Measurement of Protein Degradation in Leaves of Zea mays Using [3H]Acetic Anhydride and Tritiated Water

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ABSTRACT

The rate of protein degradation in Zea mays leaves has been estimated by using tritiated water and [3H]acetic anhydride as the labeling agents. Both methods circumvent many of the problems usually associated with measuring protein degradation in plants. The half-life of ribulose-1,5-bisphosphate carboxylase protein in second leaves of 13-day-old seedlings under continuous light was found to be 7.8 ± 0.9 days by the tritiated water technique and 6.5 ± 0.8 days by the [3H]acetic anhydride method. The half-lives determined under a 14-hour-light, 10-hour-dark photoperiod are 6.2 ± 0.8 days with tritiated water and 5.4 ± 0.4 days with [3H]acetic anhydride. Whereas the values obtained by the two methods do not differ significantly, the use of either method for the determination of protein half-life can be recommended.

The demonstration of the continuous synthesis and degradation of plant proteins (26) has led to the realization that protein turnover is an integral part of cell differentiation and function. Unfortunately, many of the rates of protein turnover reported in the literature are inaccurate, due to the compartmentalization of plant cells and the recycling of labeled precursors (10). These problems can be minimized by using the tritiated water technique of Humphrey and Davies (11), and this method has been applied to obtain more precise estimations of the rate of protein degradation in Lemma minor under various conditions (5, 6, 7).

The problem of precursor reutilization can be circumvented also by the use of [3H]acetic anhydride, a technique originally used in mammalian tissue culture (21). This compound labels preexisting protein by acetylating primary amino groups, including the amino-terminus and the e-amino group of lysine. This method has been adapted for use with intact Zea mays plants by Simpson (23), who reported that the half-life of RuBPCase from the third leaves of 27-day-old maize plants growing under a 14-h-light, 10-h-dark photoperiod and high nutrient conditions was 3 days.

The synthesis and degradation of RuBPCase have also been studied by other workers in different plants and by using different techniques with conflicting results. Peterson et al. (20) reported that, in the first leaf of 6-day-old barley seedlings kept in continuous light following [14C]O2-labeling, the amount of RuBPCase protein and the radioactivity of this protein remained constant, indicating that no degradation was occurring. In continuous darkness, there was a loss of RuBPCase protein and a low level of incorporation of 13C02 into the enzyme. These workers suggest that the majority of the RuBPCase is synthesized in the light and degraded in the dark, and that any turnover (i.e. concomitant synthesis and degradation) occurs. In separate experiments, Kannangara et al. (13) reported a net loss of RuBPCase protein after completion of expansion of Perilla leaves, indicating that degradation exceeds synthesis at this time. Similar findings were reported for wheat leaves (2). In senescing leaves, RuBPCase protein is lost (9, 19), and its synthesis is depressed (2, 4). These findings, which report different values for the turnover or degradation of RuBPCase protein, may be due to species-specific differences, or they may reflect the different methods used for measurement.

In this paper, we describe two independent methods for studying protein degradation which avoid reutilization of labeled amino acids into newly synthesized proteins. Using labeled acetic anhydride and tritiated water to label proteins, we have measured the rate constants of degradation and half-lives of soluble protein and RuBPCase protein in Z. mays leaves.

MATERIALS AND METHODS

Biochemicals. With the following exceptions, all reagents were obtained from Sigma and were analytical grade: reagents for gel electrophoresis (Bio-Rad); NCS solubilizer, D,L-2(n)-[3H]glutamic acid (20 mCi/μmol), and [3H]acetic anhydride (500 mCi/mmol; Amersham-Searle); l-1-[U-14C]glutamic acid (225 mCi/mmol; New England Nuclear); and Freund's adjuvant (Difco).

Plant growth. For the experiments comparing the half-life of protein degradation by the two different methods, Z. mays (cv. Kelvedon 33) seedlings were grown in vermiculite in continuous light at 25°C for 9 days. Seedlings were then carefully removed to 2-liter beakers containing 600 ml half-strength mineral medium (24) (20 seedlings/beaker) and grown with continuous aeration for an additional 4 days, either under continuous light or under a 14-h-light, 10-h-dark photoperiod. Respiration and photosynthesis of acetylated and control leaf sections were measured both in the light and in the dark with an O2 electrode and recorder.

