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Xylulose 1,5-Bisphosphate Synthesized by Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase during Catalysis Binds to Decarbamylated Enzyme[1]

Genhai Zhu and Richard G. Jensen*

Departments of Biochemistry and Plant Science, University of Arizona, Tucson, Arizona 85721

ABSTRACT

Xylulose 1,5-bisphosphate (XuBP) is synthesized from ribulose 1,5-bisphosphate (RuBP) at carbamylated catalytic sites on ribulose 1,5-bisphosphate carboxylase (Rubisco) with significant amounts of XuBP being formed at pH less than 8.0. XuBP has been separated by high performance liquid chromatography and identified by pulsed amperometry from compounds bound to Rubisco during catalysis with the purified enzyme and from celery (*Apium graveolens* var *Utah*) leaf extracts. XuBP does not bind tightly to carbamylated sites, but does bind tightly to decarbamylated sites. Upon incubation of fully activated Rubisco with 5 micromolar XuBP, loss of activator CO_2 occurred before XuBP bound to the enzyme catalytic sites, even in the presence of excess CO_2 and Mg^{2+}. Binding of XuBP to decarbamylated Rubisco sites was highly pH dependent. At pH 7.0 and 7.5 with 10 millimolar $MgCl_2$ and 10 millimolar $KHCO_3$, the apparent dissociation constant for XuBP, K_d, was 0.03 micromolar, whereas at pH 8.0 and 8.5, the apparent K_d was 0.35 and 2.0 micromolar, respectively. This increase in K_d with pH was a result of a decrease in the association rate constant and an increase in the dissociation rate constant of XuBP bound to decarbamylated sites on Rubisco. The K_d of 2-carboxyarabinitol 1-phosphate binding to carbamylated sites was only slightly pH dependent.

The enzyme Rubisco is carbamylated by addition of an activator CO_2 followed by binding of Mg^{2+} to become catalytically active. Rubisco activity in plants is regulated by changing its carbamylation state dependent on light and/or by binding an inhibitor such as CA1P,[2] a potent Rubisco inhibitor (3, 15, 20, 24). A second chloroplast protein, Rubisco activase, is also involved (23).

Rubisco can be spontaneously activated *in vitro* by incubation with CO_2 and Mg^{2+} at alkaline pH (18). Upon addition of RuBP the catalytic rate decreases with time in a first order manner to a final steady state rate as activated Rubisco fixes CO_2. The time-dependent Rubisco kinetics have been described (1, 8–11, 18, 25) and the term "fallover" was used to describe this die-off phenomenon (9). During the preparation of this paper, Edmondson *et al.* (8–11) demonstrated that the products of RuBP isomerization, including XuBP, could inhibit Rubisco activity during catalysis. The inhibitors formed by Rubisco *per se* during catalysis could explain the fallover phenomenon. They proposed that the loss of Rubisco activity during catalysis occurs without loss of carbamate CO_2 and that it is due to the tight binding of an unknown inhibitor, not XuBP, to carbamylated sites.

In this study we have used a pulsed amperometric detector following HPAE-PAD separation to separate and identify XuBP bound to Rubisco during catalysis. The observations of Edmondson *et al.* were corroborated, but we found that their conclusions were only part of the story. We report that the formation of XuBP is pH dependent and that XuBP is bound slowly but tightly to inactive sites of Rubisco. Lower pH increases the rate of synthesis as well as the binding affinity of Rubisco for XuBP. The decarbamylation of Rubisco during fallover does occur at low pH values (pH 7.5), but was much less or nonexistent at high pH (pH 8.5) (29). A preliminary report of this work has appeared elsewhere (28).

We have separated and identified XuBP from celery leaf extracts, indicating that XuBP occurs *in vivo*. From these and other results (4, 7, 17), it appears that the regulation of Rubisco catalysis does occur by decarbamylation induced by RuBP or XuBP binding to inactive Rubisco. Our *in vitro* experiments suggest that when the pH of the chloroplast stroma drops below 8.0, binding of XuBP could be significant.

MATERIALS AND METHODS

RuBP was synthesized and purified as previously described (2, 27) and shown to be free of other sugar phosphates, including XuBP, by HPAE-PAD. CABP was synthesized and purified as described elsewhere (21, 27). Rubisco was purified from spinach leaves (16). XuBP was synthesized by Alan Smrcka by aldolase-catalyzed condensation of glycolaldehyde phosphate and dihydroxyacetone phosphate (5, 19). Xylulose was obtained from Sigma.

