Inhibition of Ribulose Diphosphate Carboxylase/Oxygenase by Xylitol 1,5-diphosphate*

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Received May 5,1975

Summary

Xylitol 1,5-diphosphate is a potent inhibitor of RuDP carboxylase/oxygenase activity. At an enzyme concentration of 400 μg/ml (less than 1 μM), RuDP carboxylase is inhibited 50% by 2 μM xylitol 1,5-diphosphate. The molar ratio of effector to enzyme is 2 to 1. The inhibition with respect to RuDP is mixed uncompetitive and noncompetitive. The response of enzyme to bicarbonate in the presence of xylitol 1,5-diphosphate is sigmoidal. The inhibition is time-dependent and pH-dependent, with a pK_a for inhibition of about 8.6. RuDP oxygenase is inhibited at pH values below 9, but at pH 9 or above, neither RuDP oxygenase nor carboxylase is significantly inhibited. Xylitol 1,5-diphosphate does not produce a differential regulation of the RuDP carboxylase and oxygenase activities.

Introduction

Ribulose 1,5-diphosphate carboxylase/oxygenase catalyzes the incorporation of carbon dioxide into RuDP** to give two molecules of 3 P-glyceric acid, or the incorporation of oxygen to give one P-glycolate and one 3 P-glyceric acid. These two reactions represent a branch point in carbon flow for photosynthesis or photorespiration. The free energy changes for both the carboxylation and the oxygenation are large and the structure of the enzyme is complex. These facts suggest that the enzymic activities should be tightly regulated. We have investigated the regulatory effects of a number of sugar phosphates and their

*This work was supported in part by NSF Grant 32040X and is published as journal article number 7253 of the Michigan Agricultural Station.

**Abbreviations: RuDP for ribulose 1,5-diphosphate
XDP for xylitol 1,5-diphosphate

analogs with respect to the RuDP oxygenase activity (1, 2), and others have reported on the effect of these compounds on the RuDP carboxylase activity (3, 4, 5, 6). Ribose-5-P and fructose diphosphate affect either activity only at concentrations approaching 1 mM and 50 μM 6 P-gluconate is required for modification of enzyme activity.

A number of analogs of RuDP have been tried as modifiers of the RuDP carboxylase/oxygenase activities (unpublished). One of these compounds, xylitol 1,5-diphosphate, is a particularly potent inhibitor. Its mode of inhibition is not simple competition with respect to RuDP; rather, it displays the properties of an allosteric effector.

Materials and Methods

RuDP carboxylase/oxygenase was purified from spinach leaves as described previously (1) with one modification. Gel filtration through a 2.6 x 90 cm column of Sepharose 4B was substituted for the sucrose density gradient centrifugation step. There was no change in the yield or purity of the isolated enzyme. The protein concentration of the purified desalted enzyme used for the assays was 10 mg/ml.

The preparation of XDP (7) and of RuDP (1) have been described previously. Other compounds were of highest commercial grade available. For assay of RuDP carboxylase activity, the reaction mixture contained in 0.25 ml: 0.25 μmoles potassium N,N-bis-(2-hydroxyethyl)-glycine at pH 8.1, 2.5 μmoles $MgCl_2$, 0.06 μmoles Na_2 ethylenediaminetetra-acetate, 0.15 μmoles dithiothreitol, 2.5 μmoles ^{14}C-$NaHCO_3$ (specific activity 2.3 x 10^5 cpm μmole HCO_3^{-1}), 0.125 μmoles RuDP, and 100 μg enzyme. All except RuDP were preincubated together for 10 minutes at 30°. One minute after the addition of RuDP the reaction was terminated by 0.25 ml 2 M HCl. The activity was measured as incorporation of ^{14}C into acid stable product. If XDP were used, it was included in the preincubation mixture.