Immuno precipitation of RuBPCase Holoenzyme. Antibodies were raised against spinach RuBPCase (purified as described by Wishniak and Lane [27]) in two female white New Zealand rabbits by subcutaneous injection of 5 mg/rabbit in complete Freund's adjuvant with boosters of 2 mg/rabbit in incomplete Freund's at 3- or 4-week intervals. Blood was collected by ear bleeding.
Protein degradation in maize (Zea mays)

Presumptions were obtained from each rabbit for controls. Sera were stored at -20°C. Antiserum precipitated corn leaf enzyme at an equivalence of 200 μg/ml serum with extract containing 80 to 100 μg homogenate determined with pure spinach homologous protein as a standard. RuBPCase from [3H]acetic anhydride-labeled or tritiated water-labeled leaf extracts (100 μl aliquots) was immunoprecipitated with 200 μl of lyophilized antiserum to RuBPCase (reconstituted as 170 mg/ml in distilled H2O). The mixtures were incubated at 37°C for 1 h and then centrifuged for 10 min at 10,000 g. The immunoprecipitates were washed in phosphate-buffered saline (5% w/v; pH 7.3), reagent and resuspended in 1 ml 0.1 N NaOH. A small aliquot (100 μl) was removed for measurement of protein (17), and, by reference to a standard curve prepared by using authentic RuBPCase, the content of RuBPCase protein was determined. The remainder of the immunoprecipitated protein and of the total extract was precipitated in cold 10% trichloroacetic acid, and radioactivity in the precipitate was measured by liquid scintillation spectrometry (Packard). SDS-polyacrylamide gel electrophoresis (see below) of the immunoprecipitate from [3H]acetic anhydride-labeled corn leaves showed labeled 550,000, 60,000, 30,000, and 16,000 dalton proteins, corresponding to the holoenzyme, large subunit, dimer of small subunit, and small subunit of RuBPCase, respectively.

Polyacrylamide Gel Electrophoresis. TCA precipitated proteins or immunoprecipitates were dissolved in electrophoresis buffer and submitted to electrophoresis in either SDS or non-denaturing native polyacrylamide gels (23). SDS gels (10% polyacrylamide) were run at 200 mg/gel (16 mm2 area) for 5 h at room temperature. Native gels (5% polyacrylamide) were run at 0.5 mg/mg overnight at 5°C. Bromophenol blue was used to mark the front. Gels were stained in Coomassie Blue R (1% in 7.5% acetic acid, 5% methanol) and scanned at 540 nm in a Gilford model 240 recording spectrophotometer to locate the bands. They were then sliced to 1 mm in a Bio-Rad electrophoresis gel slicer (model 190), digested with NCS tissue solubilizer (Amersham/Searle) at 50°C for 2 h, cooled, and counted in toluene cocktail in a Packard Tri Carb liquid scintillation spectrometer.

Labeling of Plant Proteins for Degradation Studies.

In Vitro Acetylation. RuBPCase, partially purified from 14-day-old maize leaves by the procedure of Lorimer et al. (16), was reacted with [3H]acetic anhydride by the addition of acetic anhydride to give 500 mol acetic anhydride/mol enzyme (18). The extent of acetylation achieved was determined by measuring radioactivity in immunoprecipitated protein (below). Enzyme activity was assayed by4CO2 fixation (1).

In Vivo Acetylation. Third leaves of 3-week-old maize plants were labeled with [3H]acetic anhydride (500 μCi/μmol) by applying 25 μCi in 2.5 ml benzene or tolune with a 5-μl Hamilton syringe. The seedlings were left for 6 h before the zero time sample was taken. Samples consisting of five leaves were taken on specified days, weighed, and frozen. Acetylated leaves were homogenized in 0.15 M K-phosphate (pH 7.5) containing 10 mM sodium acetate. This extract was filtered through cheesecloth and centrifuged at 30,000 g for 15 min, and the supernatant was passed through an Amicon filter (PM10; 10,000 mol wt exclusion limit) to remove low molecular weight compounds which interfere with immunoprecipitation. The high molecular weight retentate was used for immunoprecipitation with RuBPCase antisera.

