Simultaneous Kinetic Analysis of Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase Activities

0032-0889/80/65/0465/04/$00.50/0

SAMUEL S. KENT AND JOSEPH D. YOUNG
Department of Botany and Range Science, Brigham Young University, Provo, Utah 84602

ABSTRACT

An assay was developed for simultaneous kinetic analysis of the activities of the bifunctional plant enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase [EC 4.1.1.39]. [1-14C,5-3H]Ribulose-1,5-bisphosphate (RuBP) was used as the labeled substrate. Tritium enrichment of the doubly labeled 3-phosphohexcrate (3-PGA) product, common to both enzyme activities, may be used to calculate \( v_v/v_o \) ratios from the expression \( 1/(A/B-A) \) where \( A \) and \( B \) represent the \(^{3}H/^{14C} \) isotope ratios of doubly labeled RuBP and 3-PGA, and \( v_v \) and \( v_o \) represent the activities of carboxylase and oxygenase, respectively. Doubly labeled substrate was synthesized from [2,4,6,3H]glucose and [6,6-2H]glucose using the enzymes of the pentose phosphate pathway coupled with phosphoribulokinase.

The kinetic properties of a commercial preparation of fully activated spinach carboxylase were studied under approximated physiological conditions of 20% \( O_2 \) (252 micromolar), 295 \( \mu l/1 \) CO\(_2\) (10 micromolar), 25 C, and pH 8.19. The \( v_v/v_o \) ratio, was, within experimental error, constant at 30 seconds and 1 minute. This double label assay method may be used to calculate \( v_v/v_o \) ratios for the Laing-Ogden-Hageman equation, \( v_v/v_o = (V_v/k_v/V_o/k_o) (ICO_2/O_2) \) where \( V_v \) and \( V_o \) represent \( v_{max} \) and \( K_v \) and \( K_o \) represent Michaelis constants for the carboxylase and oxygenase activities, respectively.

The plant enzyme RuBP\(^1\) carboxylase/oxygenase (EC 4.1.1.39) represents nature's major port of entry of CO\(_2\) into the biosphere. The same enzyme also appears to be primarily responsible for photosynthetic inefficiency in C\(_3\) plants, namely the process of photorespiration whereby a fraction of the newly fixed carbon is lost to the atmosphere as CO\(_2\) (29). In the carboxylation reaction, CO\(_2\) condenses with RuBP to form 2 mol 3-PGA (28). The oxygenase reaction catalyzes the O\(_2\)-dependent cleavage of RuBP to yield 1 mol each of 3-PGA and PG (3, 6, 7, 15). The former product supports photosynthesis, but PG enters the carbon oxidation cycle, thus diminishing photosynthetic yield.

At the molecular level, CO\(_2\) and O\(_2\) are indeed competitive substrates in the carboxylase/oxygenase mechanism (4–6, 12, 14). Relative amounts of the two substrates define the ratio of products and hence (to a degree) the level of photorespiration. Although of limited practical application, photorespiration may be controlled in the field by a slight elevation of the partial pressure of CO\(_2\) (1, 6, 8). Alternatively, the problem of reducing photosynthetic inefficiency due to photorespiration must be attacked through principles of mutation. Detection of mutants by CO\(_2\) compensation point criteria has been a favored method of many investigators (13, 20, 22, 23; citations in ref. 13). A survey of gene pools of oats and soybeans by this method has revealed no cultivars with diminished photorespiratory activity (23), and several hundreds of plant species analyzed by Moss' group (13) produced (with two exceptions) narrow \( \Gamma \) values characteristic of either C\(_3\) or C\(_4\) plants. Bona fide evidence for the existence of mutant RuBP carboxylase/oxygenase enzymes is now forthcoming (9, 10, 18). Garrett (10) has correlated low rates of photorespiration with mutant enzyme activity in four tetraploid cultivars of rye grass. Compared to the (normal) diploid cultivars, the \( K_m \) (CO\(_2\)) of the tetraploid enzyme was significantly lower, and the oxygenase \( K_o \) (O\(_2\)) was unchanged. Garrett's report of increased tiller size and leaf extension rates in the tetraploid cultivars is reminiscent of Hardman and Brun's finding (11) that a 4-fold increase in atmospheric CO\(_2\) (which level represses photorespiration) could significantly boost the harvest index of soybeans. There is probably little dispute that RuBP carboxylase/oxygenase represents the major molecular-kinetic event which controls the balance between net photosynthesis and photorespiration. If modification of this event can be proven a significant and practical factor in enhancing crop yields, the detection of naturally occurring or induced mutants could prove invaluable to plant breeders or to the manipulations of genetic engineering. The present report describes a technique which is capable of detecting variations in the basic kinetic parameters of both enzyme activities monitored simultaneously in a single assay.

