Plant Physiol. (1987) 84, 1451–1456
0032-0889/87/84/1451/06/$01.00/0

# Photosynthetic Characteristics of a Photoautotrophic Cell Suspension Culture of Soybean[1]

Received for publication March 16, 1987 and in revised form May 19, 1987

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## ABSTRACT

**A soybean suspension culture (SB-P) which can grow photoautotrophically in 5% $CO_2$ will not grow in ambient $CO_2$ levels. This elevated $CO_2$ requirement seems to be due to the additive effects of a number of factors. The *in vivo* activity of ribulose-1,5-bisphosphate carboxylase (RuBPcase) is much lower in the SB-P cells, compared to soybean plants. This may be due to the low light intensity used to culture the cells, which has been shown to decrease both the amount and activity in whole plants, resulting in a low rate of net photosynthesis. The RuBPcase activation level is also lowered in air $CO_2$ levels. The presence of the liquid medium raises the cells $CO_2$ compensation concentration (the $CO_2$ concentration reached when the rates of $CO_2$ fixed by photosynthesis and the $CO_2$ respired by the cells are equal). These factors, coupled with the high respiratory loss of $CO_2$ all contribute to reduced net photosynthesis in air, resulting in a photosynthetic capacity that is inadequate for cell survival. Active cell division, low photosynthetic capacity, elevated respiration, and a low ratio of RuBPcase(initial)/phosphoenolpyruvate carboxylase are traits that SB-P cells share with young leaf cells, indicating SB-P cell physiology may be comparable to that of young expanding leaves rather than to that of mature leaves.**

Photoautotrophic cell suspensions are easily manipulated systems for studying the physiological and biochemical aspects of photosynthetic cells. Few in-depth studies have been done on photoautotrophic cultures due to the limited number of such cell lines. They are potentially useful in the selection for photosynthetic herbicide resistance, herbicide metabolism studies, and the production of secondary plant products. These cultures can be repeatedly bleached in the dark and greened in the light and are therefore useful for differentiation and dedifferentiation studies. Erdos and Buetow (6) successfully used the SB-P[2] to investigate the production of the small subunit of RuBPcase during greening.

Unlike intact leaves, photoautotrophic cultures require elevated levels of $CO_2$ for growth, have ratios of $CO_2$ fixation enzymes different from those in $C_3$ plants, and incorporate a large portion of the $CO_2$ via PEPcase (reviewed in Ref. 7). The purpose of this present work was to more fully investigate the photosynthetic parameters of a photoautotrophic suspension culture of soybean in order to attempt to determine why such cultures have several unique characteristics not found in $C_3$ plants. The results indicate that the cells inability to grow in air is due to low photosynthetic efficiency. The physiological state of these cultured cells is most comparable to that of young dividing leaf cells (16).

## MATERIALS AND METHODS

**Photoautotrophic Culture Conditions.** SB-P, initiated from *Glycine max* (L.) Merr var Corsoy, was established in 1982 by Horn *et al.* (8). The cells were cultured in KN°, a modified KT° medium that contained only thiamine (0.1 mg/L) and hormones as the sole organic components, in a 5% $CO_2$ atmosphere under continuous light (250–300 $\mu E\ m^{-2}\ s^{-1}$). The cells were shaken on a gyratory shaker (130 rpm) at 28 ± 1°C, and were subcultured at 14 d intervals with 0.5 g fresh weight of cells inoculated into 80 ml of medium.

**Chl Determination.** Chl was extracted from the cells with 80% acetone (v/v) and was measured spectrophotometrically (2).

**Photosynthetic Oxygen Evolution.** Oxygen evolution in the light was measured with a Clark type electrode at 26°C. A stirring bar, 0.03 to 0.09 g fresh weight of cells in KN° medium, 50 mM MES (pH 5.8), and 1.5 mM $NaHCO_3$ in a final volume of 1 ml was added to the water-jacketed vessel and illuminated at a light intensity of 400 $\mu E\ m^{-2}\ s^{-1}$. The evolution rate measurements were repeated three times.

**$^{14}CO_2$ Incorporation Experiments.** Duplicate flasks of cell suspensions (pH 4.5) were combined and aliquots (950 $\mu$l) of cells, (7.0–11.4 mg fresh weight, 6–18 $\mu$g Chl) and a stir bar were placed in serum stoppered 16 × 55 mm glass scintillation vials. Each assay was run in triplicate. The cells were stirred and gassed with $CO_2$ free air at 26°C under a light intensity of 400 $\mu E\ m^{-2}\ s^{-1}$ for 5 min. The assays were initiated by the simultaneous injection of 25 $\mu$l of 205 mM bicarbonate (pH 9.3, 0.89 $\mu Ci\ \mu mol^{-1}$) and 50 $\mu$l of 1.025 M Mes (pH 1.05) to adjust pH. The final concentrations were 5 mM bicarbonate and 50 mM Mes, at a pH of 4.5 which is near that of the culture conditions. After 5 min assays were terminated by the injection of 0.5 ml of 6 N acetic acid. Incorporation of $^{14}CO_2$ in the dark was carried out in the same manner with the vials being wrapped in aluminum foil. Samples were dried at 60°C, 0.5 ml of 0.5 N HCl was added to dissolve the residue, 5 ml scintillation cocktail added, and the dpm were determined by liquid scintillation spectrometry.

