Slow Inactivation of Ribulosebisphosphate Carboxylase during Catalysis Is Not Due to Decarbamylation of the Catalytic Site

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ABSTRACT

An investigation was made of the proposal that the slow inactivation of ribulosebisphosphate carboxylase (Rubisco) activity, which occurs during in vitro assays, is due to decarbamylation of the enzyme. The level of carbamylation was compared with catalytic activity during assay conditions in which activity was both increasing and decreasing. Carbamylation level was measured using the reaction-intermediate analogue 2'-carboxy-D-arabinitol-1,5-bisphosphate (carboxyarabinitol-P2). A dual isotope procedure was used in which [14C]carboxyarabinitol-P2 measured total active sites and 13CO2 reported the level of carbamylation. The efficacy of the procedure was verified both in the presence and in the absence of the substrate d-ribulose-1,5-bisphosphate (ribulose-P2). These measurements showed that changes in activity during assays were not correlated with carbamylation status. Inactivation during assays initiated with both fully and partially carbamylated enzyme was not associated with any change in carbamylation level. This implies that the loss of activity during assays is not due to ribulose-P2 binding and sequestering the E form of the enzyme. Ribulose-P2 did not alter to appear to alter the equilibrium between carbamylated and uncarbamylated enzyme, but it did slow the rate at which enzyme was both decarbamylated and carbamylated. The most likely explanation for the loss of activity during assays appears to be the sequestration of carbamylated, Mg2+-bound active sites by an inhibitor.

Slow loss of Rubisco activity occurs during in vitro assays when reactions are initiated by the addition of the substrate, ribulose-P2, to CO2- and Mg2+-activated enzyme. This phenomenon, to which we have applied the term 'fallower,' is manifested as an apparent, first-order decline in catalytic rate to a lower final steady state rate, with a half-time of about 7 min (6). An understanding of what causes this decline in activity has generally been sought in terms of interactions occurring during the activation and inactivation of Rubisco by the reversible binding of CO2 and Mg2+ at the catalytic site (13). It has generally been assumed that this fallower is due to ribulose-P2 binding to and sequestering the noncarbamylated form of Rubisco (E) (12, 14, 16), thereby reducing the concentration of the catalytically competent CO2- and Mg2+-bound form (ECM). This theory has been based on experimental findings which show that phosphorylated sugars, including ribulose-P2, may bind to both the E and ECM forms of the enzyme at the catalytic site (2, 14). When bound to the ECM form, the activating CO2 and Mg2+ are stabilized on the enzyme. When bound to the E form, carbamylation is retarded. Depending on the relative affinity of these compounds for E and ECM, they may shift the equilibrium between these forms of the enzyme.

There have been several indications that ribulose-P2 binds tightly to the E form of Rubisco. For example, Jordan and Chollet (9) measured a dissociation constant for the E-ribulose-P2 complex of 21 nM at 2 °C, and Laing and Christeller (11) showed that activation of E was very slow in the presence of ribulose-P2. With such tight binding of ribulose-P2 to E, it could readily be expected that there should be a decline in the level of ECM during carboxylation, and conversely, that there should be very little increase in the level of ECM when E is added to ribulose-P2 in the presence of CO2 and Mg2+. In fact, it is thought that the enzyme, Rubisco activase, is required in vivo to promote activation in the presence of ribulose-P2 (20). Contrary to this, there are a number of reports of ribulose-P2 promoting carboxylation of Rubisco. Vater et al. (22) showed that ribulose-P2 induced a decrease in the dissociation constant for the EC complex similar to that seen with sugar phosphate activators such as 6-phosphogluconate, and NADPH (2, 5, 14). Additionally, Laing and Christeller (12) proposed that enhanced carbamylation by ribulose-P2 was responsible for a deviation from Michaelis-Menten kinetics at high ribulose-P2 concentrations.

Although the proposition that fallower is caused by tight binding of ribulose-P2 to E is consistent with most of the experimental data, it has yet to be proven. Indeed, there are times in vivo when the activity of Rubisco is not a direct reflection of its carbamylation level. For example, extracts of darkened leaves of some species show low levels of Rubisco activity, yet it can be demonstrated that the Rubisco is largely in the ECM form. The anomaly arises because of the presence of a phosphorylated inhibitor, 2'-carboxy-D-arabinitol-1-phosphate, which binds tightly at the catalytic site of the ECM form of Rubisco, rendering it inactive (21). This same possibility exists during fallower, where an inhibitor might bind tightly to the carbamylated active site, thus reducing activity

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2 Abbreviations: Rubisco, ribulose-P2, carboxylase-oxygenase (EC 4.1.1.39); ribulose-P2, d-ribulose-1,5-bisphosphate; carboxyarabinitol-P2, 2'-carboxy-D-arabinitol-1,5-bisphosphate.
without a decline in the carbamylation level. Indeed, the prevention of fallover by Rubisco activase (19) can be explained most readily in terms of the removal of such an inhibitor.