The oxygenase assay was monitored by a Ranke Brothers oxygen electrode (1). The reaction mixture was the same for the carboxylase except that the pH of the N,N-bis-(2-hydroxyethyl)-glycine buffer was 8.6, and 500 μg of enzyme was used in 0.5 ml final assay volume. The oxygenase reaction proceeded at 25° in air.

Results

XDP at very low concentrations inhibited RuDP carboxylase activity. The non-linear Dixon plot shown in Figure 1A suggests that there are two modes of action for this effector. In the presence of approximately 1 μM enzyme (100 μg per 0.25 ml) activity was reduced 50% by 2 μM XDP at pH 8.1. The molar ratio of effector to enzyme at this point is approximately 2 to 1. However, there are 8 catalytic subunits per molecule of enzyme, so the molar ratio of effector to active site is

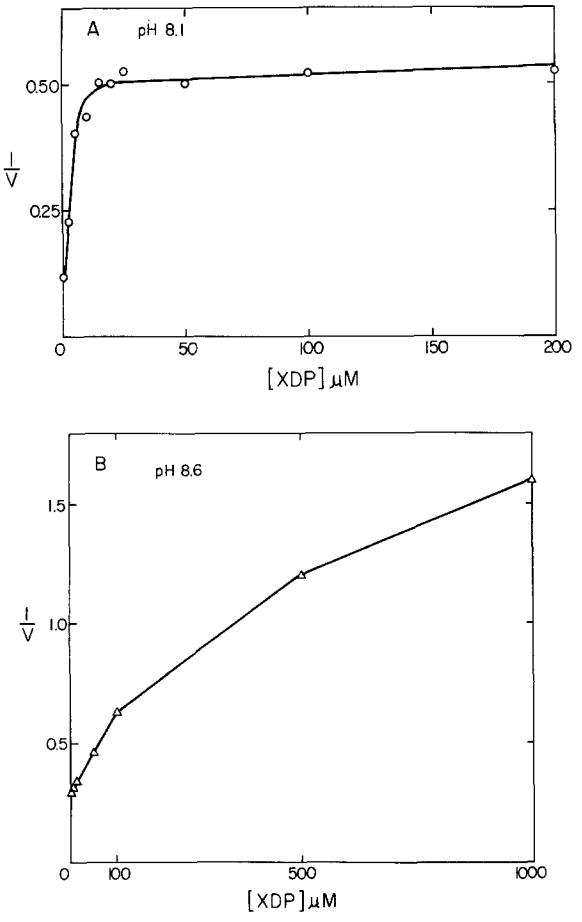

Figure 1.
Dixon plot for inhibition by XDP. Velocity is expressed as 10^3 cpm/min/assay. A. Assay carried out at pH 8.1. B. Assay carried out at pH 8.6.

1 to 4. Thus the site of action of the effector would appear to be other than the catalytic site. In Figure 1B is shown a Dixon plot for the inhibition at pH 8.6. Again, the plot is non-linear.

When RuDP and XDP were added to the enzyme simultaneously, no inhibition was noted in the one minute assay. The inhibition was time-dependent (Figure 2A): the change is initially rapid but there is little change after 10 minutes. The inhibition was not complete but had characteristics expected from the conversion of the enzyme from an active form to a less active form. One can estimate the activity of the less

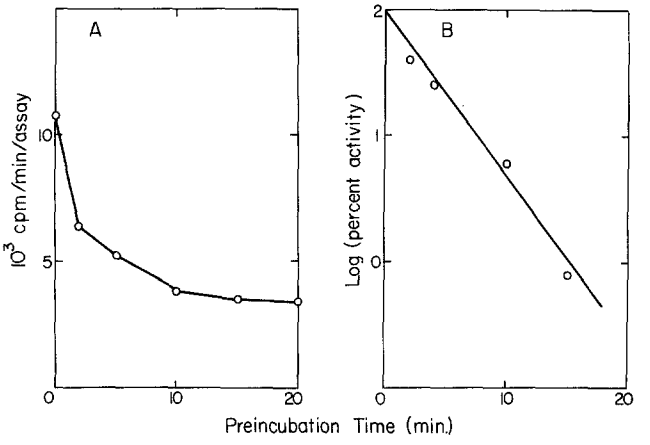

Figure 2.
A. Time dependence of inhibition by 5 μM XDP at pH 8.1.
B. First order plot of the inhibition, assuming that there is an active and less active form of the enzyme.