In Vivo Double Labeling with Amino Acids. To double label RuBPCase in vivo, individual excised leaves were fed through the transpiration stream as follows: 25 μCi [14C]glutamic acid (225 mCi/μmol; New England Nuclear) for 5 h; then 1 mm glutamic acid overnight; then deionized water until the conclusion of the experiment when 25 μCi [2,3-3H]glutamic acid (20 mCi/μmol; Amersham/Searle) was given to each leaf for 5 h. Seventeen hours after the initiation of [14C]glutamate labeling, five of the 10 leaves were given unlabeled acetic anhydride (10.5 μmol, as determined by reaction with hydroxylamine (15), per leaf in 2.5 μl benzene) applied to the upper leaf surface. The interval between this modification of the leaf protein with cold acetic anhydride and exposure to the second 3H label was 24 h in one experiment and 72 h in another. At the conclusion of the [3H]glutamine labeling period, each leaf was individually homogenized in 0.15 M K-phosphate (pH 7.5) containing 10 mM sodium acetate and 40 mM glutamate (3 ml/g fresh weight). This extract was then processed and immunoprecipitated as described above.

In Vivo Labeling with Tritiated Water. Nine-day-old maize seedlings were transferred to mineral medium containing tritiated water (0.5 mCi/ml), labeled for 3 days, and then washed in several changes of unlabeled medium for one more day. Preliminary experiments indicated that one day was sufficient to chase excess 3H2O from the leaves. Samples consisting of five leaves were taken on successive days, weighed, and frozen until required. The leaves were ground in 100 mM K-phosphate (pH 7.5); the extracts were centrifuged at 10,000 g for 10 min, and the supernatants were used as a source of total soluble protein. Low molecular weight compounds were removed by passage through 12.0 × 3.5-cm columns of Sephadex G25, and the extracts were concentrated to a volume of 1.0 ml by using Aquacide (Calbiochem). Aliquots (100 μl) of extracts were removed for determination of total protein and for immunoprecipitation. The 2-[3H]-content of the hydrolyzed protein amino acids was determined by racemization with acetic anhydride as described by Humphrey and Davies (11).

RESULTS

In Vivo Labeling of Corn Leaves with [3H]Acetic Anhydride Results in Modification of Many Proteins in All Cell Types. When 25 μCi of [3H]acetic anhydride (500 μCi/μmol) were applied to a corn leaf, 1% of the label was recovered as TCA-precipitable material. Immunoprecipitation of RuBPCase with RuBPCase antisera showed that the enzyme contained 30,000 cpm/mg or an average modification of one out of every 200 RuBPCase molecules in each leaf. Examination of extracts of labeled leaves by polyacrylamide gel electrophoresis showed that many salt-extractable (0.15 M K-phosphate [pH 8.0]) and SDS-soluble proteins are acetylated to about the same extent (data not shown). The [3H]acetic anhydride applied to the upper leaf surface penetrates across the leaf and labels all cell types as shown by light microscope autoradiography (Fig. 1).

Antibody to Spinach RuBPCase Holoenzyme Specifically Precipitates Both Acetylated and Nonacetylated Corn Leaf Holoenzyme. Antisera to spinach RuBPCase holoenzyme was characterized for its cross-reactivity with corn holoenzyme in leaf extracts. It was necessary to filter all leaf extracts through Sephadex G-25 gel filtration columns or Amicon filters (PM10, 10,000 mol wt exclusion limit) to remove low molecular weight compounds contributing to nonspecific precipitation of radioactivity (10-20% of total radioactivity precipitated).

To determine whether acetylation of RuBPCase interferes with its precipitation by antibody, partially purified corn leaf enzyme was acetylated to different extents in vitro with [3H]acetic anhydride, and the amount of protein precipitated was determined at each level of modification. Acetylation of greater than 50 residues per holoenzyme molecule, far above the in vivo level of modification, did not interfere with antibody precipitation (Fig. 2).

Acetylated RuBPCase is Enzymically Active. To determine the effect of acetylation on enzymatic activity of RuBPCase, acetylation was performed in the presence and absence of its substrate, RuBP. Enzymic activity was assayed by4CO2 fixation (1). The reaction rate was linear for 2 min and dependent on RuBP and on enzyme (data not shown). Partially purified RuBPCase, modified in the presence of 5 mM RuBP, bound 26 mol acetic anhydride per mol enzyme and showed enzymic activity to be 74% of that of the
Application of label to the upper leaf surface has resulted in even labeling across the leaf. (Top, × 530; bottom, × 1860)

Acetylation in the absence of RuBP resulted in approximately the same extent of acetylation (22 mol acetic anhydride per mol enzyme), but enzymic activity was much lower, 32% that of the control (unmodified, −RuBP). RuBP apparently protects a critical site, possibly the active site, of the enzyme from acetylation. The protection of RuBPCase enzymic activity by substrate levels of RuBP during modification by a lysine-reactive reagent was previously shown by Schloss and Hartman (22) using the affinity label 3-bromo-1,4-dihydroxy-2-butanone, 1,4-bisphosphate. Since the level of RuBP in the chloroplast in the light is about 0.5 mm (14), in vivo acetylation also occurs under RuBP excess, analogous to the in vitro conditions which result in modified, active RuBPCase. In vivo acetylation results in an average of one mol acetic anhydride per mol modified RuBPCase; this is, those RuBPCase molecules which are modified are acetylated on one residue only, on average.

