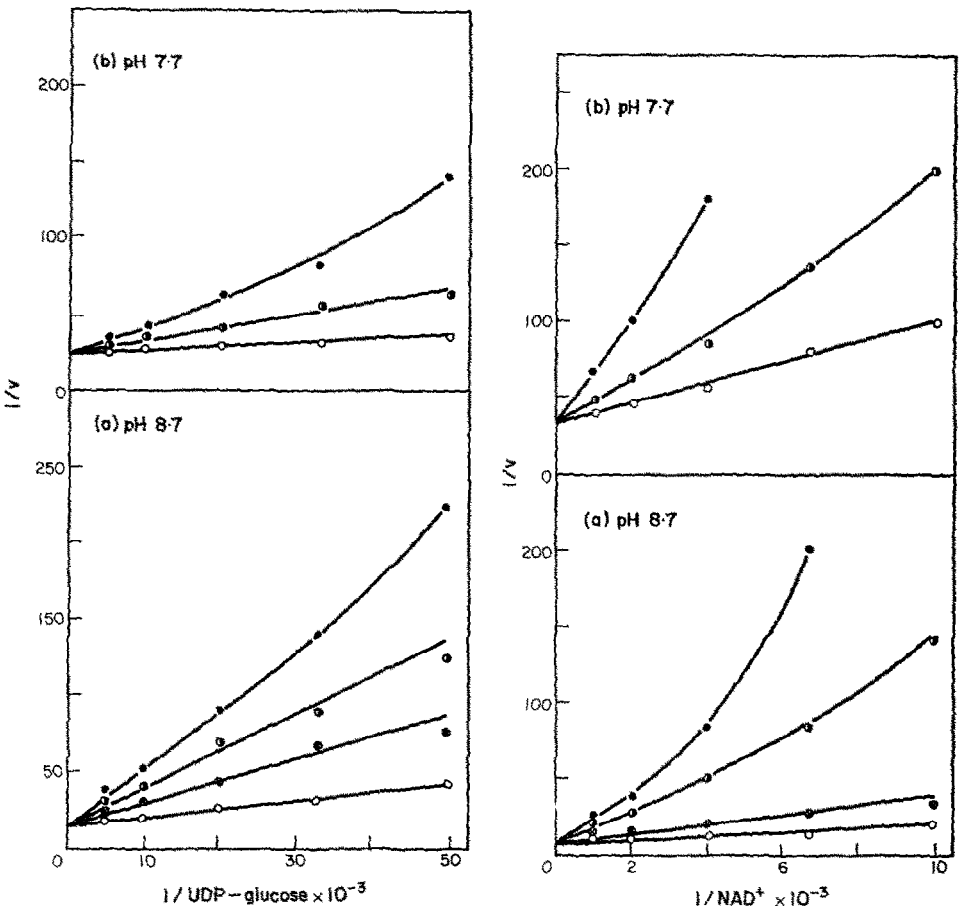


FIG. 2. Inhibition of UDP-glucose dehydrogenase by UDP-xylose with respect to UDP-glucose. (a) Reaction mixtures (pH 8·7) contained, in 1 ml, $2·0 \times 10^{-3}$ M $NAD^+$, $5 \times 10^{-2}$ M tris-acetate, 70 units of enzyme (sp. act., 20,300 units/mg), and the indicated amount of UDP-glucose. Initial reaction velocity was determined in the absence (○) and presence of $1 \times 10^{-5}$ M (⊗), $2 \times 10^{-5}$ M (◐) and $3 \times 10^{-5}$ M (●) UDP-xylose. Velocity (v) is expressed in terms of change in $A_{340}$ per min. (b) Reaction mixtures (pH 7·7) had the same composition as in (a). Initial reaction velocity was determined in the absence (○) and presence of $2 \times 10^{-6}$ M (◐) and $4 \times 10^{-6}$ M (●) UDP-xylose

FIG. 3. Inhibition of UDP-glucose dehydrogenase by UDP-xylose with respect to $NAD^+$. (a) Reaction mixtures (pH 8·7) contained, in 1 ml, $5 \times 10^{-4}$ M UDP-glucose, $5 \times 10^{-2}$ M tris-acetate, 70 units of enzyme (sp. act., 20,300 units/mg) and the indicated amount of $NAD^+$. Initial reaction velocity was determined in the absence (○) and presence of $1·25 \times 10^{-5}$ M (⊗), $2·5 \times 10^{-5}$ M (◐) and $3·75 \times 10^{-5}$ M (●) UDP-xylose. (b) Reaction mixtures (pH 7·7) had the same composition as in (a). Initial reaction velocity was determined in the absence (○) and presence of $5·0 \times 10^{-6}$ M (◐) and $1·0 \times 10^{-5}$ M (●) UDP-xylose.

the $y$ axis than the $x$ axis, indicating a mixed-type of inhibition.[22] The apparent $K_i$ values were estimated by the method of Webb[22] and are summarized in Table 2. Deviations from linear reciprocal plots were also observed at low substrate and high analogue concentrations (Figs. 4b, 6a and 8).