To test the various explanations for fallover, it is necessary to be able to measure the carbamylation state of the enzyme under various assay conditions. An effective measure of carbamylation level can be made using carboxyarabinitol-P2, an analogue of the carboxylation intermediate, 2-carboxy-3-keto-D-arabinitol-1,5-bisphosphate, to trap and measure the amount of Rubisco in the ECM form (15). We have modified and tested methods for reliable estimation of carbamylation level both in the presence and absence of ribulose-P2. Using these procedures, this paper examines the carbamylation state of Rubisco during fallover and other conditions. It is clear from the results that fallover does not occur because of a reduction in the level of carbamylated, Mg2+-bound Rubisco during catalysis.

**MATERIALS AND METHODS**

**Materials**

Rubisco, ribulose-P2, and phosphoribulokinase were prepared as described previously (6). [5-3H]carboxyarabinitol-P2 was prepared from [5-3H]ribulose-P2, as described by Pierce et al. (18). The [5-3H]ribulose-P2 was first prepared from [D-6-3H]glucose as described by Kuehn and Hsu (10). Bicine, 1,3-bis[tris(hydroxymethyl)methylamino]propane, bovine albumin, ATP, NADP, creatine phosphate, creatine kinase, and carbonic anhydrase were obtained from Sigma Chemical Co., and all other enzymes from Boehringer Mannheim. NaH[14]CO3 came from Amersham, [3H]-glucose from New England Nuclear, Sephadex from Pharmacia, and Chelex from Bio-Rad. All other chemicals were of the highest purity commercially available.

**Methods**

Rubisco assays were carried out generally as described previously (6). Specific details are given in the legends to the Figures. When necessary, Rubisco was decarbamylated by gel filtration through a PD-10 column of Sephadex G-25, equilibrated with CO2- and Mg2+-free 100 mM Bicine-NaOH (pH 8.3). This buffer was prepared with Chelex 100-treated water and sparged overnight with CO2-free N2, before adjusting the pH with CO2-free, saturated NaOH solution.

**RESULTS**

**Validation of the Method Used for Measuring the Carbamylation Level of Rubisco in the Presence of Ribulose-P2**

Carboxyarabinitol-P2 binds stoichiometrically to Rubisco. It shows biphasic binding to ECM, rapidly forming a loose complex, which undergoes a slow transition to a very tight complex (Kd < 10−11 M [18]), rendering the carbamate-CO2 non-exchangeable. Carboxyarabinitol-P2 binds much less tightly to E and EC (8, 18). If [14]CO2 is used in the assay, the label which remains with the high mol wt fraction following carboxyarabinitol-P2 trapping and gel filtration gives a measure of the proportion of carbamylated, Mg2+-bound enzyme (9, 14, 17). Since carboxyarabinitol-P2 binds more tightly to ECM than to E, it promotes carbamylation. To avoid measuring falsely high carbamylation levels, it is necessary to dilute the concentration and/or the specific radioactivity of the [14]CO2 in the trapping solution. An alternative method employs labeled carboxyarabinitol-P2, and relies on the observation of Pierce et al. (18) that carboxyarabinitol-P2 binds irreversibly only to the ECM form of Rubisco. Labelled carboxyarabinitol-P2 bound to E and EC can be exchanged for unlabelled carboxyarabinitol-P2.

The dual isotope method described in the legend to Figure 2 was used throughout these studies to measure the total number of Rubisco catalytic sites present and the fraction of them that were carbamylated during fallover. Slow inactivation was followed in the presence of [14]CO2, so that the active-site carbamate would be labeled with 14C. At intervals, a trapping solution containing [3H]carboxyarabinitol-P2 was added, together with a large excess of 12CO2 to ensure that any further carbamylation promoted by the presence of car- boxyarabinitol-P2 would be silent. The [14]CO2 bound to the enzyme measures the carbamylation state at the time of trapping, while the [3H]carboxyarabinitol-P2 measures the total number of catalytic sites. Therefore, the 14C/3H ratio of the bound label directly reflects the carbamylation status at the time of trapping. This approach assumes: (a) that the [3H] carboxyarabinitol-P2 will bind, eventually irreversibly, to all Rubisco sites present, displacing any other sugar phosphates (such as ribulose-P2) already bound; and (b) that this exchange of sugar phosphates is not accompanied by exchange of the 14C-carbamate.