Acetylated Enzyme Is Not Degraded Faster Than Unmodified Enzyme in Vivo. To determine whether acetylated RuBPCase is degraded faster than unmodified enzyme, double labeling experiments were carried out. Excised maize leaves were labeled with [14C]glutamate and then, after an interval of 24 or 72 h, with [3H]glutamate. Half of the leaves were treated with unlabeled acetic anhydride (10.5 μmol/leaf) 12 h after [14C]glutamate feeding. Degradation of RuBPCase would result in a loss of label, so preferential degradation of acetylated RuBPCase would be detected as a higher [3H]:[14C] ratio of RuBPCase from acetylated leaves relative to that from unmodified leaves. Application of 10.5 mol of acetic anhydride (AcAn) per mol of RuBPCase protein.
μmol acetic anhydride per leaf results in modification of 33% of the RuBPCase molecules, based on labeling of leaves at several levels of acetic anhydride (data not shown). With the observed standard deviation in the [H] [C] ratio, this level of acetylation would be sufficient to reveal differential degradation of RuBPCase if it occurs. The observation (Table I) that the [H][C] ratios of immunoprecipitated RuBPCase protein from control and acetylated leaves are not significantly different indicated that acetylated RuBPCase is not degraded faster than unmodified enzyme. Taken with the evidence given above that enzyme acetylated in the presence of RuBP is not inactivated, these results show that acetylation does not induce degradation of RuBPCase; therefore, the loss of radioactivity from the enzyme over time may be used as an assay of in vivo physiological degradation.

Measurement of Protein Degradation. Table II shows the changes in soluble protein and RuBPCase protein which occur in second leaves of 13-day-old maize over the 6-day time course of a typical experiment, both under conditions of continuous light and under a 14-h-light, 10-h-dark photoperiod. In continuous light, the soluble protein remains essentially constant, but it falls slightly under the light/dark conditions. Under both light regimes,

![Graph](https://via.placeholder.com/150)

**Fig. 3.** Enzymic activity of in vitro modified (acylated) and unmodified RuBP carboxylase. Partially purified RuBP carboxylase was reacted with [H]acetic anhydride (500 μCi/μmol) to achieve a level of modification of 5 acetylated residues per enzyme molecule. There were four treatments: 1) -RuBP, unmodified RuBPCase; 2) +RuBP, unmodified enzyme incubated in the presence of 5 mM RuBP; 3) -RuBP + acetic anhydride, enzyme acetylated in the absence of 5 mM RuBP; and 4) +RuBP + acetic anhydride, enzyme acetylated in the presence of 5 mM RuBP. Acetylation was performed on ice in Hepes buffer (18). Enzyme activity was assayed by [C] fixation (1) and is expressed as cpm of [C]CO₂ fixed at each time point.

Table I. Double Labeling of Excised Leaves with U[14C]/[Glutamic Acid and [2,3-3H]/[Glutamic Acid, with and without Unlabeled Acetic Anhydride

<table>
<thead>
<tr>
<th>Lag Interval</th>
<th>1H/[14C] Ratio Control Leaves</th>
<th>1H/[14C] Ratio Acetylated Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.42 ± 0.04</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>72</td>
<td>0.55 ± 0.03</td>
<td>0.51 ± 0.05</td>
</tr>
</tbody>
</table>

The [H] [C] ratio of immunoprecipitated RuBPCase is given for control (unacetylated) and treated (acylated) leaves for each of two experiments. In the 1st experiment, there was a 24-h lag between application of unacetylated acetic anhydride and feeding of [H] glutamate. In the 2nd experiment, there was a 33-period. Each value is the mean ± SD of separate determinations on five leaves.

