The Mechanism to Suppress Photosynthesis Through End-Product Inhibition in Single-Rooted Soybean Leaves during Acclimation to CO₂ Enrichment

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Single-rooted soybean leaves were used to investigate the suppression of photosynthesis through end-product inhibition during acclimation to CO₂ enrichment. The photosynthetic activity was greater in leaves cultured at a CO₂ partial pressure of 70 Pa (high-CO₂) than that in the leaves cultured at 35 Pa CO₂ (control) during the initial exposure to CO₂ enrichment but then decreased rapidly with a large accumulation of starch, to well below the level of the control leaves. The response curve of photosynthesis (A) to the intercellular CO₂ concentration (Ci) in the high-CO₂ leaves cultured long-term exhibited a significantly low initial gradient. However, on exposure to darkness for 48 h, the initial gradient of the A to Ci curve and rate of photosynthesis were completely restored, and almost all of the accumulated starch was expended. The ribulose bisphosphate carboxylase (RuBPcase) content and activation ratio in the high-CO₂ leaves remained high and roughly constant during the experiment, and were unchanged by the exposure, while this enzyme was slightly inactivated or inhibited after long-term exposure to CO₂ enrichment. The lower rate of photosynthesis in the high-CO₂ leaves could be linearly increased to a rate approaching the control level by increasing the external atmospheric [CO₂], which thereby compensated for a reduced CO₂ transfer diffusion from the intercellular space to the stroma in chloroplasts. It is consequently concluded that, during the acclimation to CO₂ enrichment, the suppression of photosynthesis through end-product inhibition was mainly caused by a lowering of the carboxylation efficiency of RuBPcase due to hindrance of CO₂ diffusion from the intercellular space to the stroma in chloroplasts brought about by the large accumulation of starch.

Key words: Acclimation to elevated CO₂ — Carboxylation efficiency — CO₂ transfer conductance — End-product inhibition — Ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) — Starch accumulation.

Abbreviation: RuBPcase, ribulose-1,5-bisphosphate carboxylase.

Introduction

Many studies have reported that CO₂ enrichment generally stimulates photosynthesis and accelerates plant growth (for reviews, see Stitt 1991, Drake et al. 1997, Makino and Mae 1999). However, the stimulation of photosynthesis caused by CO₂ enrichment decreases on subsequent exposure to elevated levels of CO₂, and then sometimes even a suppression of photosynthesis occurs. Stitt (1991) suggested that the suppression of photosynthesis during acclimation to enhanced levels of CO₂ is related to an inadequate demand for carbohydrates in the remainder of the plant. Furthermore, he suggested that differences in the long-term response of photosynthesis to CO₂ enrichment may be explained by differences in the sink-source status of plants, depending upon the species, the developmental stage and the developmental conditions. Thus, it is difficult to generalize the long-term response of photosynthesis to elevated CO₂.

Two mechanisms have been proposed whereby the accumulation of carbohydrates during the acclimation to elevated levels of CO₂ could lead to a direct inhibition of photosynthesis (for review, see Stitt 1991, Makino and Mae 1999). First, a large accumulation of starch may physically disrupt the chloroplasts and decrease CO₂ diffusion from the intercellular space to the stroma in chloroplasts. Second, high levels of carbohydrates could lead to a feedback inhibition of carbohydrate synthesis, with the result that photosynthesis is inhibited because P_i is not recycled rapidly enough. Stitt (1991), furthermore, suggested the possibility of additional feedback mechanisms in which carbohydrates could indirectly cause a decrease in the levels of proteins and other components of the photosynthetic apparatus.

To study the source-sink status of plants, Sawada et al. (1986) have developed a simple source-sink model plant, a single-rooted soybean leaf, with one leaf as the sole source organ and the root system as the sole sink organ. They have used this model plant to clarify the regulatory mechanism of photosynthetic metabolism that is responsible for the end-product inhibition operating in sink-limited plants (Sawada et al. 1986, Sawada et al. 1987, Sawada et al. 1989, Sawada et al. 1990, Sawada et al. 1992, Anwaruzzaman et al. 1995).

The purpose of the present study was to investigate the mechanism to suppress photosynthesis through end-product inhibition in plants cultured long-term in a high CO₂ atmosphere. The effects of CO₂ enrichment on photosynthetic characteristics and levels of carbohydrates and proteins in the photosynthetic apparatus were examined for the single-rooted soybean leaves in order to determine the contribution of direct and indirect factors to the suppression of photosynthesis.
Photosynthetic suppression by high CO$_2$ through end-product inhibition during the acclimation to CO$_2$ enrichment.