*Effect of pH on $K_m$ and $K_i$.* The apparent $K_i$ (for UDP-xylose) and $K_m$ (for UDP-glucose) values were calculated to be $0·53 \times 10^{-5}$ M and $3·33 \times 10^{-5}$ M, respectively,

TABLE 2. APPROXIMATE $K_i$ VALUES AT pH 8·7

| Analogues | $K_i \times 10^5$ M | |
|---|---|---|
| | With respect to UDP-glucose | With respect to $NAD^+$ |
| UDP-xylose | 0·5 | 0·4 |
| $H_2N$-UDP-xylose | 0·4 | 0·5 |
| HO-UDP-xylose | 2·0 | 9·0 |
| $H_2$-UDP-xylose | 2·0 | 7·0 |

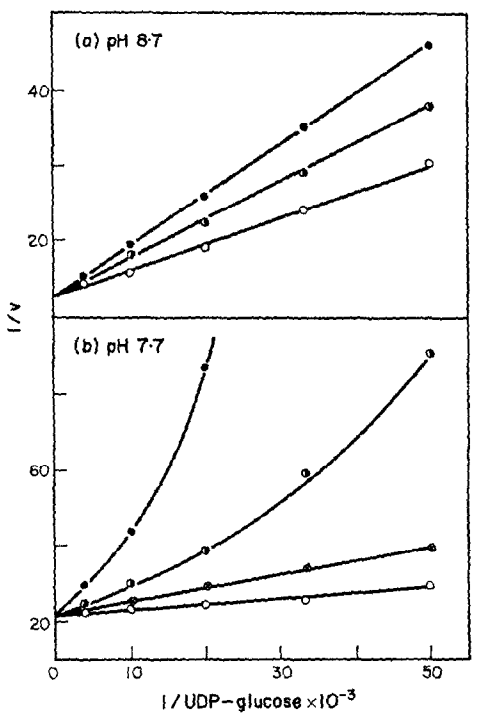


FIG. 4. Inhibition of UDP-glucose dehydrogenase by $H_2$UDP-xylose with respect to UDP-glucose. (a) Reaction mixtures (pH 8·7) contained, in 1 ml, $1 \times 10^{-3}$ M $NAD^+$, $5 \times 10^{-2}$ M tris-acetate, 50 units of enzyme (sp. act., 2400 units/mg) and the indicated amount of UDP-glucose. In (a), the initial reaction velocity was determined in the absence (○) and presence of $1 \times 10^{-5}$ M (◐) and $2 \times 10^{-5}$ M (●) $H_2$UDP-xylose. In (b), the initial reaction velocity was determined in the absence ○) and presence of $5 \times 10^{-6}$ M (⊗), $2{\cdot}5 \times 10^{-5}$ M (◐) and $5 \times 10^{-5}$ M (●) $H_2$UDP-xylose.

at pH 8·7, whereas at pH 7·7 the corresponding values were $0{\cdot}06 \times 10^{-5}$ M and $1{\cdot}07 \times 10^{-5}$ M. With $NAD^+$ as the variable substrate, the $K_i$ and $K_m$ (for $NAD^+$) were $0{\cdot}36 \times 10^{-5}$ M and $12{\cdot}5 \times 10^{-5}$ M at pH 8·7, and $0{\cdot}35 \times 10^{-5}$ M and $20{\cdot}8 \times 10^{-5}$ M at pH 7·7 respectively. The $K_m$ (for UDP-glucose) and $K_i$ values were lower at pH 7·7 than at pH 8·7, and the change of $K_i$ was significantly greater than that of $K_m$ (for UDP-glucose). With respect to $NAD^+$, lowering of the pH did not affect the $K_i$ and caused a 2-fold increase in the $K_m$ (for $NAD^+$).

## DISCUSSION

The procedure for purification of UDP-glucose dehydrogenase employed is the same as that reported by Wilson,[15] except that hydroxylapatite was used in place of DEAE-cellulose, resulting in improved purity and an increased yield of enzyme.