**Infrared Gas Exchange Analysis.** Steady state photosynthesis and respiration of SB-P suspensions from the same flasks of cells used for $^{14}CO_2$ fixation experiments, was measured with an IR gas analyzer (UNOR-2, Bendex Corp.) in an open system. Aliquots (5 ml) of cells buffered with 50 mM Mes (pH 4.5), and a stir bar were placed in a 50 ml side arm test tube. A glass tube was submerged in the cell suspension, and the test tube was placed in a 26°C glass water bath, over a stir plate. Humidified

[1] Supported in part by funds from the Illinois Agricultural Experimental Station and by the McKnight Interdisciplinary Photosynthesis Research Program.

[2] Abbreviations: SB-P, photoautotrophic soybean line; Γ, the $CO_2$ compensation concentration, which is the $CO_2$ concentration reached when the rates of $CO_2$ fixed by photosynthesis and $CO_2$ respired by cells are equal; $C_3$, photosynthesis by direct fixation of $CO_2$ with ribulose 1,5-bisphosphate; PEPcase, phosphoenolpyruvate carboxylase; RuBPcase, ribulose 1,5-bisphosphate carboxylase; PEP, phosphoenolpyruvate.

gas mixtures were bubbled at 400 to 475 ml $min^{-1}$ through the illuminated (400 $\mu E\ m^{-2}\ s^{-1}$) stirred cell suspensions. To measure dark respiration the tube containing the cells was covered with aluminum foil. All measurements were taken on duplicate aliquots of SB-P, repeated twice, and the numbers averaged.

**$CO_2$ Compensation Concentration (Γ).** The $CO_2$ compensation concentration of the cells was measured in a closed system containing an IR gas analyzer (Horiba PIR-2000), a peristaltic pump (Cole-Parmer No. 7018), a flask of water with an aerator tube, a 125 ml flask containing 50 ml of cell suspension (1.7–3.0 g fresh weight cells) fitted with the stopper assembly used for growth, and an ice finger desiccant tube. All connections were with 6 mm i.d. plastic tubing, and the flow rate was 550 ml $min^{-1}$. The cells (in KN° medium, pH 4.5, 29.5°C) were shaken at 130 rpm at a light intensity of 250 to 300 $\mu E\ m^{-2}\ s^{-1}$. The $CO_2$ concentration, at 21% $O_2$, was monitored until equilibrium was reached. Measurements were repeated three times and then averaged.

The Γ was also measured on SB-P without the culture medium. Cells (1.50–2.25 g fresh weight were spread on the bottom of a Petri dish without media, which was placed in the system as described above, and the system was allowed to reach a constant $CO_2$ concentration.

***In Vitro* RuBPcase and PEPcase Activity Assays.** The shaker, containing the flasks of cells was turned off, the cells were allowed to settle for 3 min, then pipetted as a thick slurry, from the bottom of the flask, directly into liquid $N_2$, and ground with a pestle in a prefrozen mortar. The homogenate was mixed with an equal volume of buffer containing 50 mM Hepes (pH 7.3), 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM D-isoascorbate, 5 mM DTT, and 2% (w/v) PVP. Aliquots were taken for Chl and soluble protein determination. Homogenates were centrifuged for 15 s in a microfuge at 5000*g* at 4°C, and the supernatants were immediately assayed for enzyme activities. Assays were conducted in glass scintillation vials fitted with serum caps. The RuBPcase assay media contained 0.45 ml; 0.1 M tricine (pH 8.1), 10 mM $MgCl_2$, 10 mM $NaH^{14}CO_3$ (3 $\mu Ci/\mu mol$), and 0.5 mM RuBP. Reactions were initiated by the injection of 50 $\mu l$ of supernatant through the serum cap. The PEPcase assays were similar except 5 mM PEP was substituted for RuBP, the specific activity of $NaH^{14}CO_3$ was increased to 4 $\mu Ci/\mu mol$, and 2 mM DTT, 0.2 mM NADH, and 5 units of Sigma malate dehydrogenase were added.

To assay for total $CO_2$ + $Mg^{2+}$ activated RuBPcase activity, 100 $\mu l$ of supernatant was incubated for 7 min at 40°C, prior to the assay in 900 $\mu l$ of buffer containing 50 mM tricine (pH 8.1), 50 mM $NaHCO_3$, and 10 mM $MgCl_2$.