Rubisco was activated by incubation with 10 mM $KHCO_3$ and 10 mM $MgCl_2$ in 50 mM Hepes (pH 8.0) for 15 to 30 min. Activity of Rubisco was measured in 50 mM Hepes, pH 8.0, 10 mM $NaH^{14}CO_3$ (1 Ci/mol), 10 mM $MgCl_2$, and 0.5 mM RuBP. After 30 s 1 mL of 1 N HCl was added and the unfixed $^{14}CO_2$ released upon heating at 80°C.

[1] This research was supported in part by the Arizona Agricultural Experiment Station.

[2] Abbreviations: CA1P, 2-C-carboxy-D-arabinitol 1-phosphate; $^{A}CO_2$, activator CO_2; CABP, 2-C-carboxy-D-arabinitol 1,5-bisphosphate; HPAE-PAD, high performance anion exchange chromatography with pulsed amperometric detection; PGA, 3-phosphoglycerate; RuBP, ribulose 1,5-bisphosphate; t_R, retention time; XuBP, xylulose 1,5-bisphosphate.

Compounds Tightly Bound to Rubisco

After 30 to 60 min CO_2 fixation with RuBP, the Rubisco reaction solution was passed through a Bio-Rad Econo-Pac 10DG gel filtration column to separate Rubisco and protein-bound compounds from unbound substrates and product. The Rubisco protein fractions were combined and $HClO_4$ added to 3%. Precipitated protein was removed, the supernatant was neutralized to pH 6.5–7.0 with 4 N KOH, and the $KClO_4$ precipitate was removed. The supernatant was put either directly on the HPAE-PAD or passed through a silica ion-exchange column (Sepralyte SAX, 1 mL syringe with a 0.6 mL bed volume), washed with 1 mL H_2O and the PGA and monophosphates eluted with 5 mL 0.05 N HCl. The bisphosphates were eluted next with 5 mL 0.15 N HCl (26). The fractions were evaporated almost to dryness under vacuum in a Savant SpeedVac concentrator to remove HCl and redissolved in a small volume of water. Large amounts of PGA in the samples on the HPAE-PAD interfered with separation of the bisphosphates.

XuBP from Celery Leaves

Celery (*Apium graveolens* var *Utah*) plants, grown in a greenhouse for 8 weeks, were adapted to either dark or low light for 1 h. The leaves were ground in 5% $HClO_4$ and the homogenate was filtered through three layers of Miracloth. The filtrate was centrifuged at 10,000*g*, 15 min. The supernatant was neutralized with 4 N KOH, the $KClO_4$ removed by centrifugation, and the supernatant passed through a cation exchange column, H^+ form (AG 50W-X4, 60 mL syringe with about 50 mL bed volume). The column eluate was adjusted to pH 7, excess 1 M barium acetate was added, and the precipitate formed was immediately discarded (mostly nucleotides and UV-absorbing material). An equal volume of 95% ethanol was added to the supernatant. The second precipitate contained the bisphosphate fraction and was dissolved by adding Bio-Rad AG 50W-X4(H^+). The filtrate was purified on a 55 × 1.5 cm column of AG 1-X8(Cl^-) and eluted with a gradient of 0 to 0.4 M of LiCl (1 mL/min) (27). The fractions that inhibited Rubisco activity were collected and concentrated, and the barium precipitate was redissolved with AG 50W-X4(H^+). The product from this step was applied to the HPAE-PAD for separation and identification.

Separation and Identification of XuBP by HPAE-PAD

The HPAE-PAD system used a Dionex CarboPac PA1 or an Ion PAC column, a Dionex pulsed amperometric detector (model PAD-2) with a pump and solvent delivery system (Spectra Physics SP8700) at 0.8 mL/min. For sugar phosphate separation,the column was equilibrated with 50 mM NaOH and 400 mM K-acetate. Five minutes after injecting the sample, a linear gradient of K-acetate from 400 to 500 mM was run. In this system, RuBP and XuBP were well separated. For sugar separation, the column was equilibrated and the sample eluted with 100 mM NaOH. Ribulose and xylulose were well separated under these conditions. XuBP in celery leaf extracts was identified by comparing the retention times of both the sugar bisphosphate and the dephosphorylated sugar with standard XuBP and xylulose. XuBP was dephosphorylated by incubation with *Escherichia coli* alkaline phosphatase.