active form by extropolating the activity of the linear portion of the Dixon plot to zero concentration of XDP in Fig. 1A. This value of about 30% (see also Fig. 2A) and the activity of the native form of the carboxylase can be used to calculate the relative amounts of fully active and less active forms of the enzyme present at any point during the time course of inactivation. A plot of the logarithm of the percentage of fully active enzyme as a function of time, Figure 2B, is a straight line indicating that the conversion is apparent first order.

A double reciprocal plot of the rate of carboxylase activity as a function of RuDP concentration in the presence of two levels of XDP as presented in Figure 3, indicates the complex nature with respect to RuDP of the interaction of the XDP effector with the enzyme. The mode of inhibition appears to be a mixture of uncompetitive and noncompetitive types. It is certainly not competitive with respect to RuDP. The most profound effect of the XDP appears to be on the affinity of the enzyme for the HCO_3^- species, as shown by a plot of rate as a function of HCO_3^- in Figure 4. The velocity was greatly reduced by the presence of even small amounts of the effector, and the hyperbolic response of enzyme to substrate has been converted to

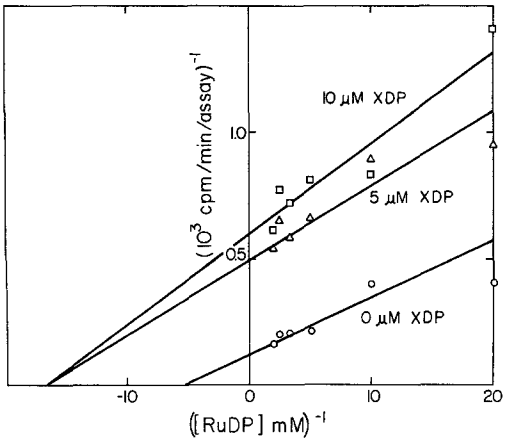

Figure 3.
Double reciprocal plots of rate of the RuDP carboxylase reaction as a function of concentration of RuDP in the presence of 0, 5 and 10 μM XDP. The kinetic parameters for each line were determined from direct linear plots (8).

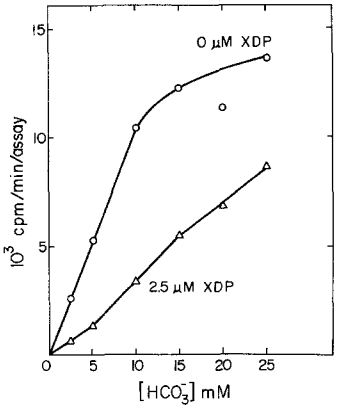

Figure 4.
Rate of the RuDP carboxylase reaction as a function of bicarbonate concentration in the presence of 0 and 2.5 μM XDP.

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a sigmoidal response. The K_m (HCO_3^-) of the inhibited form of the enzyme must be very high.

The inhibition of RuDP carboxylase by XDP was pH dependent (Fig. 5). With 10 μM XDP in the standard assay, the inhibition decreased from 80% at pH 8 or less to zero inhibition at pH 9 or above. The shape of the curve is reminiscent of a titration curve for an amino group. The pK_a is about 8.6.