All enzyme assays were conducted at 26°C for a period of 1 min and terminated by the injection of 0.2 ml of 6 N acetic acid. Samples were dried at 60°C, 0.5 ml of 0.5 M HCl added to dissolve the residue, 5 ml scintillation fluid added, and radioactivity in acid stable products determined by scintillation spectrometry.

Soluble protein was determined by the method of Spector (25), using BSA as the standard.

## RESULTS AND DISCUSSION

**Present Characteristics of the SB-P Cells.** The SB-P cells, initiated in 1982 and characterized by Horn *et al.* (8) currently have several characteristics which remained unchanged including: doubling time, cell clump size, ability to rapidly regreen in the light after being bleached in the dark, an absolute requirement for an atmosphere of elevated $CO_2$, a constant linear growth habit for at least 3 weeks. The medium has been modified by removing the complex vitamin stock. Thiamine (0.1 mg/L) has been found to be the only vitamin required. The only characteristics which have changed since the previous publication are the ability to evolve $O_2$ under culture conditions and the Chl content which has increased from an initial level of about 400 $\mu g\ g^{-1}$ fresh weight to just under 2000 $\mu g\ g^{-1}$ fresh weight (Fig. 1A). This is currently the highest Chl level reported for photoautotrophic cultures.

When the soluble protein content per g fresh weight of the cells is measured over time, the levels peak early in the growth period (Fig. 1B) indicating a cell synthetic phase, which is characterized by increased respiration (Fig. 2A) and the elevated soluble protein content. The peak respiration rate is apparently due to a high demand for carbon skeletons in synthetic reactions (Fig. 2A). This is followed by a decline in accumulated protein, a pattern which has also been observed in a *Chenopodium* photoautotrophic culture (9). Ticha *et al.* (28) and Thornley (26) have demonstrated that developing leaves have high dark respiration due to energy requirements for growth. These requirements decrease as the leaf matures to a level needed for maintenance only.

**Oxygen Exchange.** Linear rates of endogenous $O_2$ evolution occurred for up to 10 min when the SB-P cells were suspended in KN° medium saturated with 5% $CO_2$ (Fig. 2B). The addition of $HCO_3^-$ (1.5 mM) stimulated $O_2$ evolution and was necessary to obtain maximum rates, as has been observed in other photosynthetic cell cultures (18, 20). Oxygen evolution became saturated at 400 $\mu E\ m^{-2}\ s^{-1}$, higher than the intensity used for cell growth (250–300 $\mu E\ m^{-2}\ s^{-1}$), a characteristic which has been observed in other plant cells (13).

The rate of photosynthetic $O_2$ evolution by SB-P cells, about 80 $\mu mol\ mg^{-1}\ h^{-1}$ (Fig. 2B), was comparable to that of photomixotrophic tobacco cells (17) and isolated spinach mesophyll

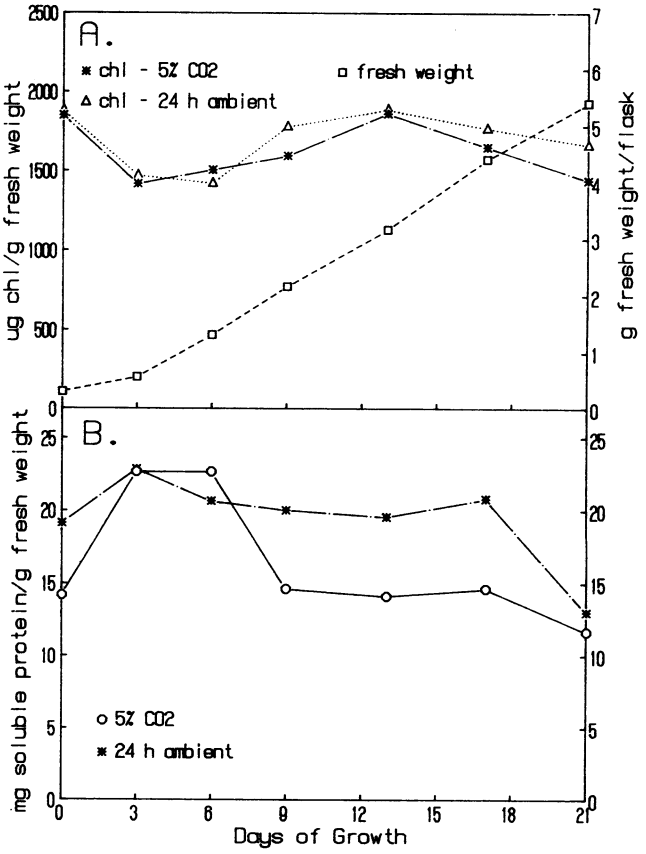


FIG. 1. Characteristics of SB-P cell growth when cultured in a 5% $CO_2$ atmosphere or in an atmosphere of ambient air (350 $\mu l/L\ CO_2$) 24 h prior to the assay. A, Fresh weight (average) and Chl content; B, soluble protein content.