The sedimentation equilibrium data show that native UDP-glucose dehydrogenase has a molecular weight of 300,000 ± 15,000 and indicate that the enzyme can be dissociated into six subunits of molecular weight 51,000 ± 2500. Dissociation by sodium dodecyl sulfate into six to eight subunits has been reported previously.[21] Sulfhydryl

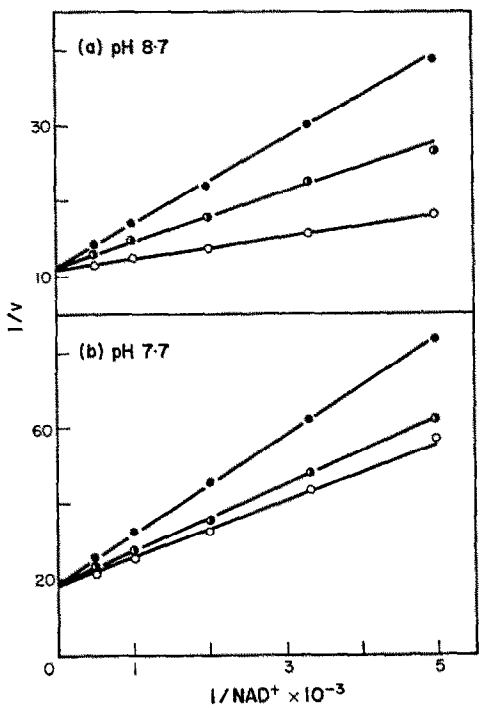


FIG. 5. Inhibition of UDP-glucose dehydrogenase by $H_2$UDP-xylose with respect to $NAD^+$. (a) Reaction mixtures (pH 8·7) contained, in 1 ml, $6 \times 10^{-4}$ M UDP-glucose, $5 \times 10^{-2}$ M tris acetate, 50 units of enzyme (sp. act., 2,400 units/mg) and the indicated amount of $NAD^+$. In (a), the initial reaction velocity was determined in the absence (○) and presence of $1 \times 10^{-4}$ M (◐) and $2 \times 10^{-4}$ M (●) $H_2$UDP-xylose. In (b), the initial reaction velocity was determined in the absence (○) and presence of $2{\cdot}5 \times 10^{-5}$ M (◐) and $5 \times 10^{-5}$ M (●) $H_2$UDP-xylose.

group analyses of UDP-glucose dehydrogenase with 5,5′-dithiobis(2-nitrobenzoic acid) indicate that 20 ± 1 SH groups are titrated, whereas in the presence of denaturant about 46 ± 1 SH groups (about eight SH groups per polypeptide chain) are titrated.

UDP-xylose and its analogues, $H_2$UDP-xylose, HO-UDP-xylose and $H_2$N-UDP-xylose, act as inhibitors of the UDP-glucose dehydrogenase of the calf liver enzyme. The inhibitory effects are reversed by UDP-glucose or $NAD^+$, the two substrates for the enzyme. The nature of the inhibition produced by UDP-xylose, $H_2$UDP-xylose, HO-UDP xylose or $H_2$N-UDP-xylose appears to be similar with respect to either

substrate. The reciprocal plots of the data deviate from linearity with increasing UDP-xylose concentrations. These data are consistent with the postulation[5] that UDP-xylose influences the function of the substrate sites of the enzyme by acting at allosteric site(s).

Neufeld and Hall[5] showed that UDP-arabinose, the 4-epimer of UDP-xylose, has one-twentieth the inhibitory activity of UDP-xylose for liver UDP-glucose dehydrogenase, and that UDP-*N*-acetylglucosamine has no significant inhibitory activity.