Results

The growth and photosynthetic characteristics of rooted leaves cultured at high CO$_2$

When single-rooted leaves were cultured at a CO$_2$ partial pressures of 35 (control) and 70 Pa (high-CO$_2$), the root and leaf dry weights per leaf area increased at almost constant rates during the experimental period of 15 d in the culture (Fig. 1). However, the rates of the increase in both organs were much greater in the high-CO$_2$ leaves than the control leaves.

The photosynthetic activity determined at a CO$_2$ partial pressure of 35 Pa in the control leaves peaked on the 9th day of the culture, and this rate was maintained thereafter (Fig. 2). On the other hand, the activity at a CO$_2$ partial pressure of 70 Pa in the high-CO$_2$ leaves reached a maximum on the 6th day much higher than that at the peak in the control leaves, then decreased rapidly, and on the 15th day reached 76% of the control value. The activity on the 15th day was just 64% of that at the peak on the 6th day in the same leaves.

Fig. 3 indicates that the response curve of photosynthesis (A) to CO$_2$ partial pressure in the intercellular space (Ci) for the high-CO$_2$ leaf, which was cultured for 8 d and had high photosynthetic activity (see Fig. 2), exhibited an initial gradient and CO$_2$ response above an intercellular CO$_2$ partial pressure of 40 Pa near to the respective values of the control leaf. However, the A to Ci curve of the high-CO$_2$ leaves which were cultured for 14 and 16 d and had significantly low photosynthetic activities exhibited very low initial gradients, being 54 and 34% of the control value, whereas the CO$_2$ responses were higher than the control value.
Photosynthetic suppression by high CO₂

Fig. 4 shows that, in rooted leaves cultured both for 10 or 11 d under the control condition and for 7 or 8 d under high CO₂ conditions, the photosynthetic activities increased with the increase in CO₂ partial pressure from 35 to 70 Pa and approached saturated values near 150 Pa. On the other hand, the activity in the high-CO₂ leaves cultured for 16 or 17 d increased linearly with the increase in CO₂ partial pressure from 35 to 150 Pa, and, at 150 Pa, approached to a certain extent both values in the former two leaves.

Fig. 5 shows that, in the high-CO₂ leaves, the starch and sucrose contents increased more rapidly and were 10- and 3.6-fold the respective values in the control leaves on the 15th day.

Fig. 6 shows that, in rooted leaves cultured under both the control and the high CO₂ conditions, the amount of RuBPcase protein increased slightly and similarly with the amount of soluble proteins, and both amounts differed little between the two leaves during the experiment. Therefore, the ratio of RuBPcase to soluble protein in both leaves was relatively constant during the experiment with a mean value of 58 and 54%, respectively.

Fig. 7 indicates that the initial and total activities of RuBPcase in the high-CO₂ leaves remained roughly constant during the period from the 6th day to 12th day and were close to the respective values in the control leaves, while on the 15th

Table 1  Effects of CO₂ and P↓ on activation of RuBPcase in the single-rooted soybean leaves cultured for 15 d under the control or high-CO₂ conditions

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Activity of RuBPcase (mol CO₂ (mol enzyme)⁻¹ s⁻¹ ± SD)</th>
<th>Activation ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial activity</td>
<td>Total activity</td>
</tr>
<tr>
<td></td>
<td>Pre-incubation with (CO₂ + Mg²⁺)</td>
<td>Pre-incubation with (P↓ + Mg²⁺)</td>
</tr>
<tr>
<td>Control</td>
<td>4.76±0.12</td>
<td>6.43±0.03</td>
</tr>
<tr>
<td>High-CO₂</td>
<td>3.93±0.05</td>
<td>5.45±0.14</td>
</tr>
</tbody>
</table>

Assays of the initial and total activities of RuBPcase are the same as those shown in Fig. 7. The concentrations of NaHCO₃, NaHPO₄ and MgCl₂ in the pre-incubation medium were 10, 10 and 10 mM, respectively. Each value is the mean ± SD of four determinations.
day both activities in the former leaves were decreased slightly to about 80% of the respective values in the latter leaves. The activation ratios of RuBPcase in both leaves were almost constant at 80% during the experiment.