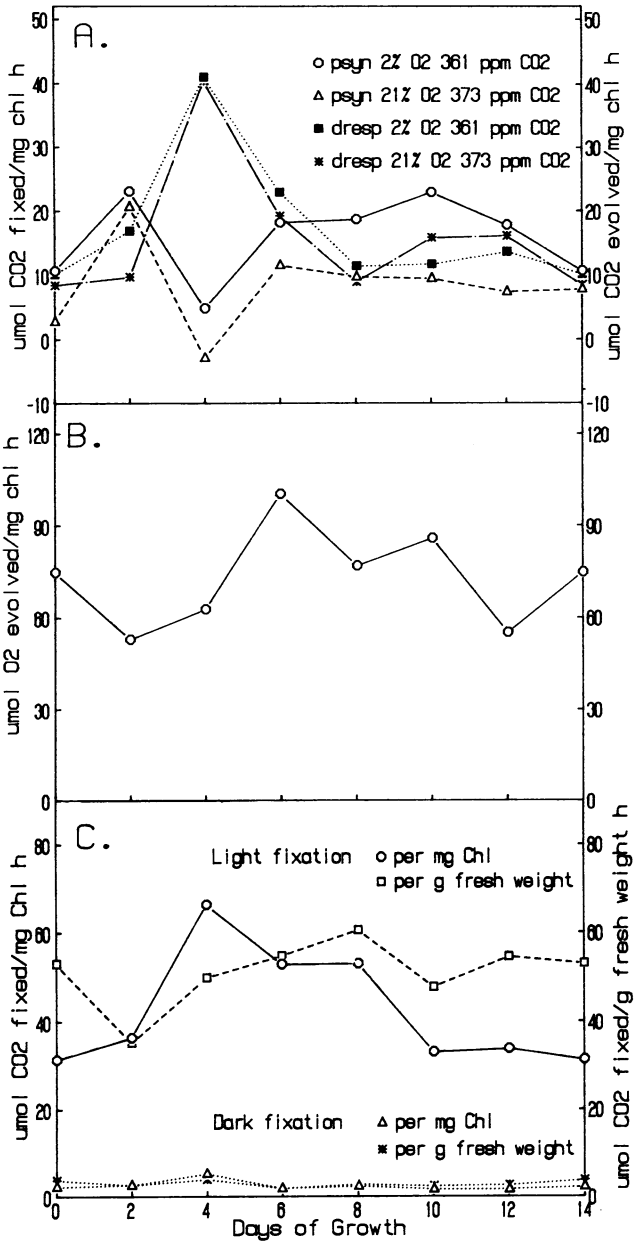


FIG. 2. Photosynthetic and respiratory characteristics of SB-P cells during growth. A, Net photosynthesis and dark respiration determined under high (21%) and low (2%) $O_2$ conditions; B, photosynthetic $O_2$ evolution; C, photosynthetic $^{14}CO_2$ fixation and dark $^{14}CO_2$ fixation.

cells (1), and was lower than that reported for photomixotrophic peanut cells (20). As has been generally observed in plants, a higher Chl content is correlated with a lower rate of $O_2$ evolution on a Chl basis (13, 20). The original SB-P cells did not exhibit net $O_2$ evolution, even with added bicarbonate (7) unless assayed at a pH greater than 7.5. The ability of the present cells to evolve $O_2$ under culture conditions may reflect an increased photosynthetic capacity of the cells due in part to increased Chl levels.

**$^{14}CO_2$ Fixation.** In order to determine rates of photosynthesis and $CO_2$ fixation in the dark the cells were exposed to $^{14}CO_2$ under cell growth conditions to measure fixation (KN° medium, pH 4.5, 26°C). Other investigators have used higher pH conditions (above 7.0) for fixation experiments (20), which does not reflect the actual growth conditions, and could result in altered photosynthetic activities. As the $H^{14}CO_3^-$ concentration was increased from 0.1 to 5 mM, the rate of incorporation reached a maximum at 1 mM, where the calculated concentration of $CO_2$ in the medium would be 0.102 mM. Since this is about 10-fold greater than the ambient $CO_2$ concentration (0.011 mM), light fixation of SB-P would be $CO_2$-limited in air. However, the 5% $CO_2$ (1.64 mM) used in SB-P culture should thus be more than adequate for maximum photosynthesis.

The highest rates of photosynthetic $CO_2$ fixation occurred on d 4 (66 $\mu$mol $CO_2$ $mg^{-1}$ Chl $h^{-1}$), then decreased to about half this by d 10 (Fig. 2C). Dark fixation, which showed little variation over the growth cycle (Fig. 2C), was never more than 8% of the rate in the light, a value which is comparable to that measured in other photoautotrophic cultures (15). The reason for the peak rates of light fixation, on a Chl basis, is presumably related to the decrease in Chl content that occurs early in the growth period (Fig. 1A). When fixation is expressed on a fresh weight basis, there are no significant changes during the growth cycle, however.