Assumption (a) was verified by observing that the amount of 3H bound under all conditions was the same and reflected the total amount of Rubisco present (data not shown). Assumption (b) must also be substantially correct because, even when a saturating concentration of ribulose-P2 was present (in the presence of high CO2 and Mg2+ concentrations), the molar ratio between the bound 14C and 3H was always 0.75 or greater (Figs. 2, A and B, and 3A). This ratio ranged between 0.75 and 0.90 in all experiments at high CO2 concentration, whether or not ribulose-P2 was present (see also Table I, D–F; Fig. 1). Since the ratio is less than unity, it is possible that some limited exchange of the activator carbamate with the unlabelled CO2 occurred during trapping before all sites were irreversibly sequestered by carboxyarabinitol-P2. Alternatively, a slight systematic error in the specific radioactivities of either 14CO2 or [3H]carboxyarabinitol-P2 might explain ratios different from unity. In any event, the ratio was not altered by the presence of ribulose-P2. However, the substitution of carboxyarabinitol-P2 for ribulose-P2 was rather slow. Samples gel filtered 45 min after addition of the trapping solution had 5 to 10% less 3H bound than samples left for 135 min before gel filtration, indicating that tight binding of carboxyarabinitol-P2 was not quite complete in 45 min when ribulose-P2 was present. Therefore, trapped samples were routinely left overnight at room temperature before gel filtration.

The necessity for inclusion of a large excess of unlabelled
CARBAMYLATION OF RUBISCO DURING CATALYSIS

Figure 1. Exchange of $^{14}$C-labeled activator carbamate after addition of carboxyarabinitol-P$_2$. Rubisco (7 $\mu$g·mL$^{-1}$) was activated at 25 °C in a solution (total volume of 10.1 mL) containing 100 mM Bicine-NaOH (pH 8.3), 10 mM NaH$^{14}$CO$_3$ (100 Bq·nmol$^{-1}$), 20 mM MgCl$_2$, and 0.1% (w/v) bovine albumin. [H]Carboxyarabinitol-P$_2$ (500 Bq·nmol$^{-1}$) was added to 1 mL aliquots at either 10 $\mu$M (C) or 0.16 $\mu$M (C) and, at various times afterward, 1 mL of a mixture of NaH$^{14}$CO$_3$ and MgCl$_2$ was added to give final concentrations of 50 and 20 mm, respectively. The solutions were left for a further 45 min before application to a 0.9 x 25 cm column of Sephadex G-50 (fine), equilibrated with 100 mM Bicine (pH 8.3), 15 mM NaHCO$_3$, 20 mM MgCl$_2$, and 0.1% (w/v) bovine albumin. Aliquots (2.5 mL) of the pooled high-mol wt fraction were added to 1 mL of 0.1 M NaOH before addition of 15 mL of ACS II scintillation fluid (Amersham). The $^{14}$C/3H molar ratio, which is a measure of the fraction of total Rubisco sites which were carbamylated, was measured by liquid scintillation spectrometry with the external standard method of quench correction. CABP: carboxyarabinitol-P$_2$.

In the trapping solution was demonstrated by experiments in which decarboxylated, metal-free Rubisco was trapped in a solution containing CO$_2$ in the trapping solution was demonstrated by experiments in which decarboxylated, metal-free Rubisco was trapped in a solution containing CO$_2$. Full incorporation of $^{14}$C occurred (Table IE), even when addition of the $^{14}$CO$_2$ (and Mg$^{2+}$) was delayed 30 min after addition of [H]carboxyarabinitol-P$_2$ (Table IF). Thus, when CO$_2$, Mg$^{2+}$, and carboxyarabinitol-P$_2$ are present, all of the enzyme is eventually converted to the quaternary complex, regardless of the order of addition. This is consistent with weaker binding of carboxyarabinitol-P$_2$ to the decarboxylated form of Rubisco (18).