Table 1 shows that, by pre-incubation with NaHCO$_3$ plus MgCl$_2$ and with NaHPO$_4$ plus MgCl$_2$, the RuBPcase in the control and high-CO$_2$ leaves cultured for 15 d were similarly activated, and the activation ratios were not much different between the two leaves. However, the initial activities and the total activities after both pre-incubations in the high-CO$_2$ leaves were about 80% of the respective values in the control leaves.

**Effects of dark treatment on photosynthetic characteristics and carbohydrate contents in the rooted leaves cultured at high CO$_2$**

In another experiment, the rooted leaves cultured for 15 d were exposed to darkness to remove the end-product inhibition by expending the carbohydrates accumulated in the leaves.

Table 2 shows that, when the high-CO$_2$ leaves cultured for 15 d were exposed to darkness for 48 h, the starch content decreased significantly to 28% of the value before the treatment, while the sucrose content decreased slightly to 72%. On the other hand, in the control leaves, the former content decreased to 54%, while the latter content was almost unchanged. Both values in the high-CO$_2$ leaves increased markedly from the 15th to 17th day, while, in the control leaves, they were practically constant.

Table 3 indicates that, on exposure of the high-CO$_2$ leaves to darkness, the suppressed photosynthetic activity was restored to a near-maximum value, while, in the control leaves, the activity was almost unchanged. In addition, the activity in the high-CO$_2$ leaves decreased from the 15th to 17th day, whereas the activity in the control leaves was almost unchanged. Furthermore, the content and the initial and total activities of RuBPcase in both rooted leaves cultured under the control and high CO$_2$ conditions were little affected by the exposure to darkness and roughly constant from the 15th to 17th day, while both activities in the high-CO$_2$ leaves were about 80% of the respective values in the control leaves. There-
Some of the control and high-CO₂ leaves cultured for 15 d were exposed to darkness for 48 h. Each value is the mean ± SD of four determinations.

**Table 2** Effects of darkness on sucrose and starch contents in single-rooted soybean leaves cultured under the control or high CO₂ conditions

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Days in culture</th>
<th>Sucrose content (g m⁻² ± SD)</th>
<th>Starch content (g m⁻² ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>0.84±0.05</td>
<td>4.02±0.08</td>
</tr>
<tr>
<td>Dark (48 h)</td>
<td>17</td>
<td>1.05±0.11</td>
<td>4.01±0.54</td>
</tr>
<tr>
<td>High CO₂</td>
<td>15</td>
<td>0.92±0.07</td>
<td>2.18±0.39</td>
</tr>
<tr>
<td>Dark (48 h)</td>
<td>17</td>
<td>2.60±0.18</td>
<td>14.99±1.28</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>3.24±0.17</td>
<td>23.46±0.79</td>
</tr>
<tr>
<td></td>
<td>Dark (48 h)</td>
<td>1.87±0.18</td>
<td>4.15±0.77</td>
</tr>
</tbody>
</table>

Some of the control and high-CO₂ leaves were little affected by the exposure.

**Discussion**

**Suppression of photosynthesis through end-product inhibition in sink-limited leaves by CO₂ enrichment**

When the single-rooted leaves were grown at the ambient CO₂ partial pressure of 35 Pa (control leaves), the photosynthetic activity determined at the CO₂ partial pressure of 35 Pa peaked on the 9th day of the culture, and this rate was maintained thereafter (Fig. 2). On the other hand, the activity at 70 Pa CO₂ in the rooted leaves grown at the high CO₂ partial pressure of 70 Pa (high-CO₂ leaves) reached a maximum at first that was much higher than the control value, but then rapidly decreased finally attaining a significantly low level. Hence, the high-CO₂ leaves cultured long-term showed a distinct suppression of photosynthesis during the acclimation to enhanced CO₂. At the same time, the starch and sucrose contents in the high-CO₂ leaves increased markedly throughout the experimental period compared to those in the control leaves (Fig. 5). Therefore, there was a clear negative correlation between starch and sucrose contents and photosynthetic activity (Fig. 8). It is consequently concluded that the suppression of photosynthetic activity during the acclimation to enhanced CO₂
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is brought about through the end-product inhibition by an accumulation of carbohydrates in the sink-limited leaves.