**$CO_2$ Exchange.** To determine if the cells were sensitive to $O_2$ concentration, as is $C_3$ photosynthesis in higher plant leaves, the effect of $O_2$ concentration on the rate of photosynthesis was measured. The $CO_2$ exchange rate could not be measured at the $CO_2$ concentration (5%) used to culture the cells because this level exceeded the $CO_2$ analyzer capacity. Photosynthesis was inhibited about 50% at 21% $O_2$ compared to photosynthesis in 2% $O_2$ (Fig. 2A) indicating that $O_2$ competitively inhibited $CO_2$ fixation. Therefore, at least the first step of photorespiration, the ability of RuBPcase to fix $O_2$, occurred at ambient levels of $CO_2$ and $O_2$ (Fig. 2A). Regardless of the $O_2$ concentration, the cells had a low level of net photosynthesis throughout the culture period, with the exception of d 4 at 21% $O_2$, where the cells had net $CO_2$ loss in the light due to the high rate of respiration which occurs at this time. Although a low level of net fixation occurred under ambient conditions, SB-P cannot be successfully cultured at this $CO_2$ concentration, indicating that the amount of net fixation is inadequate for continued cell maintenance and growth.

The peak rate of dark respiration per unit Chl in SB-P cells (40 $\mu$mol $CO_2$ $mg^{-1}$ Chl $h^{-1}$), which was more than 10 times the level in mature soybean leaves (3.6 $\mu$mol $CO_2$ $mg^{-1}$ Chl $h^{-1}$) (15), occurred during the synthesis period (time of highest protein accumulation) (Fig. 2A). During this period, because of the high rate of protein synthesis which apparently occurs at this time (Fig. 1B), respiratory activity would presumably increase due to the increased demand for carbon skeletons.

High rates of dark respiration in photoautotrophic cell lines, reported here and in other studies (10), may reflect the high energy requirement of dividing cells for growth and maintenance (27). In leaves, this need decreases with maturity, lowering overall dark respiration to a constant value (27). As SB-P cells never reach stationary phase under culture conditions, the respiration required for growth would continuously contribute to the high dark respiration rate.

Gas exchange analysis revealed that the cells generally had net photosynthesis in air (350 ppm $CO_2$, 21% $O_2$) (Fig. 2A). This indicates that the $CO_2$ compensation concentration ($\Gamma$) generally was below the ambient $CO_2$ concentration (361–373 ppm). Indeed $\Gamma$ was 230 ± 25 ppm in SB-P cells (7–14 d old) under liquid culture conditions, a level more than three times higher than the $\Gamma$ of soybean leaf discs (23). However, this does not mean the $\Gamma$ of the cells is always below air $CO_2$ levels. Young cells (4 d old) have very high respiration, and have net $CO_2$ evolution in the light at air $CO_2$ levels. This indicates that at this time in the culture period the $\Gamma$ is above the ambient $CO_2$ concentration. The net photosynthesis rate in air, over the entire culture period, must be inadequate to support growth since the cells will not grow under such conditions.

When the cells were removed from the culture medium, spread

as a thin layer in a Petri plate and analyzed, they were found to have a Γ of 90 ± 15 ppm $CO_2$. This value was less than half that of cells in liquid medium, indicating that the liquid medium affects the photosynthetic efficiency of the cells. This is in contrast to the findings of Servaites and Ogren (21) who reported the Γ of isolated soybean leaf cells in liquid media (1 ml, pH 7.8) was unchanged from that in leaves based on $^{14}CO_2$ fixation kinetics and direct measurement. This seems to indicate that SB-P cells differ from isolated leaf cells in regard to the influence of liquid media on the Γ.

While we cannot explain the reason for this difference in Γ it should not be due to differences in diffusion rates since the Γ was determined in a closed system at equilibrium. As such diffusion rates would only affect the time to achieve equilibrium, and not the final value reached.

**RuBPcase Activity.** The RuBPcase activation state could be very important because it has been correlated with the photosynthetic rate (29). Since SB-P cells have low net photosynthesis, initial (*in vivo*) and total (that which is present after preincubation with $CO_2$ and $Mg^{2+}$ to fully activate the enzyme) RuBPcase activity was measured over time. RuBPcase activity, (total or initial) did not vary much during the growth cycle except when related to variations in Chl and protein content (Fig. 3, A and B). In the early part of the culture period, the time of lowest Chl and highest soluble protein content, RuBPcase activity is somewhat elevated when expressed on a Chl basis (Fig. 3A) or decreased when expressed on a soluble protein basis (Fig. 3B). Although this is a time of high protein synthesis, RuBPcase is apparently not preferentially synthesized. Total RuBPcase levels (Fig. 3A) were compared to that found in leaves of soybean plants grown hydroponically in a growth chamber at a light intensity twice that used to culture the cells. The cell activity was only 15% of the total activity in leaves (516 μmol $CO_2$ $mg^{-1}$ Chl $h^{-1}$). The low total activity in SB-P can be partially explained by the low light intensity, about one-tenth full sun used in culture. The amount of RuBPcase activity has been shown to be directly correlated with light intensity (29).