The trapping procedure used in these studies measures any carbamylated enzyme that exists in the absence of Mg$^{2+}$ as well as if it were uncarbamylated enzyme (Table IB) and this is not altered when ribulose-P$_2$ is present (Table IC). If any $^{14}$CO$_2$ is bound to metal-free sites, it must be able to exchange completely with the medium during subsequent binding of Mg$^{2+}$ and carboxyarabinitol-P$_2$.

Although the binding of carboxyarabinitol-P$_2$ to Rubisco is ultimately very tight, a preliminary loosen binding precedes a slow isomerization ($t_{1/2}$ = 17 s) to the ultimate tight complex (18). The concentration of carboxyarabinitol-P$_2$ used in the trapping procedure must be sufficient to saturate the preliminary, loose-binding step, which has a K$_1$ of 0.4 to 0.6 $\mu$M (18). Otherwise, any sites which remain uncomplexed during this loose-binding phase are free to exchange their labeled carbamate with unlabeled CO$_2$ in the trapping medium. This exchange at a low carboxyarabinitol-P$_2$ concentration (0.16 $\mu$M) can be readily demonstrated, as can its prevention by use of the saturating carboxyarabinitol-P$_2$ concentration (10 $\mu$M) used in all other experiments (Fig. 1).

The carbamyl-trapping procedure was used to determine whether the slow decline in activity during catalysis reflected

Carbamylation Levels During Fallover

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Table I. Effectiveness of the $^{14}$CO$_2$/[3H]Carboxyarabinitol-P$_2$ Procedure for Measuring Rubisco Carbamylation Levels

<table>
<thead>
<tr>
<th>Preincubation Solution Composition</th>
<th>Trapping Solution Components</th>
<th>$^{14}$CO$_2$/[3H]CABP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. E$^{14}$C NaH$^{14}$CO$_3$</td>
<td>[3H]CABP, NaH$^{14}$CO$_3$</td>
<td>0</td>
</tr>
<tr>
<td>B. E$^{14}$C NaH$^{14}$CO$_3$</td>
<td>[3H]CABP, NaH$^{14}$CO$_3$, MgCl$_2$</td>
<td>0.03</td>
</tr>
<tr>
<td>C. E$^{14}$CR NaH$^{14}$CO$_3$, ribulose-P$_2$</td>
<td>[3H]CABP, NaH$^{14}$CO$_3$, MgCl$_2$</td>
<td>0.02</td>
</tr>
<tr>
<td>D. E$^{14}$CM Na$^{14}$HCO$_3$, MgCl$_2$</td>
<td>[3H]CABP, NaH$^{14}$CO$_3$, MgCl$_2$</td>
<td>0.88</td>
</tr>
<tr>
<td>E. E No additions</td>
<td>[3H]CABP, NaH$^{14}$CO$_3$, MgCl$_2$</td>
<td>0.81</td>
</tr>
<tr>
<td>F. E No additions</td>
<td>[3H]CABP, then NaH$^{14}$CO$_3$, MgCl$_2$</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Effect of Ribulose-P$_2$ on Carbamylation Level

When fully carbamylated Rubisco was diluted into a CO$_2$ free, but otherwise standard, assay solution at pH 8.3, decarbamylation was very slow (Fig. 3A, filled circles). Omitting the ribulose-P$_2$ under these conditions resulted in a much more rapid rate of decarbamylation (Fig. 3A, open circles). Similar effects were obtained when the pH of the assay solution was 7.3, except that the declines in carbamylation level were more rapid both in the presence and absence of ribulose-P$_2$ (Fig. 3B).

Carbamylation Levels during Activation

When the standard assay for fallover was carried out at saturating CO$_2$, but commencing with fully decarbamylated Rubisco (E form) instead of the fully carbamylated (ECM) form, an exponential increase in rate was observed. Eventually, a final steady state rate was achieved which was the same as that attained after fallover of the ECM form (Fig. 4). During this slow activation, the carbamylation level progressively increased in parallel, but it did not stop increasing when the steady state catalytic rate was attained. Instead, it continued to increase slowly throughout the remainder of the assay period (Fig. 5). Probably, given sufficient time, full carbamylation would be eventually reached. When ribulose-P$_2$ was omitted, the carbamylation level increased very rapidly ($t_0 < 30$ s) to full carbamylation (Fig. 5).