PRINCIPLE

Where [1-14C,5-3H]RuBP is used as the labeled substrate, the ratio of enzyme activities \( v_v/v_o \) may be calculated from the change in the isotope ratio \( (H_h/^{14C}) \) of the 3-PGA product relative to the original RuBP substrate. Reaction with CO\(_2\) yields equimolar amounts of [\(^1H\)J3-PGA] and [\(^14C\)J3-PGA], such that in the absence of any oxygenase activity the \( H_h/^{14C} \) ratio of the 3-PGA product remains unchanged relative to the RuBP substrate (Fig. 1). The oxygenase reaction generates only [\(^1H\)J3-PGA] and equimolar amounts of [\(^14C\)JPG]. The contribution of additional irradiated 3-PGA to the total 3-PGA pool by oxygenase turnover may be described mathematically permitting an exact calculation of the ratio of enzyme activities for the defined conditions of the assay. PG need not be analyzed. Derivation: let the \( H_h/^{14C} \) ratio of RuBP equal \( A \) such that

\[
A = y/x
\]

where \( y \) = the specific activity of [\(^1H\)JRuBP] and \( x \) = the specific activity of [\(^14C\)JRuBP].

Where \( v_v \) and \( v_o \) represent oxygenase and carboxylase velocities, respectively, the contribution of \(^1H\) and \(^14C\) to the 3-PGA product...
enzymes of establish Thus, the PGDH.

of enzymes. (carboxylase) (oxygenase) oxygenase powder), (3-PGA) (RuBP)

Fig. 1. Label mechanism describing 14C and 3H isotope contributions to the 3-PGA pool resulting from carboxylase and oxygenase turnover. Double label method defines the mode of carbon-carbon cleavage for the PgDH reaction. PG must be derived either from C-1,2 or C-3,4 of RuBP (excluding an unusual rearrangement reaction). A tritium enrichment of 3-PGA supports a 1,2 origin while a 14C enrichment would support a 4,5 origin. Former case is found in agreement with expectations.

of simultaneous reactions may be defined by the functions \( y(v_c + v_o) \) and \( x(v_o) \), respectively. Substituting \( v_c = v_o/R \) where \( R \) is the ratio of enzyme activities \( (v_c/v_o) \) yields the relationship:

\[
B = \frac{y(v_c + v_o/R)}{x(v_o)} \tag{2}
\]

where \( B \) is the measured ratio of 3H/14C in the 3-PGA product. Solving for \( y/x \) in equation 2 and substituting in equation 1 gives the relationship:

\[
\frac{v_c}{v_o} = \frac{A}{B - A} \tag{3}
\]

Thus, under defined conditions of pH, temperature, CO2, and O2 concentration, the ratio of enzyme activities \( (v_c/v_o) \) may be calculated from the 3H/14C isotope ratios of the RuBP substrate and the 3-PGA product.

MATERIALS AND METHODS

Enzymes and Substrates. D-[6-3H]Glucose (5,000 \( \mu \)mol) and D-[2-14C]glucose (30 \( \mu \)Ci/\( \mu \)mol) were purchased from Amersham/Searle. Hexokinase (EC 2.7.1.1) (type V from yeast, 45 units/mg lyophilized powder) 6-PGDH (EC 1.1.1.44) (type IV from yeast, 370 units/mg protein, in a 3.1 mM ammonium sulfate suspension-0.2 M glycylglycine (pH 7.4)), GDH (EC 1.1.1.49) (type IV from yeast, 220 units/mg protein crystallized and lyophilized sulfate-free powder), and mixed enzyme preparation of yeast hexokinase and GDH (150–175 units hexokinase and 75–90 units GDH crystallized and lyophilized), PRK (EC 1.1.1.19), ATP, and NADP were obtained from Sigma.