The elevated $CO_2$ levels may also be a factor in the reduced RuBPcase levels in SB-P since soybean plants grown at elevated $CO_2$ (800 ppm) were consistently found to have lower RuBPcase levels, especially during the earlier growth stages (30). In *Nerium oleander* a doubling of ambient $CO_2$ reduced the amount of RuBPcase protein (5). Although these studies used elevated $CO_2$ (about 2 times ambient), the levels were low compared to the 5% $CO_2$ (140 times ambient) used to culture SB-P. Our very high level of $CO_2$ may cause a large depression of RuBPcase levels therefore also contributing the low values of the enzyme observed in the photoautotrophic cells.

Since soybean RuBPcase is not always fully activated *in vivo* (22) the initial activity in SB-P, as a percentage of total activity, was compared to the activities in soybean plants. RuBPcase activity was found to be about 50% activated in SB-P cells, which is somewhat lower than the 70 to 95% activation measured in growth chamber and greenhouse grown soybean plants, respectively (22, 29). However, Torisky and Servaites (29) observed that RuBPcase activation was 95% and 74% in plants grown at 100% and 20% full sunlight, respectively, an indication that an increase in the light intensity used to culture SB-P (culture intensity 10% full sunlight) may increase the extent of RuBPcase activation.

To determine the effect of low $CO_2$ on RuBPcase activation, cells were exposed to ambient air prior to the assays. This treatment resulted in a 57% decrease in initial RuBPcase activity and a 37% increase in total activity (Figs. 2C; 3, A and B). These changes mean that the initial activity under ambient $CO_2$ levels, as a percentage of total activity, was only 14%, compared to 50%, in the cells maintained on 5% $CO_2$ (Fig. 3C), demonstrating

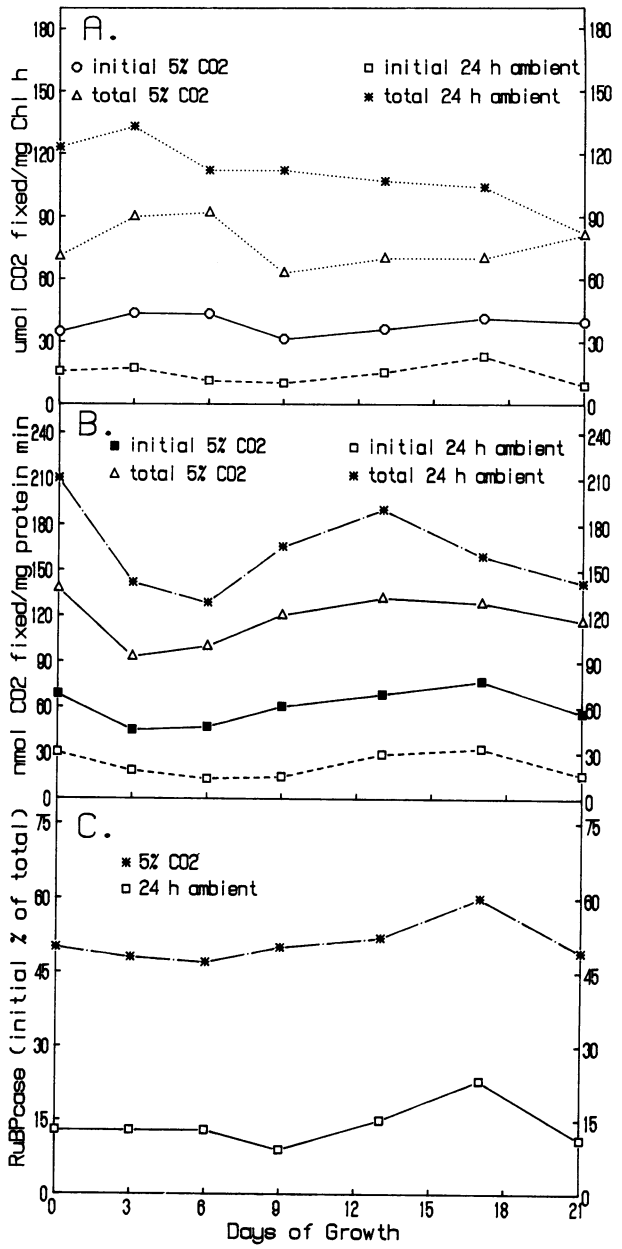


FIG. 3. Initial (*in vivo*) and total ($Mg^{2+}$ and $CO_2$ activated) RuBPcase activity of SB-P cells when cultured in a 5% $CO_2$ atmosphere or in an atmosphere of ambient air (350 μl/L $CO_2$) 24 h prior to the assay. A, Activity on a per mg Chl basis; B, initial and total RuBPcase activity on a per mg protein basis; C, ratio of initial to total RuBPcase activity.

that the enzyme was deactivating at low $CO_2$. The higher total activity in SB-P cells exposed to ambient $CO_2$, compared to cells at 5% $CO_2$ is comparable to the effects seen with soybean plants where RuBPcase levels are depressed by high $CO_2$ levels.