The RuBPCase protein falls by about 25% in the course of the experiment, most of the loss occurring between days 4 and 6. With each of the methods used to estimate degradation in the present study, a plot of log specific activity of [H] in protein against time should yield a straight line, the slope of which multiplied by ln 10 should give the first-order rate constant of degradation, kd. The half-life (t₁/₂) can be calculated from the equation t₁/₂ = ln 2/kd. Figure 4 shows typical semilogarithmic plots for both the tritiated water (8A) and the [H]acetic anhydride (8B) methods, the data representing soluble protein degradation in continuous light. The slopes of these lines, determined by least-squares analysis, lead to the values for kd and t₁/₂ in Table III which also contains data for soluble protein degradation under conditions of 14-h-light, 10-h-darkness, and for RuBPCase degradation under both light regimes. Table III shows that there is no statistically significant difference between the values for t₁/₂ determined by the tritiated water method and those obtained by using [H]acetic anhydride.

Unlabeled Acetic Anhydride Does Not Affect Degradation Rate

Table II. Total Soluble Protein and RuBPCase Protein per Gram Fresh Weight in 13-Day-Old Second Leaves of Z. mays Reared under Continuous Light or 14-h-Light, 10-h-Dark

<table>
<thead>
<tr>
<th>Day</th>
<th>Total Protein</th>
<th>RuBPCase Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>L/D</td>
</tr>
<tr>
<td>0</td>
<td>18.3 ± 1.9 (7)</td>
<td>19.3 ± 1.1 (7)</td>
</tr>
<tr>
<td>1</td>
<td>20.0 ± 2.9 (5)</td>
<td>21.9 ± 0.7 (6)</td>
</tr>
<tr>
<td>2</td>
<td>18.4 ± 1.3 (5)</td>
<td>19.2 ± 2.9 (7)</td>
</tr>
<tr>
<td>3</td>
<td>17.6 ± 1.5 (6)</td>
<td>17.9 ± 2.4 (6)</td>
</tr>
<tr>
<td>4</td>
<td>17.4 ± 2.3 (4)</td>
<td>17.3 ± 2.2 (3)</td>
</tr>
<tr>
<td>5</td>
<td>17.3 ± 1.0 (2)</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>16.3 ± 1.9 (4)</td>
<td>15.5 ± 1.8 (3)</td>
</tr>
</tbody>
</table>

* L, treated with continuous light; L/D, treated with 14-h-light, 10-h-dark photoperiod.

**b ND, not determined.**

![Graph](https://via.placeholder.com/150)

**Fig. 4.** Measurement of total soluble protein degradation in second leaves of 13-day-old maize in continuous light using A, the tritiated water method of Humphrey and Davies (11), or B, the [H]acetic anhydride method of Simpson (23). Radioactivity per mg of TCA precipitable protein was measured daily for 6 days and is expressed as percentage of total at zero time.
Determined by Using Tritiated Water as Labeling Agent. To check whether that acetic anhydride does not induce enhanced degradation of protein to any significant extent, maize leaves were treated either with an amount of unlabeled acetic anhydride (in toluene) or with 10.5 μmol acetic anhydride (in toluene) per leaf. Degradation of soluble protein was measured under continuous light by the tritiated water method. The \( t_{1/2} \) obtained (mean of 3 experiments ± se) was 8.1 ± 0.9 days in the first case and 7.7 ± 0.8 days in the second case. These values do not differ significantly from those obtained without applied acetic anhydride (cf. Table III).

Oxygen Uptake and Evolution of Leaves are Unaffected by Acetic Anhydride Treatment. To determine whether acetic anhydride or benzene (the solvent in which it is stored and applied to leaves) affects the respiration or photosynthetic rates of treated leaves, the capacities of control and acetylated leaves to evolve \( O_2 \) in the light and to consume \( O_2 \) in the dark were measured. These results showed that acetylation did not affect the rate of \( O_2 \) consumption or release (data not shown). It is probable that this treatment did not cause any basic damage to cell structure.

**DISCUSSION**

The foregoing results indicate that acetylation of maize leaves in vivo with \([^{3}H]\)acetic anhydride results in the specific labeling of RuBPCase as well as a large range of other proteins in all cell types. Acetylated RuBPCase retains enzymatic activity (Fig. 3) and is not degraded more rapidly than nonacetylated proteins (Table I). This latter conclusion is substantiated by the fact that the two methods used to estimate protein degradation provide values of \( t_{1/2} \) which do not differ significantly for RuBPCase protein (Table III). In addition, \( t_{1/2} \) values for degradation of total soluble protein, as determined by both procedures, agree well (Table III). Although in general it is believed that modified proteins are catalyzed faster (8), the extent and nature of the modification in this case appears insufficient to allow the acetylated protein to be recognized as abnormal. Also, in certain systems, N-acetylation confers stability and protection against proteolysis (12, 25), although there is evidence to the contrary (3).