Synthesis and Isolation of Labeled Substrate. In a modification of the method of Wishnick and Lane (28), D-[1-14C,5-3H]RuBP was synthesized in two steps from labeled glucose using the enzymes of the pentose phosphate pathway coupled with PRK. To establish optimal conditions for the synthesis of Ru5P (step 1), reactions were monitored initially by following the manometric evolution of CO2. Final reaction conditions were as follows: 100 mM Tris (pH 7.84), 1.67 mM \( \alpha \)-d(+)-glucose, 3.33 mM ATP(Na)2, 6.67 mM NADP, 30 mM MgCl2, 2 mM KCl; 2 mM DTT, 0.4 mM EDTA(Na)2, 5–15 units hexokinase-GPDH mixture, and 1 unit GDPDH. The total reaction volume was 3 ml; the reaction was maintained at 35 C. Labeled glucose (10–50 \( \mu \)l) was added directly from stock to the reaction mixture with a Hamilton syringe. Final specific radioactivities for both isotopes were approximately 500,000 dpm/\( \mu \)mol. For routine syntheses 6.2 mg ATP(Na)2, 15.6 mg NADP, and 1 mg DTT were dissolved in 2 ml composite buffer and 50 \( \mu \)l 0.1 M carrier glucose, and adjusted to a total volume of 2.98 ml with filtered, glass-distilled, deionized H2O. The reaction was initiated with 5–15 units solid, lyophilized hexokinase-GPDH enzyme mixture. The GDPH from bakers' yeast is sensitive to sulfite ion (50% inhibition at 10 mM sulfite). Since 6-PGDH is stored as a suspension in 3.1 M ammonium sulfate and dialysis is impractical, 1 unit enzyme (20 \( \mu \)l) was added 10 min after initiating the reaction with hexokinase-GPDH. Under these conditions yields were consistently 95 ± 2%.

Attempts to synthesize labeled RuBP from starting substrate in a single, coupled reaction have met with unsatisfactory results. Due to contaminating enzymes in the crude, commercial preparation of PRK, the side products depressed yield; the two-step synthesis described herein increases yield slightly. This problem may be circumvented ultimately with purified enzyme preparations.

Ru5P was isolated as described below and used as the substrate for the synthesis of RuBP (step 2). Final reaction conditions were as follows: 100 mM Tris (pH 8.09), 5 mM ATP(Na)2, 5 mM MgCl2, 1 mM EDTA, 2 mM DTT, 2–7 \( \mu \)mol Ru5P, and 10 units PRK in a total reaction volume of 2 ml. At the end of a 4h reaction starting with 5 \( \mu \)mol of substrate, the pH typically dropped from 8.09 to 7.41 due to the generation of H+ upon hydrolysis of ATP. The reactions were quenched with 0.5 ml 12% HClO4 and neutralized with 0.180 ml 6 M KOH. Precipitated protein and KClO4 were removed by centrifugation and, in the case of RuBP, samples were chromatographed immediately. Otherwise, RuBP is stable in acid and may be stored prior to neutralization and chromatography.

Sephadex QAE-25 Chromatography. Ru5P and RuBP were isolated from contaminating components on a column (30 × 0.9 cm) of Sephadex QAE-25 with a stepwise gradient of 0.2 M and 0.3 M NH4HCO3. The flow rate was 0.4 ml/min. Substrates and products were identified by radioactivity profiles. Nucleotide by-products, detected by A profiles at 366 nm, did not contaminate the labeled sugar phosphates. Since RuBP slowly decomposes at alkaline pH, the ammonium bicarbonate buffers were adjusted to pH 7.4 with HCl. The resin was preswashed with 2 M ammonium bicarbonate. Pooled fractions of doubly labeled Ru5P product were exhaustively lyophilized to remove the ammonium bicarbonate which volatilizes as NH3 and CO2. Precautions must be taken to prevent loss of labeled substrate during lyophilization (caused by sudden microexplosions and escape of solid ammonium bicarbonate from the vessel). The [1-14C,5-3H]RuBP product (500,000 dpm/\( \mu \)mol in each isotope) was taken up in a volume such that the addition of 100 \( \mu \)l to a 0.5 ml reaction volume for the RuBP carboxylase/oxygenase assay would give the final RuBP concentration of 0.6 mM. Aliquots were removed with a 1-\( \mu \)l Hamilton syringe for counting. Specified volumes were transferred to conical mini-vials (Applied Science Labs., State College, Pa.) for storage at –86 C.