*In vitro*, full activation of RuBPcase requires supraphysiological concentrations of $CO_2$ and $Mg^{2+}$, while *in vivo* the enzyme can be completely activated at air levels of $CO_2$ (24). However, the RuBPcase in SB-P, which is cultured with 5% $CO_2$, may, unlike whole plants, deactivate when the cells are cultured at low $CO_2$, because the cells may be adapted to high $CO_2$.

A specific chloroplast enzyme. RuBPcase activase, has been observed to be required for the activation of RuBPcase *in vivo* (17). The isolation of an *Arabidopsis* mutant which requires elevated $CO_2$ for growth, demonstrated that the mutants inability to activate RuBPcase upon illumination was correlated with the

absence of RuBPcase activase (17). Western blots on protein extracts from SB-P revealed that RuBPcase activase is present (JM Werneke, unpublished data), although the activity of this enzyme in the cells has not yet been determined.

The elevated $CO_2$ atmosphere and the low light intensity used to culture SB-P may both contribute to the lower RuBPcase levels, resulting in decreased net photosynthetic rates. The large deactivation of RuBPcase which occurs at ambient $CO_2$ results in further severely decreased photosynthetic capacity, and could be a major factor in the cells inability to grow in air. Attempts to gradually adapt SB-P cells to these low $CO_2$ conditions have not produced SB-P cells which can survive.

**PEPcase Activity.** In SB-P cells, PEPcase capacity peaked early in the growth period (about 85 $\mu$mol $CO_2$ $mg^{-1}$ Chl $h^{-1}$) before declining to the initial value (about 27 $\mu$mol $CO_2$ $mg^{-1}$ Chl $h^{-1}$). This characteristic pattern of PEPcase activity has been observed in other photosynthetic cultures (14). The peak of PEPcase activity in SB-P (Fig. 4A) coincides with the synthetic phase, which is the time of highest protein content (Fig. 1B). This phenomenon indicates that protein synthesis seems to favor PEPcase in actively dividing cells. It has been suggested that PEPcase contributes significantly to $CO_2$ fixation by the carboxylation of PEP to yield oxaloacetic acid and subsequently malate which is used in synthetic reactions in the tricarboxylic acid cycle. This would allow the cycle to continue during times of high demand for synthesis precursors. Short-term labeling studies of photosynthetic cultures have revealed considerable labeling of $C_4$ carboxylic acids, particularly malate (9, 19). Malate is significantly higher in dividing cells as compared to cells from stationary phase (9). This further supports the proposed role of PEPcase in anaplerotic $CO_2$ fixation which supplies carbon skeletons to the tricarboxylic acid cycle.

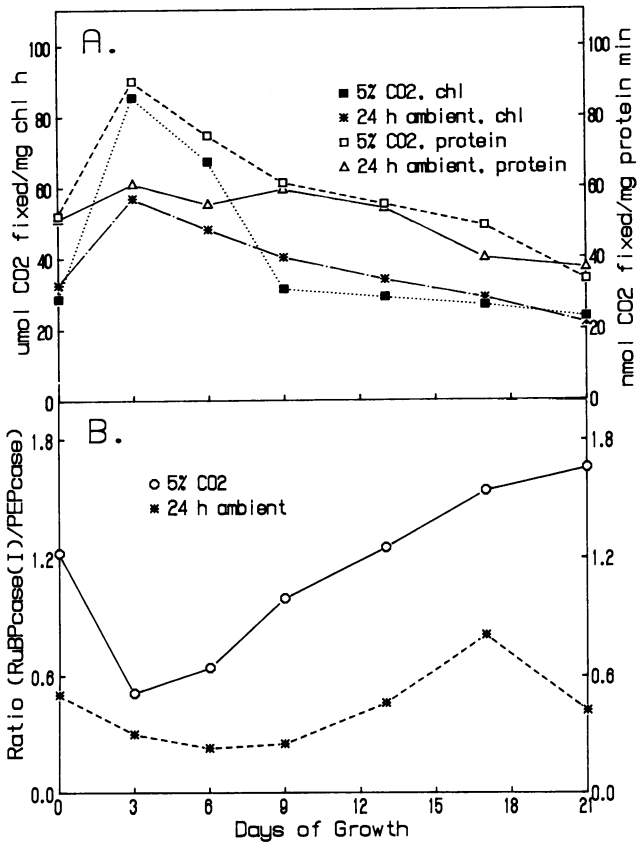


FIG. 4. PEPcase activities in SB-P cells cultured in a 5% $CO_2$ atmosphere or in an atmosphere of ambient air (350 $\mu$l/L $CO_2$) 24 h prior to the assay. A, Activity on a per mg Chl basis and on a per mg protein basis; B, ratio of RuBPcase(initial) activity to PEPcase activity.