It seems that \([^{3}H]\)acetic anhydride provides a valuable tool for the measurement of protein degradation, and we recommend either this method or the tritiated water method for the more accurate estimation of rates of proteolysis in plants, depending on circumstances. For example, the free-floating habit of *Lemma minor* makes it ideally suited for use with the tritiated water method and totally unsuitable for use with the \([^{3}H]\)acetic anhydride technique. Our attempts to measure protein degradation in *Lemma* by using \([^{3}H]\)acetic anhydride failed, as the plant is very sensitive to even small volumes of toluene and acetic anhydride.

On the other hand, acetic anhydride labeling is potentially of great value for studies of protein degradation in other situations. This method can be applied to whole plants and, thus, be used in field situations. Labeling of tissue with acetic anhydride containing different isotopes just before and just after a developmental change occurs (e.g., germination of pollen [Roberts, R. M., unpublished observations] or seeds, exposure of leaf tissue to light) and observation of changes in labeled proteins should give information on the turnover, cleavage, or association of proteins during the new developmental state. Acetic anhydride may be particularly useful for dry seeds, since it can be applied as a vapor and, thus, not affect the water status of the seeds.

It is of interest that RuBPCase is degraded with a \( t_{1/2} \) of approximately 7 days (Table III) under conditions in which the level of RuBPCase protein remains constant (day 0–4 of a typical experiment; Table II). This implies that, even under continuous light, there must be concomitant degradation and synthesis (i.e., turnover) of the protein. Peterson *et al.* (20) reported no turnover of RuBPCase in barley leaves in the light and concluded that, although the enzyme can be both degraded and synthesized, the processes are not concomitant, degradation taking place during periods of darkness. Preliminary experiments indicate that there is incorporation of tritiated water into RuBPCase protein (i.e., synthesis) occurring over the same time period used to estimate degradation, both in continuous light and under a 14-h-light, 10-h-dark photoperiod.

Previous reports (23) on the use of the \([^{3}H]\)acetic anhydride method on maize leaves give a \( t_{1/2} \) value of 3 days for RuBPCase degradation. However, in this work a different cultivar of maize (cv. Earlking) was grown under high nutrient conditions (added N, P, K, and Fe EDTA), and leaves of a different age and developmental stage were used. The different \( t_{1/2} \) of degradation obtained in the present study by the same method is, thus, expected. When plants of the same cultivar are grown under identical conditions and treated with either \([^{3}H]\)acetic anhydride or tritiated water and labeled acetic anhydride, the resulting values for \( t_{1/2} \) of RuBPCase protein are the same, validating the use of the \([^{3}H]\)acetic anhydride and tritiated water methods.

We have demonstrated that \([^{3}H]\)acetic anhydride provides a valuable tool for the reliable measurement of protein breakdown in plants and that the tritiated water method for estimating protein degradation can be successfully applied to plants other than *Lemma*. We have also obtained evidence for the concomitant synthesis and degradation (i.e., turnover) of RuBPCase in leaves of *Z. mays*.

**Acknowledgments**—The suggestions and advice of Joe Varner are gratefully acknowledged. We thank Jane Oliver for providing valuable technical assistance and John Jacobs for reviewing the manuscript.

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*Table III. The Half-Life and Rate Constant of Degradation of Soluble Protein and RuBPCase Protein in 13-Day-Old Second Leaves of Z. mays under Continuous Light or 14-h-Light, 10-h-Dark.*

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Method</th>
<th>Total Protein</th>
<th>RuBPCase Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( t_{1/2} )</td>
<td>( t_{1/2} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days</td>
<td>( \text{day}^{-1} )</td>
</tr>
<tr>
<td>L(^a)</td>
<td>( ^{3}H_{2}O )</td>
<td>7.5 ± 0.7</td>
<td>-0.10 ± 0.01</td>
</tr>
<tr>
<td>L</td>
<td>([^{3}H] acetic anhydride)</td>
<td>6.7 ± 0.3</td>
<td>-0.10 ± 0.01</td>
</tr>
<tr>
<td>L/D</td>
<td>( ^{3}H_{2}O )</td>
<td>6.9 ± 0.4</td>
<td>-0.10 ± 0.01</td>
</tr>
<tr>
<td>L/D</td>
<td>([^{3}H] acetic anhydride)</td>
<td>6.2 ± 0.9</td>
<td>-0.11 ± 0.02</td>
</tr>
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</table>

\(^a\) L, treated with continuous light; L/D, treated with 14-h-light, 10-h-dark photoperiod.
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