Trapping of Activator CO_2 with CABP

Rubisco was first activated by incubation with 10 mM $KH^{14}CO_3$ (10 Ci/mol) and 10 mM $MgCl_2$ in 50 mM Hepes (pH 8.0) for 15 min. After addition of 5 μM XuBP, 0.2 mL was removed and CABP and $KHCO_3$ added so that the solution (0.60 mL) contained 50 μM [^{12}C]CABP to trap the [^{14}C]$^A CO_2$ plus 0.17 M $KH^{12}CO_3$ to dilute the unbound radiolabel. The Rubisco-CABP complex was separated by passing it through a Bio-Rad gel filtration column. The amount of Rubisco, which eluded the column, was estimated (mg/mL) by absorbance at 280 nm multiplied by 0.61. The $^A CO_2$ trapped was determined by counting the Rubisco-[^{14}C]$^A CO_2$-[^{12}C]CABP complex (27).

RESULTS

pH and Formation of XuBP during Fallover

Purified spinach Rubisco was activated in 10 mM $NaHCO_3$ and $MgCl_2$. Freshly prepared RuBP was added at pH 6.6, 7.0, 7.7, and 8.2 and samples analyzed after 30 min catalysis (Fig. 1). Different amounts of XuBP were formed depending on the pH. The pH values indicated in Figure 1 were measured at the end of the reaction. A lower pH favored XuBP formation during fallover. The relative amounts of XuBP determined by the integrator were 7.7, 5.3, 3.0, and 1 for pH 6.6, 7.0, 7.7, and 8.2, respectively.

Binding of XuBP to Rubisco Causes Loss of Activator CO_2

The inhibition of Rubisco by XuBP is a slow process: maximum inhibition was reached only after incubation of

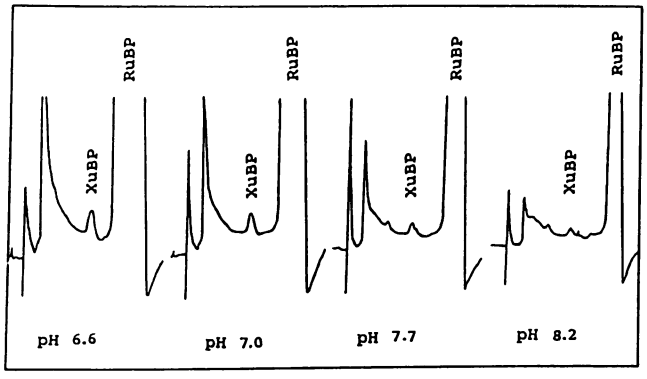

Figure 1. HPAE-PAD analysis of the bisphosphate fraction indicating more XuBP was formed during catalysis at lower pH by purified Rubisco. Rubisco (44 μM catalytic sites) was activated by incubation in 2.0 mL of 10 mM $KHCO_3$, 10 mM $MgCl_2$, and 50 mM Hepes. CO_2 fixation was started by addition of 330 μL of 30 mM RuBP, and after 30 min perchloric acid to 5% was added directly to the reaction solution. The final pH of the reaction solutions is given. The samples were adsorbed on a silica ion exchange column, the PGA separated from the bisphosphate fraction, and the bisphosphate fraction (100 μL) added to the HPAE-PAD.

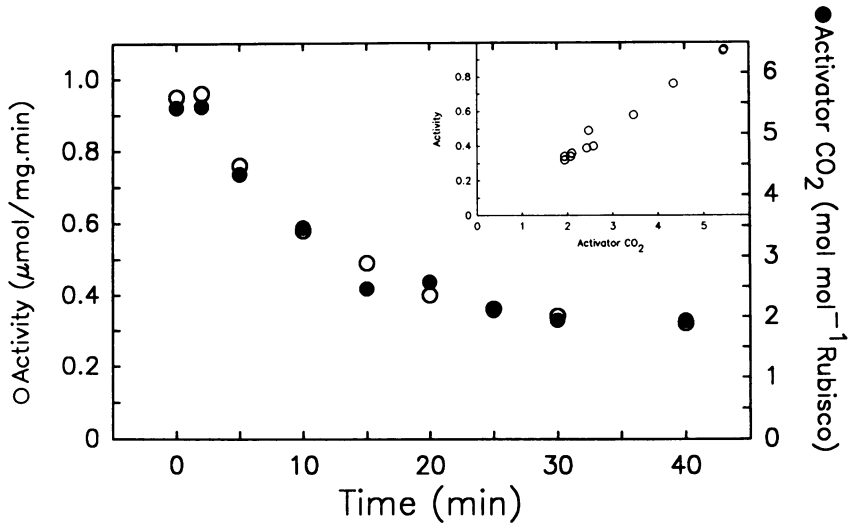

Figure 2. Loss of $^{A}CO_2$ and activity of Rubisco during incubation with XuBP. Two tubes containing Rubisco (1 μM catalytic sites), 50 mM Hepes (pH 7.8), 10 mM $MgCl_2$, and either 10 mM $NaHCO_3$ or 10 mM $KH^{14}CO_3$ (10 Ci/mol) were incubated with 5 μM XuBP. For CO_2 fixation activity (○) 50 μL of the unlabeled reaction mixture was taken. For assay of the mol $^{A}CO_2$ per mol Rubisco (●), 0.2 mL of the labeled reaction mixture was used.