**DISCUSSION**

**Efficacy of the Carboxyarabinitol-P$_2$ Trap**

The carboxyarabinitol-P$_2$ trapping method for the determination of ECM concentration has been shown here to give an accurate measure of ECM level, even in the presence of ribulose-P$_2$, providing that the mode in which carboxyarabinitol-P$_2$ binds tightly is considered and experimental procedures are adjusted to preclude possible artifacts (Table I; Fig. 1). The conditions of the trap should be as follows:

- The carboxyarabinitol-P$_2$ concentration must be many-fold greater than the dissociation constant for the initial loose binding of carboxyarabinitol-P$_2$ to ECM, to prevent exchange of carbamate-CO$_2$ during the loose-binding phase which could cause a false low reading (Fig. 1). Carboxyarabinitol-P$_2$ concentrations in excess of 10 $\mu$M should be used. High concentrations of carboxyarabinitol-P$_2$ are also required when high levels of ribulose-P$_2$ are carried over and the carboxyarabinitol-P$_2$ must compete with it during the initial binding phase. Otherwise, irreversible trapping may require an unacceptably long incubation with the trapping reagents. Even 10 $\mu$M carboxyarabinitol-P$_2$ cannot be considered a saturating concentration for the loose-binding phase when ribulose-P$_2$ is present to compete with it. However, ribulose-P$_2$ binding also prevents exchange of activator carbamate (Figs. 2 and 3). Even so, the trapping incubation should be prolonged beyond
The concentration or specific radioactivity of the \(^{14}\)CO\(_2\) used to label the carbamate-CO\(_2\) must be greatly diluted on addition of carboxyarabinol-P\(_2\), to prevent any E-form Rubisco acquiring \(^{14}\)CO\(_2\), since carboxyarabinol-P\(_2\) promotes carboxylation.

Providing these guidelines are followed, we have demonstrated that only Rubisco present as E\(^{14}\)CM will retain its \(^{14}\)C on incubation with carboxyarabinol-P\(_2\). Rubisco in any other form (E, E\(^{14}\)C, E-ribulose-P\(_2\), or E\(^{14}\)C-ribulose-P\(_2\)) did not retain \(^{14}\)C after formation of the quaternary ECM-carboxyarabinol-P\(_2\) complex.

**Carbamylation Levels During Fallover**

The results presented (Figs. 2 and 3) show clearly that fallover is not associated with a reduction in the level of carbamylated, Mg\(^{2+}\)-bound enzyme. No carbamate-CO\(_2\) was lost during fallover under the usual conditions (Fig. 2A) and, even under the more extreme conditions where the CO\(_2\) concentration was lowered at the time of addition of ribulose-P\(_2\), little or no loss occurred (Figs. 2B and 3A). Obviously, the presence of ribulose-P\(_2\) (and Mg\(^{2+}\)) at the active site prevents or greatly retards loss of the carbamate-CO\(_2\). Whatever effect ribulose-P\(_2\) may have on the carbamylation equilibrium (discussed below), loss of the carbamate-CO\(_2\) is much too slow to explain fallover. Ribulose-P\(_2\) may restrict loss of the carbamate-CO\(_2\) simply by steric effects. Its presence (together with Mg\(^{2+}\)) at the active site may hinder exchange of the carbamate-CO\(_2\) with the external medium. This concept is supported by crystallographic observations of the manner in which carboxyarabinol-P\(_2\) and, by inference, ribulose-P\(_2\), bind at the carbamylated active site (1).

**Effect of Ribulose-P\(_2\) on the Carbamylation Equilibrium**

Extensive carbamylation occurred, albeit slowly, when uncarbamylated Rubisco was exposed to CO\(_2\) and Mg\(^{2+}\) in the presence of ribulose-P\(_2\). This was shown both by an increase in catalytic activity and by direct measurement of carbamylation status (Figs. 4 and 5). The fact that carbamylation occurred at all when ribulose-P\(_2\) was present seems inconsistent with observations that ribulose-P\(_2\) binds 1000-fold more tightly to the uncarbamylated enzyme \((K_D = 21 \, \text{nm})\) than it does to the carbamylated form \((K_D(\text{ribulose-P}_2) = 20 \, \text{nm})\) (6). We suggest that a resolution of this apparent conflict might be sought in one of, or a combination of, the following possibilities:

The extremely tight binding of ribulose-P\(_2\) to uncarbamylated Rubisco was measured at 2 °C (9). Perhaps, in view of Rubisco’s known conformational sensitivity to temperature (3, 4, 23), the binding might be looser at 25 °C.