Although the cells to have high PEPcase activity, the enzyme may be inactive in the dark as shown by the low rate of dark $^{14}CO_2$ fixation exhibited by many photosynthetic cultures. Bender (3) has shown that light stimulates PEPcase activity in *Daucus carota* resulting in increased $^{14}C$ incorporation into malate in the light as compared to dark fixation. This may indicate that the low rate of dark fixation could be due to PEPcase deactivation.

The high PEPcase levels in photosynthetic cultures may be analogous to the elevated levels seen in the very young leaves of $C_3$ plants (11). Both the cultures and photosynthetic cells have very active cell division, and as young leaves mature, or the cells reach stationary phase, the PEPcase level decreases.

**Ratio of RuBPcase/PEPcase.** The changes in the RuBPcase(initial)/PEPcase ratio observed during the growth cycle are primarily due to fluctuations in PEPcase. The initial peak in PEPcase activity (Figs. 3, 4A), followed by a 75% decrease results in a RuBPcase/PEPcase ratio of about 0.5 in the early part of the growth period and about 1.3 in the latter phase (Fig. 4B). Similar changes have been observed in the ratio of RuBPcase(initial)/PEPcase activity over time in other cultures. Nato and Mathieu (12) reported that not only do these two enzymes change in activity, but they also change in concentration. Utilizing densitometric profiles of soluble proteins run on polyacrylamide gels, they observed a marked increase in PEPcase protein during the active phase of cell division, while RuBPcase showed only slight variations over the growth cycle.

The activity of the two carboxylation enzymes over time in SB-P cells resembles that observed in young developing leaves of $C_3$ plants (11) whereas the leaves mature the RuBPcase(initial)/PEPcase ratio increases. Mature soybean leaves have a much higher ratio of RuBPcase(initial)/PEPcase, about 7, due primarily to the increased RuBPcase activity (15). This is in contrast to the maximum ratio of about 1.6 in cells in 5% $CO_2$ and only about 0.8 in cells exposed to ambient $CO_2$. The lower ratios are due primarily to decreased RuBPcase activity. Due to deactivation of RuBPcase in the cells in ambient $CO_2$, RuBPcase activity was much lower than the control, and could be a major contributing factor in the low net photosynthesis rate exhibited by SB-P cells.

## CONCLUSIONS

A rapidly growing photoautotrophic soybean cell line, cultured at high $CO_2$ has been established. The characterization may help us understand why photoautotrophic cultures have certain unusual attributes. A unique characteristic of this and other photoautotrophic lines, with respect to whole plants, is the cells inability to grow at ambient levels of $CO_2$ (4, 8, 10).

The *in vivo* (initial) activity of RuBPcase, the predominant $CO_2$ fixation enzyme in $C_3$ plants, is much lower in SB-P cells compared to soybean leaves. This may be attributed to the low light intensity used to culture SB-P cells, since low light has been shown to decrease both the amount of RuBPcase protein and the level of RuBPcase activation in $C_3$ plants (29). In addition, net $CO_2$ fixation rates are further decreased due to the greatly elevated rates of dark respiration in SB-P cells compared to leaves. During the time of peak respiration, at ambient $CO_2$, the cells exhibit net $CO_2$ loss in the light because respiratory activity is greater than photosynthesis.

Growing immature leaves have lowered RuBPcase and high PEPcase activity as observed in SB-P. Young leaves show $^{14}C$-labeling of $C_4$ dicarboxylic acids indicating that active $CO_2$ fixation by PEPcase contributes to photosynthesis, as has been suggested by labeling studies on photosynthetic cultures (10, 14). However, PEPcase is apparently inadequate in supplementing RuBPcase $CO_2$ fixation activity in meeting the carbon needs of

SB-P cells at ambient air as the cells have a very low level of net $CO_2$ fixation in air and cannot grow under such conditions.

The culture shares several characteristics with young leaves such as active cell division, elevated respiration, low photosynthetic efficiency, an elevated Γ, low RuBPcase activity, and elevated PEPcase activity. The inability of SB-P cells to grow in air may be caused by aspects of both the culture conditions and the physiological state of the cells. The low light intensity and the elevated $CO_2$ used to culture SB-P cells may both decrease photosynthetic rates in SB-P cells. RuBPcase activity decreases at ambient $CO_2$, resulting in low $CO_2$ fixation rates which are further reduced by the high respiratory $CO_2$ loss.

While elevated $CO_2$ is still a growth requirement, the SB-P cells demonstrate that rapidly growing photoautotrophic cultures are easily manipulated for physiological and biochemical studies, and may be particularly useful in investigating aspects of actively dividing photosynthetic plant cells.

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