enzyme with XuBP for 20 to 30 min (17). This delay would be consistent with the catalytic site losing Mg^{+2} and $^{A}CO_2$. To examine this XuBP was added to carbamylated Rubisco after it had been activated with $^{14}CO_2$ and Mg^{+2}. Samples were treated with [^{12}C]CABP to trap the radiolabel and $KH^{12}CO_3$ was added to dilute the unbound $^{14}CO_2$. The Rubisco-$^{14}CO_2$-Mg^{+2}-[^{12}C]CABP complex was separated from the unbound $^{14}CO_2$ by gel filtration (27). The binding of XuBP induced loss of the $^{A}CO_2$ linearly with inhibition of Rubisco activity (Fig. 2). Without XuBP no loss of carbamylation occurred. While XuBP is a competitive inhibitor to RuBP at carbamylated sites (17), it appears that XuBP bound tightly only to inactive, decarbamylated sites and not to carbamylated sites on Rubisco.

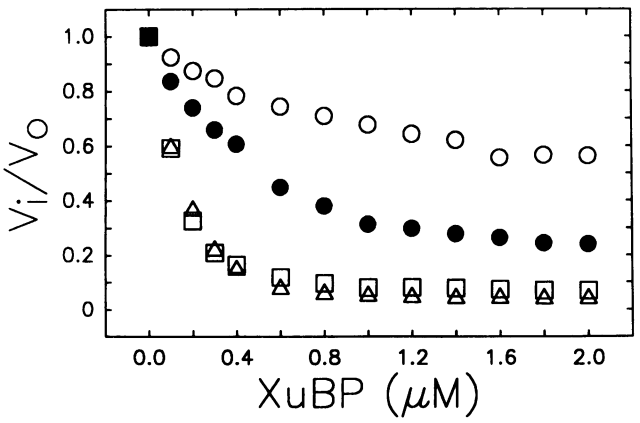

Figure 3. Effect of pH on inhibition of Rubisco activity by XuBP. Activated Rubisco (0.34 μM catalytic sites) was equilibrated with various concentrations of XuBP for 30 min in 10 mM $KH^{14}CO_3$ (1 Ci/mol), 10 mM $MgCl_2$ and 50 mM Tris for pH 8.5 (○) and pH 8.0 (●) or 50 mM Hepes for pH 7.5 (△) and pH 7.0 (□), then CO_2 fixation activities were measured for 30 s at the same pH by addition of 0.5 mM RuBP. V_o is the activity in the absence of added XuBP (1.0 μM CO_2 fixed/mg Rubisco × min) and V_i is the activity after incubation with XuBP.

XuBP Inhibition Increases at Lower pH

Activated Rubisco was incubated 30 min with up to 2 μM XuBP at various pH followed by CO_2 fixation assay at that pH. The binding affinity for XuBP increased at lower pH. At pH 8.5, the maximum inhibition by XuBP was less than 40% in spite of saturating amounts of inhibitor (2 μM, Fig. 3). Inhibition was 70% at pH 8. About 90% of the Rubisco activity was inhibited at 0.8 μM XuBP at pH 7.5 and 7.0.

Using similar methods to titrate Rubisco activity with CA1P, there was little change in binding with pH. Only at pH 8.5 could a slight difference be observed (data not shown). CA1P is known to bind tightly to carbamylated Rubisco (24) with a K_d of 0.032 μM (4). This was also the case in our hands as can be seen by the similar K_d estimates (Table I).

Not only did XuBP binding to Rubisco increase but XuBP bound faster to Rubisco at pH 7.5 than at pH 8.0 and 8.5. The effect of pH on the rate of XuBP binding was studied (Fig. 4). By using Multiple Exponential Decay Analysis Software (MEDAS, EMF Software; Knoxville, TN), the data were best fitted with a first order decay equation containing a single exponential term, and the k_{on} values were estimated as 8.3×10^{-4} s^{-1}, 3.3×10^{-3} s^{-1}, and 7.5×10^{-3} s^{-1} at pH 8.5, 8.0, and

Table I. *Effect of pH on the Binding Affinity of RUBISCO for XuBP and CA1P*

Assay	Apparent K_d			
	pH 8.5	pH 8.0	pH 7.5	pH 7.0
	μM			
XuBP				
Titration[a]	2.0	0.35	0.03	0.03
k_{off}/k_{on}[b]	2.1	0.23	0.024	
CA1P				
Titration[c]	0.10	0.035	0.029	

[a] Calculated from Figure 3 according to Berry et al. (3). [b] Derived from Figures 4 and 5. [c] Calculated from titration experiments similar to Figure 3, where carbamylated Rubisco was equilibrated with 0 to 1 μM CA1P.