Possible causes for suppression of photosynthesis by CO₂ enrichment in the sink-limited leaves

The A to Ci curve for the high-CO₂ leaves cultured for just a few days exhibited an initial gradient close to the high value for the control leaves (Fig. 3). The high-CO₂ leaves cultured long-term, however, exhibited significantly lower initial gradients. This response of A to Ci and the results mentioned above indicate that the suppression of photosynthesis during the acclimation to elevated CO₂ levels is caused by the lowering of the carboxylation efficiency of RuBPcase through end-product inhibition in the sink-limited leaves.

Previous studies have suggested that the lowering of the carboxylation efficiency of RuBPcase along with the accumulation of carbohydrates in the sink-limited leaves caused by CO₂ enrichment has four possible causes (for review, see Makino and Mae 1999): (1) physical damage of chloroplasts by extreme enlargement of starch grains (Cave et al. 1981, DeLucia et al. 1985, Yelle et al. 1989, Pritchard et al. 1997); (2) limitation of P₇-regeneration capacity in chloroplasts (Sharkey 1985, Sivak and Walker 1986, Sage and Sharkey 1987, Stitt and Quick 1989, Sawada et al. 1989, Sawada et al. 1990); (3) loss of RuBPcase capacity as proposed from the initial gradient of the response curve of photosynthesis to the intercellular CO₂ concentration (Sage 1994) and; (4) reduction in CO₂ diffusion from the intercellular space to the stroma in chloroplasts caused by a large accumulation of starch (Nafziger and Koller 1976, Makino et al. 1994, Nakano et al. 2000).

Physical damage of chloroplasts

When the high-CO₂ leaves cultured for 15 d were exposed to darkness for 48 h, the starch content decreased significantly to 28% of the value before the treatment and the suppressed photosynthetic activity was perfectly restored to a near-maximum value (Table 2). This result clearly indicates that the physical damage to chloroplasts caused by the extreme enlargement of starch grains as well as the disruption to electron transport resulting from this damage did not contribute to the lowering of the carboxylation efficiency of RuBPcase in the high-CO₂ leaves.

Limitation of P₇-regeneration capacity in chloroplasts

The clear negative correlation between the sucrose content and photosynthetic activity recognized in the high-CO₂ leaves (Fig. 8) suggests that one possible mechanism for the suppression of photosynthesis is related to the end-product inhibition of sucrose synthesis caused by the high sucrose level (Stitt et al. 1988, Stitt and Quick 1989, Foyer 1990). This inhib-
Photosynthetic suppression by high CO₂ is thought to impose a \( P_i \)-regeneration-limitation on photosynthesis because \( P_i \) is not recycled rapidly enough. There are some reports that low \( P_i \) contributes directly to the lowering of activity of RuBPcase (Machler and Nosberger 1984, Parry et al. 1985, Sawada et al. 1989, Sawada et al. 1990, Sawada et al. 1992). Subsequently, Anwaruzzaman et al. (1995) reported that the inactivation of RuBPcase caused by lowering the level of \( P_i \) is due to a decrease in the extent of binding of the activator CO₂ to activation sites on this enzyme. However, the present study shows that the activation ratios of RuBPcase in the high-CO₂ leaves remained roughly constant and high during the experiment and were close to the respective values in the control leaves (Fig. 7). In addition, the effects of CO₂ and \( P_i \) on the activation of RuBPcase were not much different between the control and high-CO₂ leaves (Table 1). These results indicate that the availability of \( P_i \) in chloroplasts for the binding of the activator CO₂ to activation sites on RuBPcase is unlimited at least in the high-CO₂ leaves.

**Lowering of RuBPcase capacity**

The initial and total activities of RuBPcase in the high-CO₂ leaves remained roughly constant during the period from the 6th day to 12th day, and were close to the respective values in the control leaves, while, on the 15th day, both activities in the former leaves decreased slightly to about 80% of the respective values in the latter leaves (Fig. 7). On the other hand, the amounts of RuBPcase protein in rooted leaves cultured under both the control and high-CO₂ conditions increased slightly during the experiment and differed little between the two leaves (Fig. 6). On exposure to darkness from the 15th day, both the decreased activities of RuBPcase and amounts of this protein in high-CO₂ leaves were almost unchanged, while both activities in the high-CO₂ leaves were about 80% of the respective values in the control leaves (Table 3). These results indicate that, in the latter experimental period, the slight lowering of RuBPcase capacity through the inactivation or inhibition of this enzyme involved a lowering of the carboxylation efficiency of RuBPcase in the high-CO₂ leaves.