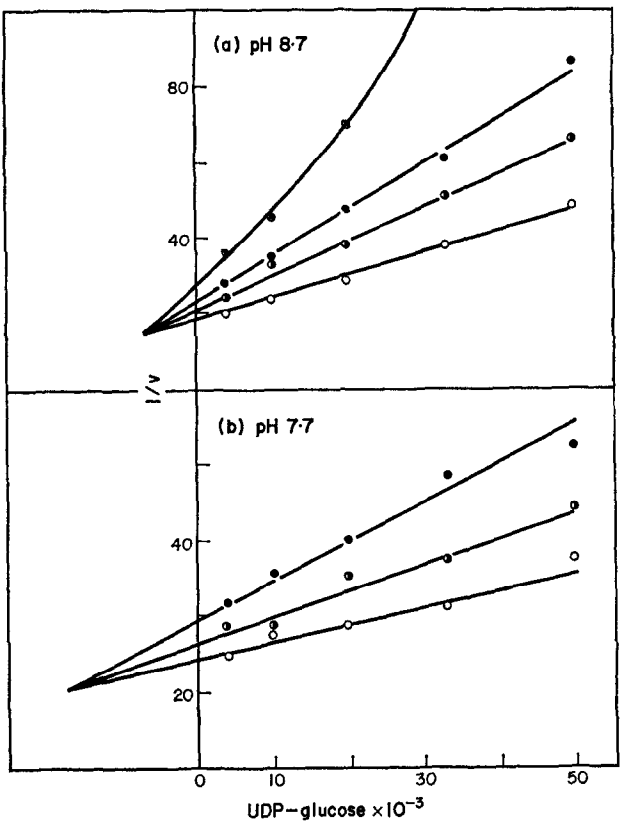


FIG. 6. Inhibition of UDP-glucose dehydrogenase by HO-UDP-xylose with respect to UDP-glucose. Reaction mixtures had the same composition as in Fig. 4. In (a) initial the reaction velocity was determined in the absence (○) and presence of $1 \times 10^{-5}$ M (◐), $2 \times 10^{-5}$ M (●) and $5 \times 10^{-5}$ M (⊗) HO-UDP-xylose. In (b) the initial reaction velocity was determined in the absence (○) and presence of $1 \times 10^{-5}$ M (◐) and $2 \times 10^{-5}$ M (●) HO-UDP-xylose.

These data show certain structural requirements of the pentosyl moiety in producing effective inhibition. The present studies provide some information regarding specificity of the uracil moiety. Hydrogenation of the 5,6-double bond of the pyrimidine portion decreases the inhibitory activity to about one-fifth to one-tenth (as estimated from the $K_i$ values) of UDP-xylose. The effect of hydrogenation is not related to changes in the dissociation of the pyrimidine moiety of the inhibitor, since the p$K_a$ of dihydrouridine[23] is higher than that of uridine.[7] It is possible that the 5,6-double bond of the uracil moiety of UDP-xylose may be involved in binding with the enzyme. Substitution of a

hydroxyl group at the C-5 position of the uracil moiety of UDP-xylose causes a decrease in the inhibitory activity to about one-fourth to one-twelfth (as estimated from the $K_i$ values) of UDP-xylose. This reduced activity may be related to the ionic form of the molecule or to the introduction of a steric factor. From the reported p$K_a$ values of uridine (9·3) and 5-hydroxyuridine (7·8), it can be assumed that, while UDP-

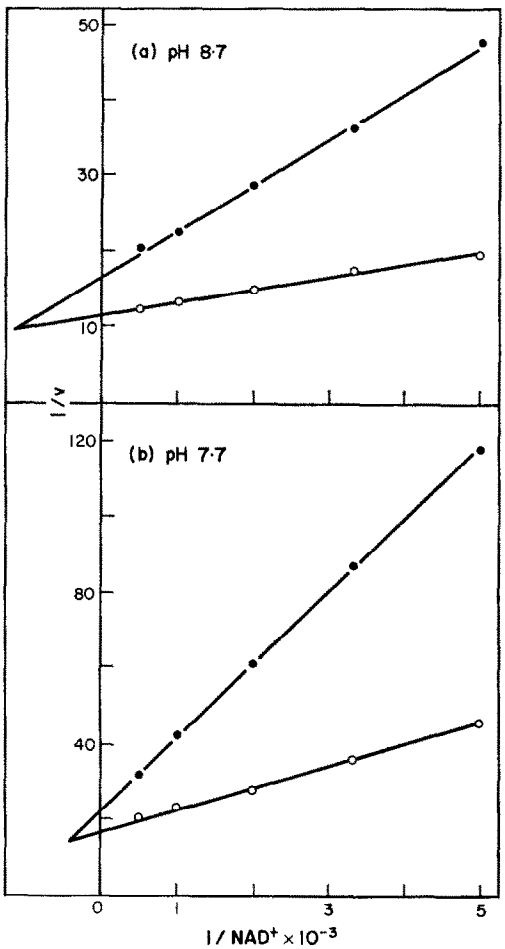


FIG. 7. Inhibition of UDP-glucose dehydrogenase by HO-UDP-xylose with respect to $NAD^+$. Reaction mixtures had the same composition as in Fig. 5. In both (a) and (b), the initial reaction velocity was determined in the absence (○) and presence (●) of $2{\cdot}24 \times 10^{-4}$ M HO-UDP-xylose.

xylose is mostly undissociated at pH 8·7, HO-UDP-xylose is almost completely ionized at pH 8·7 and partially ionized at pH 7·7. In contrast to HO-UDP-xylose, the substitution of an amino group at the C-5 position does not change the inhibitory activity of the p$K_a$ or the pyrimidine.[12] Thus, it is likely that the undissociated form of the inhibitor is more important for inhibitory function than is the nature of the 5-substituent.