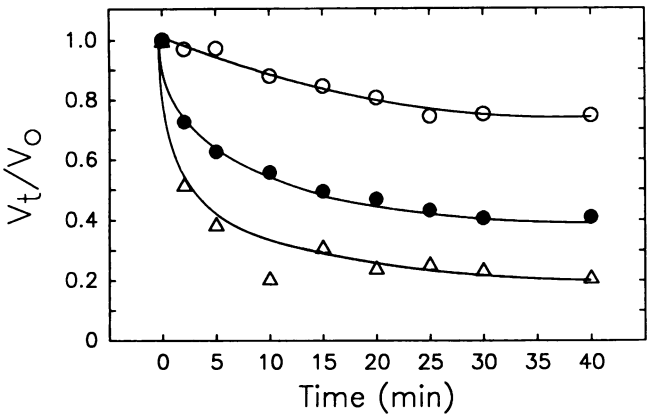

Figure 4. Rate of XuBP binding to Rubisco at various pH. Rubisco (1 μM catalytic sites) was first activated in 50 mM Tris, pH 8.5 (○) and pH 8.0 (●) or 50 mM Hepes, pH 7.5 (△), 10 mM $KHCO_3$, and 10 mM $MgCl_2$. XuBP was added (5 μM) and 50 μL samples were removed at the times given and activity measured in 0.45 mL 50 mM Hepes (pH 8.0), 10 mM $KH^{14}CO_3$ (1 Ci/mol), 10 mM $MgCl_2$, and 0.5 mM RuBP. V_t/V_o is the relative activity or the activity after XuBP incubation divided by the activity without XuBP which was 1.0 μmol CO_2 fixed/mg Rubisco × min. The data fit best a first order decay process from which the k_{on} values were determined.

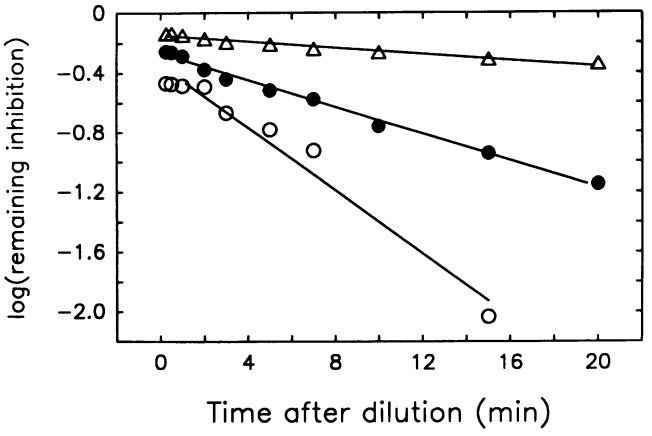

Figure 5. Rate of dissociation of XuBP from Rubisco at various pH. Rubisco was first deactivated by dialysis overnight in 50 mM Hepes (pH 8.0), 0.2 mM EDTA under CO_2-low conditions. The deactivated enzyme (14 μM catalytic sites) was then added to 50 mM Tris, pH 8.5 (○) and pH 8.0 (●) or Hepes, pH 7.5 (△) and incubated with 20 μM XuBP or without XuBP for 30 min at 24°C. Samples (5 μL) were diluted into 1.0 mL of the same buffer and pH plus 10 mM $MgCl_2$, 10 mM $KH^{14}CO_3$ (1 Ci/mol) for each time point. The 200-fold dilution of XuBP was sufficient so that the dissociation of XuBP occurred with little contribution from the rebinding of XuBP. At the times given, Rubisco activities were measured at the time given by addition of RuBP (0.5 mM) and CO_2 fixation (30 s). The "remaining inhibition" was $V_{control} - V_{XuBP}/V_{control}$ where V is the activity of the control (without XuBP) or of XuBP-incubated Rubisco at the same time point and pH. The control V_{max} was 1.2 μmol/mg Rubisco × min. The slope of the lines was the dissociation rate constant, k_{off}, and was determined by linear regression.