Assay Conditions. The reaction cell was gassed with O2 and CO2 to give final concentrations of 252 and 10 \( \mu \)m, respectively. Assay components were injected into the reaction cell, and the reactions were terminated with HClO4. The internal pressure was maintained at 760 ± 1 mm Hg, and temperature was regulated to within ±0.1 C at 25 C.

RuBP Carboxylase/Oxygenase Assay. A commercial preparation of spinach carboxylase was purchased from Sigma. The enzyme was supplied as a lyophilized preparation and for these assays had the advantage of functioning as a single, reproducible source. Prior to using [1-14C,5-3H]RuBP as the assay substrate, routine 14C fixation assays were used to define the conditions for linear reaction kinetics and 100% activation of the enzyme over the time span of the assay. The stated specific activity of the commercial preparation was 0.014 units/mg lyophilized solid.
(protein). A unit was defined as the fixation of 1 μmol CO₂ fixed/min at pH 7.8 and 25 C. The commercially defined units apparently refer to the turnovers of inactivated enzyme. Enzyme was activated according to the principles described by Andrews et al. (3) and Lorimer et al. (16, 17); 5 mg was suspended in 2 ml O₂-free 100 mM Tris (pH 8.6) containing 20 mM MgCl₂, 10 mM bicarbonate, and 1 mM DTT. After a preincubation period of 15 min at 25 C, aliquots were assayed in O₂-free 100 mM Tris (pH 8.19) containing 0.6 mM RuBP, 20 mM MgCl₂, 5 mM DTT, and variable amounts of [¹⁴C]bicarbonate. At saturating levels of 20 mM [¹⁴C]bicarbonate, 12.5 μg enzyme in a reaction volume of 0.5 ml yielded a rate of 14.7 nmol [¹⁴C]CO₂ fixed/min. This rate corresponds to a specific activity of 1.1 unit/mg solid (protein). For the enzyme range studied, this rate was proportional to the enzyme concentration. The rate was strictly linear for the 1st 2 min of the reaction decreasing to 15% of linearity at 4 min. The rate was similarly linear at limiting CO₂ concentrations, particularly at the 10-μM level of CO₂ used in the double label assay.

For the assay of carboxylase/oxygenase, the final reaction conditions were as follows: 80 mM Tris (pH 8.2), 20 mM MgCl₂, 0.650 mM bicarbonate (in equilibrium with 10 μM CO₂), 0.6 mM [¹⁴C]bicarbonate and 0.05 nCi of [¹³C]bicarbonate, and 5 mM DTT. The reaction (in 490 μl) was gassed with 20% O₂ and 295 μl/1 CO₂ (approximate atmospheric conditions) for 15 min under a pressure of 760 mm Hg and at a temperature of 25 C. The reaction volume was contained in a conical cell and was continuously mixed with a triangular stirring bar. Activated enzyme (12.5 μg) in 0.1 ml was injected into the closed system, and the reaction was terminated after 1 min with 250 μl of 12% HClO₄. After neutralizing with 90 μl 6 M KOH and removing the precipitated KClO₄, an aliquot was chromatographed on a Dowex 2 (chloride) column (0.9 x 30 cm) developed with 0.05 M HCl-0.1 M NaCl (5).

**Double Label Counting.** Aliquots from the Dowex 2 column were counted in ACS scintillation-counting fluid (Amersham/Searle) with a Beckman 100-C scintillation counter. A series of quenched standards was used to establish quench correction curves for double label counting. Linear regression curves describing quench corrections and efficiency were combined with calculations for A/(B-A) and programmed for a direct printout of v, v₀ ratios.

**RESULTS**

[²H] /[¹⁴C]Isotope ratios for the intermediates of the sequence (α-δ-glucose to Ru5P to RuBP) were typically 1.09, 1.14, and 1.13, respectively. The slightly lower value of 1.09 for the starting substrate, α-δ-glucose, does not involve hydrogen exchange but was correlated with a trace contaminant in the α-δ[²H]glucose stock. Under the conditions of the RuBP carboxylase/oxygenase assay described above, the [²H]/[¹⁴C] ratio in 3-PGA increased to values of 1.61 ± 0.05. Tritium enrichment reflects the contribution of oxygenase activity over that of carboxylase; carboxylase turnover alone generates equal amounts of [²H]3-PGA and [¹⁴C]3-PGA.