**Lowering of CO₂ diffusion from the intercellular space to stroma in chloroplasts**

The high-CO₂ leaves showed markedly decreased levels of photosynthetic activity according to the large accumulation of carbohydrates (Fig. 2, 5). In this case, significantly more starch than sucrose was accumulated. However, exposure of the high-CO₂ leaves to darkness restored completely the photosynthesis (Table 3), and simultaneously expended almost all of the accumulated starch but not so much of the sucrose (Table 2). Therefore, the changes in the starch content corresponding to those in photosynthetic activity caused by CO₂ enrichment and the exposure to darkness were greater than the respective changes in sucrose content. There have been several reports describing that the increase in starch on CO₂ enrichment is relatively greater than that in soluble sugars (Morin et al. 1992, Den Hertog et al. 1996, Nakano et al. 1997, Poorter et al. 1997, Tissue et al. 1997, Moore et al. 1997). In addition, reversibility of photosynthetic suppression with changes in the starch content has been observed (Thorne and Koller 1974, Mayoral et al. 1985, Nakano et al. 2000). Some studies, furthermore, suggested that starch accumulation hinders CO₂ diffusion in the
chloroplast (see above). Nakano et al. (2000) reported that the suppression of photosynthesis by excess starch is predominantly observed at ambient CO$_2$ partial pressure where CO$_2$ diffusion is limited but it decreases or disappears under conditions of saturated CO$_2$. They suggested that a morphological modification of chloroplasts reserving excess starch may be an important factor in CO$_2$ transfer conductance because the conductance strongly depends on the chloroplast surface area adjacent to the plasma membrane (von Caemmerer and Evans 1991).

The A to Ci curves of the high-CO$_2$ leaves cultured long-term show that the CO$_2$ response above the intercellular CO$_2$ partial pressure of 40 Pa is more sensitive than the value in the control leaf (Fig. 3). At the same time, the rate of increase in photosynthetic activity in the high-CO$_2$ leaves accompanying the elevation of CO$_2$ partial pressure from 35 to 150 Pa on the leaf surface was linear and higher than that in the control leaves (Fig. 4). These results indicate that, in the high-CO$_2$ leaves, the elevation in CO$_2$ partial pressure in the intercellular space and on the leaf surface efficiently enhanced the CO$_2$ diffusion from the intercellular space to stroma in chloroplasts, because the CO$_2$ diffusion resistance might be high. This result supports the suggestion by Nakano et al. (2000). This and the results mentioned above indicate that, in the high-CO$_2$ leaves, the accumulation of starch is related directly to the hindering of the diffusion of CO$_2$ from the intercellular space to stroma in chloroplasts. On the other hand, the photosynthetic activities in the high-CO$_2$ leaves exposed to darkness also linearly increased with the increase in CO$_2$ partial pressure from 30 to 150 Pa on the leaf surface and, at 150 Pa, reached values slightly higher than those in the control leaves, while in the former leaves, both activities at 35 and 70 Pa CO$_2$ were slightly lower than the respective values in the latter leaves (Fig. 4). This result suggests that a part of the suppression of the activity at 35 and 70 Pa CO$_2$ was also caused by the continuous lowering of stomatal conductance.

Conclusions

The clear negative correlation between the starch content and the initial gradient of the A to Ci curve for the control and high-CO$_2$ leaves and the latter leaf exposed to darkness strongly (Fig. 9) supports that the lowering of carboxylation efficiency of RuBPcase was largely caused by the depression of CO$_2$ transfer conductance due to the accumulation of starch rather than physical damage to chloroplasts caused by the enlargement of starch grains and limitation of P$_1$-regeneration capacity in chloroplasts. In addition, in the latter stage of the acclimation to CO$_2$ enrichment, the lowering of RuBPcase capacity through the inactivation of this enzyme and the lowering of stomatal conductance also involved the lowering of the carboxylation efficiency. We concluded that the suppression of photosynthesis through end-product inhibition during the acclimation to elevated CO$_2$ was largely caused by the hindrance of CO$_2$ diffusion due to starch accumulation in the chloroplast.