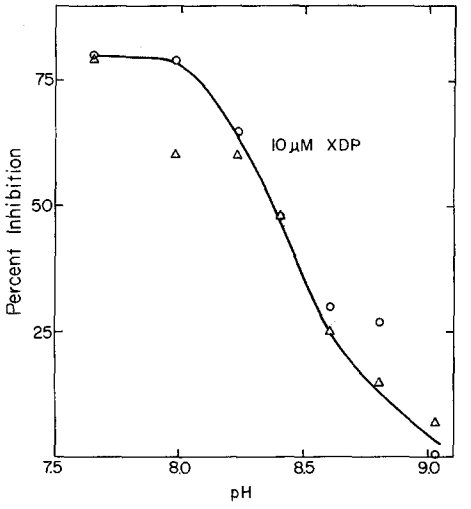

Figure 5.
Inhibition of RuDP carboxylase by XDP as a function of pH. The inhibition is expressed as percentage of the control activity (no XDP) measured at the same pH. Data from two experiments with 10 μM XDP are combined.

The RuDP oxygenase activity was also altered by XDP. The pH optimum of the oxygenase after a 10 minute preincubation is about 9.1 with bicine buffer so that oxygenase experiments with XDP were run at 8.6 to obtain measurable activity. Also the less sensitive oxygenase assay required 7.5 times more protein than in the carboxylase assay, so that more XDP was required for inhibition. A plot of rate of RuDP oxygenase activity at pH 8.6 as a function of XDP is shown in Fig. 6. At pH 9, XDP had no effect on the RuDP oxygenase activity, similar to its ineffectiveness on the carboxylase at this pH.

Discussion

The natural occurrence of XDP has not been reported, nor has it been considered to be a regulatory compound. Because it is an effective regulator of RuDP carboxylase/oxygenase at concentrations a thousand-fold lower than levels of naturally occurring sugar phosphates which affect this enzyme *in vitro*, one must assume either that XDP occurs at very low concentrations *in vivo*, or, more likely, that it is a structural analog of a naturally occurring effector of RuDP carboxylase/oxygenase. XDP can be

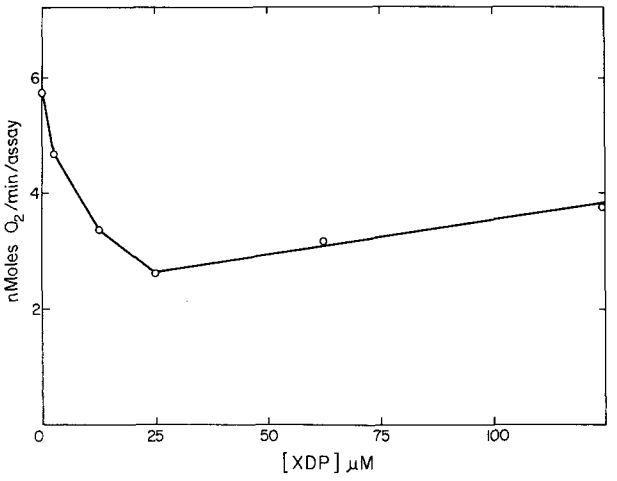

Figure 6.
Activity of RuDP oxygenase as a function of concentration of XDP. This assay was carried out at pH 8.6.

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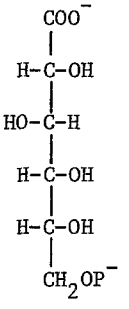
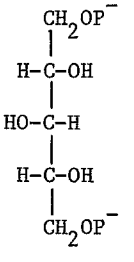
considered as a structural analog of 6-phosphogluconate, an effector of the enzyme (2-6)



6-Phosphogluconate



Xylitol-1,5-diphosphate

The pH dependence for the effector activity of XDP indicates that a group with a pK_a of approximately 8.6 is involved in the manifestation of the effect. Since the phosphates of XDP should be completely ionized at pH 8, the unknown group may be an amine at the binding site for the effector. This is the first indication of the chemical nature of any of the postulated allosteric regulatory sites of RuDP carboxylase/oxygenase. XDP probably does not produce a differential regulation of

the RuDP carboxylase/oxygenase activities. What appears to be differential regulation is simply the result of the different pH's employed in the two assays. Preliminary experiments indicated that XDP had no effect on the amount of oxygen inhibition of the RuDP carboxylase activity, an effect which would be a necessary consequence of differential regulation of the activities.

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