The conditions of these experiments approximate the normal physiological state, namely 25 C, 252 μM O₂ (20% O₂), 10 μM CO₂ (295 μl/l CO₂), and pH 8.2 (higher than normal stromal pH). For essentially comparable conditions (25 C, 20% O₂, 10 μM CO₂, and pH 8.5), Laing et al. (14) have calculated from kinetic constants a v/v₀ range of 0.3–0.5 (or a v/v₀, range of 2–3.3). The v/v₀ ratios in this study have varied from 2.1 to 2.6.

The problem of RuBP instability was circumvented by storage of substrate at −86 C. Using a single stock of RuBP (stored in separate aliquots), the v/v₀ values over a 9-month period were (in sequence) 2.3, 2.1, 2.4, 2.6, and 2.4. Where steady-state initial velocities are maintained over the course of the assay, the v/v₀ ratios should remain constant and independent of the time of the reaction. This condition appears to have been satisfied; the v/v₀ ratios were 2.4 and 2.1 after 30 and 60 s, respectively. Table 1 demonstrates the theoretical variation in v/v₀ and Kₑ as a function of B, assuming the constancy of other kinetic parameters. Although several sources of error are probable in these initial determinations, it is possible that ultimate refinements may be able to distinguish variations in Kₑ on the order of ±5 μM or less.

**Table 1. Hypothetical Variance of Kₑ with B and v/v₀, with B**

<table>
<thead>
<tr>
<th>B</th>
<th>v/v₀</th>
<th>Kₑ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.35</td>
<td>5.14</td>
<td>23</td>
</tr>
<tr>
<td>1.40</td>
<td>4.19</td>
<td>29</td>
</tr>
<tr>
<td>1.45</td>
<td>3.53</td>
<td>34</td>
</tr>
<tr>
<td>1.50</td>
<td>3.05</td>
<td>39</td>
</tr>
<tr>
<td>1.55</td>
<td>2.69</td>
<td>45</td>
</tr>
<tr>
<td>1.60</td>
<td>2.40</td>
<td>50</td>
</tr>
<tr>
<td>1.65</td>
<td>2.17</td>
<td>55</td>
</tr>
<tr>
<td>1.70</td>
<td>1.98</td>
<td>61</td>
</tr>
<tr>
<td>1.75</td>
<td>1.82</td>
<td>66</td>
</tr>
<tr>
<td>1.80</td>
<td>1.69</td>
<td>71</td>
</tr>
</tbody>
</table>

Error in determination of v/v₀, and in Kₑ, is expressed as a function of B, [²H]/[¹⁴C] ratio for 3-PGA. Other variables have been held constant arbitrarily for purpose of analyzing error. True values assumed are: A = 1.13, B = 1.65, v/v₀ = 2.4, [O₂] = 252 μM, [CO₂] = 10 μM, V,Kₑ/v₀ = 3024 μM, and Kₑ = 50 μM.

**DISCUSSION**

One of the inherent problems in the assay of RuBP carboxylase by [¹⁴C]CO₂ fixation has been the inability to maintain controlled concentrations of [¹⁴C]CO₂ in solution in an open system at low levels of substrate. In the present assay system, problems attending volatile [¹⁴C]CO₂ (and constant concentration) are overcome by constant gassing with [¹²C]O₂ and O₂ under controlled conditions of temperature and pressure. Carboxylase/oxygenase enzyme activity ratios are measured directly and calculated from isotope ratios of a nonvolatile substrate and product. Enzyme activities are measured simultaneously such that pH, temperature, and enzyme preparation (and degree of activation) error are obviated. Variations on the concept of the double (or a single) label assay system encounter alternate technical problems and/or problems of expense. In a single label assay system using [¹⁴C]RuBP, v/v₀ may be calculated directly from the ratio of labeled PG and 3-PGA products; the products, however, must be obtained quantitatively. Combinations of labeling with [³²P], [¹⁴C], or [³H] encounter difficulties of synthesis and additional decay calculations in the case of [³H]. The use of [¹³C]CO₂ in conjunction with [⁵²P]RuBP is identical in concept and calculations to the present system and circumvents the problems of expense, but sacrifices precision obtainable with a nonvolatile-labeled substrate.