Materials and Methods

Preparation of single-rooted soybean leaves

Single-rooted leaves of soybean (Glycine max L. Merr. cv. Tsurunoko), referred to as "source-sink model plants", were prepared as described previously (Sawada et al. 1986). When roots of single-rooted leaves reached about 30 mm in length, leaves of a uniform size were transferred to two growth boxes. Air containing CO$_2$ at a partial pressure of 35.0±3.5 Pa (hereafter referred to as "control CO$_2$") or 70.0±7.0 Pa ("high CO$_2$") was introduced at a flow rate of 18 liter min$^{-1}$ into the upper part of the growth boxes. The difference in CO$_2$ in the air between the inlet and outlet of both boxes was kept at less than 20% of the concentration in the air at the inlet. The lower part of the boxes contained a 10-fold diluted Epstein solution (Epstein 1972) which was renewed weekly.

The rooted leaves were cultured under a regimen of 10 h of light and 14 h of darkness. The irradiance at the leaf surface was on average 300 μmol photons m$^{-2}$ s$^{-1}$ (400–700 nm). The air temperature during the light and dark periods was kept at 27±1°C and 20±1°C, respectively. The temperature of the culture solution during both periods was adjusted to 22±1°C by thermostats. In some experiments, the rooted leaves were kept in the dark for 48 h from the 15th day after the start of the culture.

Measurement of the rate of gas exchange

The rooted leaves were transferred to assimilation chambers, and CO$_2$ and water vapor concentrations at the inlet and outlet of the chamber were measured simultaneously in an open two-way system equipped with two gas analyzers and an electronic dewpoint hygrometer, according to the methods of Sawada et al. (1982). The air tempera-
ture in the chambers was 25±1°C and the relative humidity was nearly 70%. The leaf temperature was monitored. The leaves were irradiated at 800 Epmol photons m⁻² s⁻¹ (400–700 nm), which was sufficient to saturate photosynthetic activities determined at an ambient CO₂ partial pressure of 35 Pa. The exchange of CO₂ in the light was determined in air that contained 35.0±5.0 Pa CO₂ for the rooted leaves cultured under the control condition, while, for the leaves cultured under the high CO₂ condition, it was first determined in air that contained 70.0±7.0 Pa CO₂ and then in air that contained 35.0±5.0 Pa CO₂. The rooted leaves after the measurement of gas exchange were divided into roots and leaves and then dried in an oven for determination of dry weight.

The partial pressure of CO₂ in the leaf intercellular space was calculated from the photosynthetic rate, conductance to CO₂ diffusion and ambient CO₂ concentration, using the equations of Farquhar and von Caemmerer (1982).

Quantifications of sucrose and starch

After determination of photosynthesis, leaf discs (ca. 15 mm in diameter) were cut out with a leaf puncher under light just after sampling of the rooted leaves from the growth boxes, dropped immediately into liquid nitrogen, and stored at −80°C until use. The procedures for the preparation of the leaf extract from frozen leaf discs and of the supernatant and for the analysis of sucrose, glucose and starch were those described by Sawada et al. (1995). The starch concentration was expressed as glucose units.

Assays of RuBPcase

The procedures for the preparation of the extract from the leaf discs stored at −80°C and of supernatant from the extract were those of Makino et al. (1988). RuBPcase in the supernatant was assayed at 25°C in a medium that contained 100 mM Bicine at pH 8.2, 5 mM MgCl₂, 10 mM NaHCO₃, 5 mM creatine phosphate, 1 mM ATP-2Na, 0.1 mM NADH, 0.3 mM RuBP, 10 units of phosphochetracein kinase, 10 units of glyceraldehyde 3-phosphate dehydrogenase and 10 units of phosphoglycerate kinase, as described previously (Sawada et al. 1990). The enzymatic activities were corrected for the decrease in absorbance at 340 mm in a control assay medium prepared without ribulose bisphosphate. The “initial” activity was measured after the addition of 50 μl of the extract to 1.950 μl of the assay medium. RuBPcase was activated for 20 min at 0°C after the preparation of the supernatant in an activation medium that contained 75 mM HEPES-KOH at pH 7.5, 10 mM MgCl₂ and 10 mM NaHCO₃, and its activity was also measured for determining “total” activity.

Protein determination

The amount of protein in the supernatant was determined by the method of Bradford (1976), using bovine serum albumin as standard. The protein amount of RuBPcase was determined by the method of Makino et al. (1986), using purified RuBPcase.

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References


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