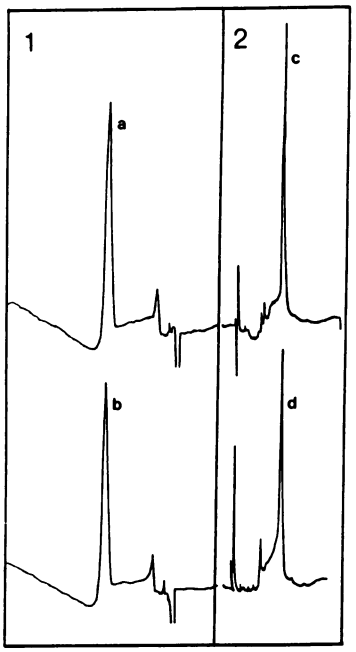

Figure 6. The HPAE-PAD profiles of known XuBP and xylulose with samples purified from celery leave. (*1*) Sugar phosphates: (a) XuBP standard, t_R = 14.3 min; (b) XuBP purified from celery leaves. (*2*) Sugars: (c) xylulose standard, t_R = 6.3 min; (d) xylulose from celery leaves after removal of the phosphates from XuBP by incubation with alkaline phosphatase. Phosphates were removed by incubation of 60 μL extract plus 25 mM ammonium bicarbonate with about 0.3 IU *E. coli* alkaline phosphatase at pH 9 for 60 min at 37°C. Protein was precipitated by perchloric acid and neutralized by KOH.

7.5 respectively. The $t_{1/2}$ was 13.9, 3.5, and 1.6 min for pH 8.5, 8.0, and 7.5.

Rubisco-XuBP Dissociation Differs with pH

To determine the k_{off}, we incubated deactivated Rubisco with 20 μM XuBP for 30 min. After incubation, a 200-fold dilution was made with simultaneously addition of 10 mM $KHCO_3$ and 10 mM $MgCl_2$. The activation was measured at different time points after dilution (Fig. 5). A control experiment without XuBP was conducted at the same time. The data were expressed as the log of the remaining inhibition and plotted *versus* time, the slope was the k_{off}. The k_{off} at pH 8.5, 8.0, and 7.5 was 1.7×10^{-3} s^{-1}, 7.5×10^{-4} s^{-1}, and 1.8×10^{-4} s^{-1}, respectively. The $t_{1/2}$ was 65.6 min for pH 7.5, 15.3 min for pH 8.0 and 6.6 min for pH 8.5. The changes in the ratio, k_{off}/k_{on}, at different pH values are similar to the K_d values from the titration experiment (Table I).

XuBP Identified from Celery Leaves

Using the HPAE-PAD system to identify sugar bisphosphates from various leaf extracts, we have identified XuBP from celery. These leaves were first adapted to low light for 1 h and then frozen in liquid N_2. The results, shown in Figure 6, indicate that celery leaves contain a sugar-P_2 which is apparently identical with known XuBP. Following hydrolysis of the phosphate groups, the sugar was xylulose.

DISCUSSION

According to the mechanism of RuBP carboxylation proposed by Calvin (6), the first step catalyzed by Rubisco is deprotonation of the C-3 of RuBP to generate a 2,3-enediol which has a nucleophilic center. This first step, *i.e.* enolization, is the same reaction as the conversion of ribulose 5-P to xylulose 5-P catalyzed by ribulose 5-phosphate-3-epimerase. The difference is that ribulose 5-P 3-epimerase puts the proton back on the same carbon (C-3) with inversion of configuration, whereas Rubisco adds CO_2 or O_2 to the enediol before going to products.

It is not surprising that XuBP can be formed by Rubisco during catalysis because enolization of RuBP occurs quite readily without carboxylation (12, 21). Paech *et al.* (19) proposed that treatment of RuBP at pH 11 without Rubisco resulted in formation of XuBP by deprotonation and reprotonation of C-3 of RuBP. When we adjusted purified RuBP to pH 9.0 and let it sit at room temperature without Rubisco for 6, 12, and 24 h or incubated it at 50°C for 30 min, RuBP was partially degraded as determined by HPAE-PAD. However, we were unable to detect XuBP as a degradation product. With Rubisco and RuBP, the formation of XuBP occurred faster at pH 7.0 than higher pH. Conditions such as low pH with carbamylated sites, which are still favorable for RuBP binding and enediol formation but less favorable for carboxylation, increased the likelihood of reprotonation of the enediol with formation of XuBP.

XuBP, as an inhibitor of Rubisco activity, has been known for many years. McCurry and Tolbert (17) observed that 20 to 30 min preincubation of Rubisco and XuBP was needed for maximum inhibition. They also noted that inhibition by XuBP was competitive with RuBP but appeared noncompetitive after 20-min preincubation. This would be consistent with the longer time needed for decarbamylation of Rubisco before tight binding of XuBP.