The equilibrium of the carbamylation reactions might be more in favor of carbamylation than previously thought, i.e. \(K_{dR} K_{MR}\), the product of the individual dissociation constants for carbamate-CO\(_2\) and Mg\(^{2+}\), might be substantially less than

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**Figure 3.** Effect of pH, ribulose-P\(_2\) and CO\(_2\) concentration on the decarbamylation of Rubisco. Two mg of fully \(^{14}\)CO\(_2\)/Mg\(^{2+}\)-activated Rubisco (130 Bq-nmol\(^{-1}\)) was added to 10 ml of a solution containing 100 mm CO\(_2\)-free 1,3-bis[(hydroxymethyl)methylamino]propanediol buffer (pH 8.3) (A) or 7.3 (B), 20 mm MgCl\(_2\), 0.1% (w/v) bovine albumin, and 1.5 mm NaHCO\(_3\) (A) or no NaHCO\(_3\) (C, D), in the presence (D) or absence (C) of 1 mm ribulose-P\(_2\). Carbamylation level was determined at intervals as described in the legend to Figure 2.

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**Figure 4.** Fallover (O, ---) of initially fully carbamylated Rubisco and activation (C, - - -) of initially fully decarbamylated Rubisco during carboxylation of ribulose-P\(_2\). For the experiment with initially fully carbamylated Rubisco, the \(^{14}\)CO\(_2\) fixation assays were as described in the legend to Figure 2, except that the reaction mixtures contained 25 mm NaH\(^{14}\)CO\(_3\) and 4 µg·mL\(^{-1}\) Rubisco. For the experiment with initially fully decarbamylated Rubisco, the reaction mixture was identical, but the order of addition was reversed: CO\(_2\) and Mg\(^{2+}\)-free Rubisco (see “Materials and Methods”) was added to an assay solution already containing ribulose-P\(_2\). The data were analyzed as described previously (6).
Figure 5. Effect of ribulose-P2 on the rate of carboxylation of initially decarbamylated Rubisco. The reaction mixtures contained 100 mM Bicine-NaOH (pH 8.3), 5 mM NaH14CO3 (100 Bq nmol−1), 20 mM MgCl2, 0.1% (w/v) bovine albumin. 0.1 mg mL−1 carbonic anhydrase, with or without 1 mM ribulose-P2, and were initiated by adding CO2 and Mg2+-free Rubisco (see "Materials and Methods") to a final concentration of 40 µg mL−1. One mL aliquots were withdrawn at intervals and added to 1 mL of a solution containing 100 mM Bicine-NaOH (pH 8.3), 20 µM [3H]carboxyarabinitol-P2, 100 mM NaH14CO3, 20 mM MgCl2, and 0.1% (w/v) bovine albumin for determination of the carboxylation level as described in the legend to Figure 2. For the reaction containing ribulose-P2, 50 µL aliquots were also withdrawn at intervals to measure acid-stable 14C, and thus the carboxylation rate (−−−), as described in the legend to Figure 2.

the value of approximately 1.5 × 103 µmol2 inferred from previous measurements (11, 13). If so, the binding of ribulose-P2 to the uncambamylated form might not be tight enough to prevent carboxylation when high concentrations of CO2 and Mg2+ are present.

The fallover phenomenon, itself, might be the cause of the anomaly. The conclusion drawn from the study described in the following paper (7) is that fallover occurs because an inhibitor derived from ribulose-P2 binds to the carboxamylated active site. If this inhibitor binds to carboxamylated Rubisco approximately as tightly as ribulose-P2 binds to the uncambamylated enzyme, the inhibitor's binding would tend to counterbalance the overall equilibrium.

These concepts obviously require further study. Nevertheless, the data of Figures 4 and 5 provide a convincing practical demonstration that carboxylation still occurs in the presence of ribulose-P2.

Cause of Fallover

These studies clearly show that fallover is a property of the fully carboxamylated enzyme. In the previous paper (6), the possibility that fallover is caused by readily reversible binding of ribulose-P2 at a non-catalytic, regulatory site was excluded. By a process of elimination, it now seems that the possibility must be considered that fallover reflects the sequestration of fully-carbamylated catalytic sites by an inhibitor. This possibility is addressed in the following paper (7).

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LITERATURE CITED

increase in bound activating CO₂: co-operativity between the subunits of the enzyme. J Expt Bot 36: 1396–1404