The activation state of the enzyme may be critical for any valid comparison of activities of enzymes from different sources. If the activation of carboxylase and oxygenase occurs as a result of identical molecular events, then, for the purposes of comparing enzymes from different sources, v/v₀, ratios, but not absolute rates, would be independent of degree of activation. If, however, carboxylase and oxygenase may be differentially activated, then the comparisons and other kinetic evaluations would be valid only for 100% activation of both enzyme activities. These alternatives are addressed somewhat by the mutually competitive nature of CO₂ and O₂ and the simultaneous inhibition of both enzyme activities by xylitol diphosphate (22) and xylulose diphosphate (21); observations suggest that the two enzymes share a single catalytic site.

Velocities of the oxygenase and carboxylase activities are directly proportional to fixed O₂ and CO₂ concentrations as described in the relationship of Laing et al. (14):

\[ \frac{v}{v_0} = \frac{V}{K_v} \times \frac{[CO_2]}{[O_2]} \]

(4)
In the double label assay system, the function \( A/(B-A) \) is similarly proportional to the gaseous substrate concentrations such that

\[
\frac{A}{B - A} = \frac{V \cdot K_a}{V \cdot K_a [CO_2]} \cdot [O_2]
\]

Of the four potentially variable kinetic parameters \( V_\infty, V, K_a, K_c \), the ratio \( V_\infty/V \) is constant as a function of temperature since activation energies of the two enzyme functions are comparable (14). \( K_c \) does not change significantly as a function of pH (21, 25) or temperature (14, 26). \( K_a \) does change significantly as a function of temperature (14). Ogren (23) has suggested that mutation would most likely be correlated with a change in \( K_c \); since \( K_c \) is a polar molecule and \( O_2 \) a nonpolar molecule, a charged amino acid substitution would (in altering the dielectric constant in the vicinfty of the active site) affect carboxylation more than oxygenation (relative to the effect of a nonpolar amino acid substitution on either enzyme activity). This prediction appears to be substantiated by the findings of Garrett (10).

In these initial determinations of \( V_\infty/V \), the potential sources of error include the double label scintillation counting procedures, the isotopic purity of substrate and product, and the composition and pressure of the gas mixtures. The latter problem may be overcome by using premixed tanks of gases. Scintillation-counting errors are greatly reduced by counting samples under identical conditions and using doubly labeled standards to monitor day-to-day variation. In the present report, the \( ^3H/^{14}C \) isotope ratios for the RuSP precursor and the RuBP product differed not more than 3%. Other available technology on the market may reduce even this error.

The immediate technical disadvantage of the double label method for the analysis of RuBP carboxylase/oxygenase is the complexity of equipment and a relatively complex set of procedures for the synthesis and storage of a relatively unstable (and expensive) substrate. Equipment, however, may be constructed with relatively streamlined features. A large batch synthesis of labeled RuBP may provide substrate of known \( ^3H/^{14}C \) ratio for long periods. RuBP has proven to be stable (with regard to isotope ratio, chromatographic properties, and reproducibility in the assay) when stored for extensive periods at \(-86^\circ\) C. Substrate probably may be maintained for indefinite periods at liquid \( N_2 \) temperatures (\(-196^\circ\) C).

The over-all advantage of the double label system is that variables are inherently under tight control. With a series of reaction cells gassed simultaneously and maintained at the same constant temperature and pressure, \( CO_2 \) and \( O_2 \) concentrations are homogeneous. Where RuBP possesses a constant \( ^3H/^{14}C \) ratio for the assay of enzymes from different sources, the only variable remaining is the variation in the \( ^3H/^{14}C \) ratio of 3-PGA which variation would signal a change in the kinetic properties of a given enzyme.

Acknowledgments—The authors wish to thank Professor John Westley of the University of Chicago for consultation on the kinetic aspects of RuBP carboxylase/oxygenase and Professor Robert H. Daines for his support and encouragement of this project.

LITERATURE CITED


4. BADGER MR, TJ ANDREWS 1974 Effects of \( CO_2 \) and temperature on a high-affinity form of ribulose diphosphate carboxylase-oxygenase from spinach. Biochem Biophys Res Commun 60: 204-210


19. MCCURRY SD, NE TOLBERT 1977 Inhibition of ribulose 1,5-bisphosphate carboxylase/oxygenase by xylulose-1,5-bisphosphate. J Biol Chem 252: 8344-8346