The tight binding of XuBP for Rubisco is greatly affected by pH, similarly to that previously reported for RuBP binding (18). Lower pH increased the binding affinity for XuBP as shown by a reduced K_d. The pH affected both the binding rate constant (k_{on}) and the dissociation rate constant (k_{off}). This pH effect on tight binding for ligands occurred only with decarbamylated sites on Rubisco. CA1P tight binding, which occurs at carbamylated sites, was little affected by pH. The difference is that decarbamylated sites have no $^{A}CO_2$ bound to lysine ($K^{L8}201$), implying that this lysine influences inhibitor binding when not carbamylated (14).

In a solution with Rubisco, CO_2, Mg^{+2}, and XuBP, the following equilibrium will be established (22):

$$EX^* \underset{k_4}{\overset{k_3}{\Leftrightarrow}} EX \underset{k_2}{\overset{k_1}{\Leftrightarrow}} E + X \Leftrightarrow E + CO_2 \underset{k_{-c}}{\overset{k_c}{\Leftrightarrow}} EC + Mg^{2+} \underset{k_{-M}}{\overset{k_M}{\Leftrightarrow}} ECM$$

where E represents a decarbamylated site on Rubisco, X is XuBP, EX^* is tightly bound XuBP to a decarbamylated site, C is CO_2, M is Mg^{2+}, and ECM is a carbamylated site. K_d, the dissociation constant for XuBP, is k_2k_4/k_1k_3. $K_c = k_c/k_{-c}$ and $K_{Mg} = k_M/k_{-M}$ are the association constants for CO_2 and Mg^{2+}, respectively.

At equilibrium the concentration of EX^* will be:

$$[EX^*] = \frac{[X][ECM]}{K_dK_cK_{Mg}[C][M]}$$

The K_d values reported here represent an apparent K_d and not the true values; they represent an equilibrium measurement of the overall binding affinity which also includes decarbamylation. Also k_{on} and k_{off} involve decarbamylation and carbamylation. As also $K_d = k_{off}/k_{on}$, the two K_d can be compared: the values are quite similar (Table I). This also indicates that pH affects both the binding and dissociation rates. The apparent K_d for XuBP depends of pH and is between 30 nM to 2.1 μM between pH 7.0 to 8.5.

In plants, the stromal pH of chloroplasts changes with the light intensity. The pH of the stroma of isolated chloroplasts in the light was approximately 8.2 (13). In low light or dark, the stromal pH could be expected to be lower than 8. Kobza and Seemann (15) have noted that the enzyme extracted from leaves of spinach adapted to low light could not be restored to full activity upon incubation with saturating concentrations of CO_2 and Mg^{2+} at ice temperatures. In contrast, enzyme extracted from leaves in the dark or at saturating PPFD could achieve full activity under these conditions. They suggested that either RuBP or an inhibitor that binds only to the decarbamylated Rubisco had bound to the enzyme at low PPFD. It might be expected from observations of the present study that XuBP may be formed in low light-adapted chloroplasts where the pH is low and plenty of RuBP is present. Confirmation of this awaits further study.

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LITERATURE CITED

1. **Badger MR, Andrews JT, Canvin DT, Lorimer GH** (1980) Interaction of hydrogen peroxide with ribulose bisphosphate carboxylase oxygenase. J Biol Chem **255:** 7870–7875
2. **Bahr JT, Jensen RG** (1978) Activation of ribulose bisphosphate carboxylase in intact chloroplasts by CO_2 and light. Arch Biochem Biophys **185:** 39–44
3. **Berry JA, Lorimer GH, Pierce J, Seemann JR, Meek J, Freas S** (1987) Isolation, identification, and synthesis of 2-carboxyarabinitol 1-phosphate, a diurnal regulator of ribulose-bisphosphate carboxylase activity. Proc Natl Acad Sci USA **84:** 734–738
4. **Brooks A, Portis AR Jr** (1988) Protein-bound ribulose bisphosphate correlates with deactivation of ribulose bisphosphate carboxylase in leaves. Plant Physiol **87:** 244–249
5. **Byrne WL, Lardy HA** (1954) Pentose phosphates formed by muscle aldolase. Biochim Biophys Acta **14:** 495–501
6. **Calvin M** (1956) The photosynthetic carbon cycle. J Chem Soc 1895–1915
7. **Cardon ZG, Mott KA** (1989) Evidence that ribulose 1,5-bisphosphate (RuBP) binds to inactive sites of RuBP carboxylase *in vivo* and an estimate of the rate constant for dissociation. Plant Physiol **89:** 1253–1257
8. **Edmondson DL, Badger MR, Andrews TJ** (1990) A kinetic

characterization of slow inactivation of ribulose bisphosphate carboxylase during catalysis. Plant Physiol **93:** 1376–1382