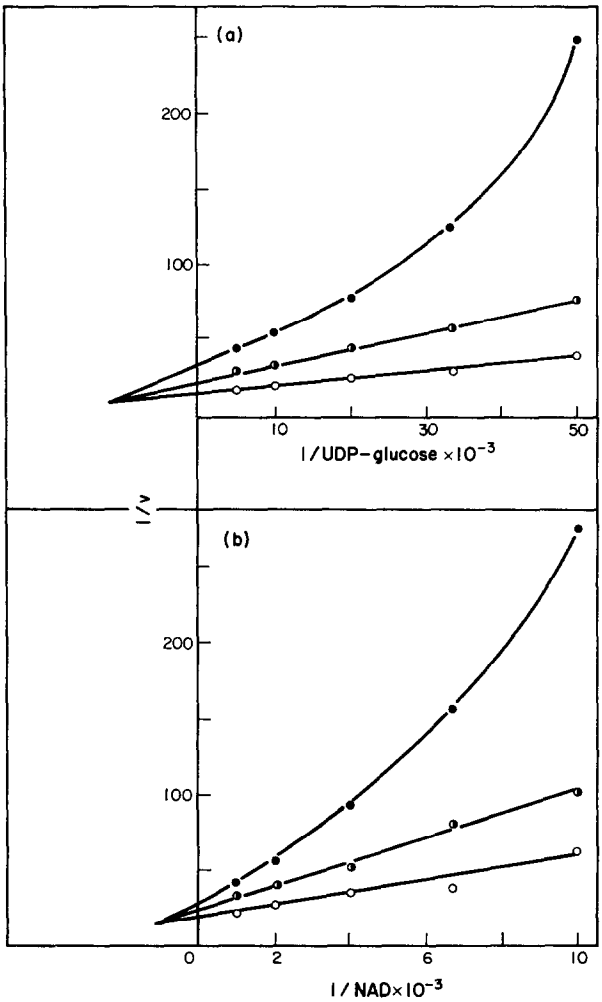


FIG. 8. Inhibition of UDP-glucose dehydrogenase by $H_2N$-UDP-xylose. (a) Reaction mixtures (pH 8·7) contained, in 1 ml, $2·0 \times 10^{-3}$ M $NAD^+$, $5 \times 10^{-2}$ M tris-acetate, 60 units of enzyme (sp. act., 12,000 units/mg) and the indicated amount of UDP-glucose. Initial reaction velocity was determined in the absence (○) and presence of $0·63 \times 10^{-5}$ M (◐) and $1·26 \times 10^{-5}$ M (●) $H_2N$-UDP-xylose. (b) Reaction mixtures (pH 8·7) contained, in 1 ml, $5 \times 10^{-4}$ M UDP-glucose, $5 \times 10^{-2}$ M tris-acetate, 60 units of enzyme and the indicated amount of $NAD^+$. Initial reaction velocity was determined in the absence (○) and presence of $0·65 \times 10^{-5}$ M (◐) and $1·26 \times 10^{-5}$ M (●) $H_2N$-UDP-xylose.

An interesting observation is that, when the pH is varied from 8·7 to 7·7, the apparent binding ability of UDP-xylose to the enzyme is increased by about 9-fold while the $K_m$ (for UDP-glucose) is decreased to about one-third. In contrast, the $K_m$ (for $NAD^+$) is increased and the $K_i$ is unaffected when the pH is lowered from 8·7 to 7·7. Thus, it appears that UDP-xylose binds more strongly at the lower pH, resulting in a preferential interference with the interaction of UDP-glucose at the catalytic site.

It is also reported here that an enzyme preparation of yeast contains UDP-xylose pyrophosphorylase activity which is known to occur in higher plants.[24] Whether or

not UDP-xylose pyrophosphorylase in yeast is distinguishable from UDP-glucose pyrophosphorylase remains to be determined. The enzyme preparation from yeast catalyzes the reaction of HO-UTP and xylose 1-phosphate to yield HO-UDP-xylose. It also catalyzes, although less efficiently, the formation of 5-aminouridine diphosphate xylose, but fails to catalyze the reaction between 6-aza-UTP and xylose 1-phosphate.

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