9. **Edmondson DL, Badger MR, Andrews TJ** (1990) Slow inactivation of ribulosebisphosphate carboxylase during catalysis is not due to decarbamylation of the catalytic site. Plant Physiol **93:** 1383–1389
10. **Edmondson DL, Badger MR, Andrews TJ** (1990) Slow inactivation of ribulosebisphosphate carboxylase during catalysis is caused by accumulation of a slow, tight-binding inhibitor at the catalytic site. Plant Physiol **93:** 1390–1397
11. **Edmondson DL, Kane HJ, Andrews TJ** (1990) Substrate isomerization inhibits ribulosebisphosphate carboxylase-oxygenase during catalysis. FEBS Lett **260:** 62–66
12. **Gutteridge S, Parry MAJ, Schmidt CNG, Feeney J** (1984) An investigation of ribulosebisphosphate carboxylase activity by high resolution ^{1}H NMR. FEBS Lett **170:** 355–359
13. **Heldt HW, Werdan K, Milovancev M, Geller G** (1973) Alkalization of the chloroplast stroma caused by light-dependent proton flux into the thylakoid space. Biochim Biophys Acta **314:** 224–241
14. **Jordan DB, Chollet R** (1983) Inhibition of ribulose bisphosphate carboxylase by substrate ribulose 1,5-bisphosphate. J Biol Chem **25:** 13752–13758
15. **Kobza J, Seemann JR** (1988) Mechanisms for light-dependent regulation of ribulose-1,5-bisphosphate carboxylase activity and photosynthesis in intact leaves. Proc Natl Acad Sci USA **85:** 3815–3819
16. **McCurry SD, Gee R, Tolbert NE** (1982) Ribulose-1,5-bisphosphate carboxylase/oxygenase from spinach, tomato or tobacco leaves. Methods Enzymol **90:** 515–521
17. **McCurry SD, Tolbert NE** (1977) Inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase by xylulose 1,5-bisphosphate. J Biol Chem **252:** 8344–8346
18. **Mott KA, Berry JA** (1986) Effect of pH on activity and activation of ribulose 1,5-bisphosphate carboxylase at air level CO_2. Plant Physiol **82:** 77–82
19. **Paech C, Pierce J, McCurry SD, Tolbert NE** (1978) Inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase by ribulose-1,5-bisphosphate epimerization and degradation products. Biochem Biophys Res Commun **83:** 1084–1092
20. **Perchorowicz JT, Raynes DA, Jensen RG** (1981) Light limitation of photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Proc Natl Acad Sci USA **78:** 2985–2989
21. **Pierce J, Tolbert NE, Barker R** (1980) Interaction of ribulosebisphosphate carboxylase/oxygenase with transition-state analogues. Biochemistry **19:** 934–942
22. **Pierce J, Lorimer GH, Reddy GS** (1986) Kinetic mechanism of ribulosebisphosphate carboxylase: evidence for an ordered, sequential reaction. Biochemistry **25:** 1636–1644
23. **Portis AR Jr, Salvucci ME, Ogren WL** (1986) Activation of Rubisco bisphosphate carboxylase/oxygenase at physiological CO_2 and ribulosebisphosphate concentrations by Rubisco activase. Plant Physiol **82:** 967–971
24. **Seemann JR, Berry JA, Freas SM, Krump MA** (1985) Regulation of ribulose bisphosphate carboxylase activity *in vivo* by a light-modulated inhibitor of catalysis. Proc Natl Acad Sci USA **12:** 8024–8028
25. **Sicher RC, Hatch AL, Stumpf DK, Jensen RG** (1981) Ribulose-1,5-bisphosphate and activation of the carboxylase in the chloroplast. Plant Physiol **68:** 252–255
26. **Smrcka AV, Jensen RG** (1988) HPLC separation and indirect ultraviolet detection of phosphorylated sugars. Plant Physiol **86:** 615–618
27. **Zhu G, Jensen RG** (1990) Status of the substrate binding sites of ribulose bisphosphate carboxylase as determined with 2-C-carboxyarabinitol 1,5-bisphosphate. Plant Physiol **93:** 244–249
28. **Zhu G, Moon JW, Jensen RG** (1990) Synthesis of xylulose bisphosphate by Rubisco (abstract No. 713). Plant Physiol **93:** S-121
29. **Zhu G, Jensen RG** (1991) Fallover of ribulose 1,5-bisphosphate carboxylase/oxygenase activity. Decarbamylation of catalytic sites depends on pH. Plant Physiol **97:** 1354–1358