Plant Physiol. (1982) 70, 1444–1448
0032-0889/82/70/1444/05/$00.50/0

Effects of Aminoacetonitrile on Net Photosynthesis, Ribulose-1,5-Bisphosphate Levels, and Glycolate Pathway Intermediates[1]

Received for publication May 11, 1982 and in revised form August 3, 1982

ELISABETH CRÉACH AND CECIL R. STEWART
Department of Botany, Iowa State University, Ames, Iowa 50011

ABSTRACT

The effects of aminoacetonitrile (a competitive inhibitor of glycine oxidation) on net photosynthesis, glycolate pathway intermediates, and ribulose-1,5-bisphosphate (RuBP) levels have been investigated at different O_2 and CO_2 concentrations with soybean (*Glycine max*)[L] Merr. cv Pioneer 1677) leaf discs floated on 25 millimolar aminoacetonitrile (AAN) for 50 minutes prior to assay.

At 2% O_2 and 200 or 330 microliters per liter CO_2, the inhibitor had no effect on the rate of net photosynthesis and RuBP levels when compared with the control levels. At 11% to 60% O_2, AAN caused a decrease in net photosynthesis in addition to the inhibition by O_2. This extra inhibition ranged from 22% to 59% depending on the O_2 and CO_2 concentrations. The levels of RuBP, however, were 1.3 to 2.7 times higher than in the control plants at the same O_2 concentrations. At 40% O_2 and 200 microliters per liter CO_2, the inhibitor caused a 6-fold increase in glycine and more than 2-fold increase in glyoxylate levels, whereas those of glycolate decreased by approximately one-half.

The decrease in net photosynthesis observed with AAN is not the result of the depletion of the RuBP pool due to the lack of recycling of carbon from the glycolate pathway to the Calvin cycle. The higher levels of RuBP caused byAAN in photorespiratory conditions, suggest that RuBP carboxylase was inhibited. Glyoxylate could be a possible candidate for the inhibition of the enzyme but what is known so far about its inhibitory properties *in vitro* may not fit the existing *in vivo* conditions. An alternative explanation for the inhibition is proposed.

Due to the catalytic properties of the enzyme RuBP[2] carboxylase, photorespiration appears to be an unavoidable process in an atmosphere containing high O_2 and low CO_2 (2). The oxygenation of RuBP leads to the formation of P-glycolate (1) and its subsequent metabolism through the photosynthetic carbon oxidative cycle is viewed as a means of recovering part of that carbon diverted from the Calvin cycle. Under physiological conditions, only 25% of the carbon entering P-glycolate is lost as CO_2 (19) and the remaining is available for the synthesis of amino acids and sucrose or reentry into Calvin cycle. In the past, it seemed feasible to increase photosynthetic productivity by inhibiting some steps of the glycolate pathway. This idea, however, has been supported by little experimental data (29, 30). It is now well substantiated that once P-glycolate has been synthesized, it must be metabolized through the complete photorespiratory pathway in order to avoid detrimental effects of photosynthesis. This has been shown through the use of inhibitors of glycolate oxidase such as BHB (7, 14, 24) and of glycine decarboxylase such as INH (9, 14, 24) and AAN (27). Also the requirement of the complete functioning of the glycolate pathway has been demonstrated in studies involving mutants deficient in specific enzymes of the glycolate pathway (25, 26). In photorespiratory conditions, these mutants are simply not viable. Earlier studies on $^{14}CO_2$ fixation with chloroplasts showed that, in conditions of low CO_2 and high O_2, glycolate was the main labeled product of photosynthesis. Its accumulation led to a decrease in net CO_2 uptake and a reduced amount of radioactivity incorporated into insoluble material and Calvin cycle intermediates (13). An analogy has been made between chloroplast preparations in which glycolate is an end product and leaves or protoplasts specifically inhibited at one step of the glycolate pathway in which glycolate or glycine accumulates. From this analogy it has been proposed that the observed decrease in the rate of net photosynthesis may be due to the shortage of Calvin cycle intermediates caused by the lack of recycling of carbon (23, 24, 27).

Our objective was to test this hypothesis using soybean leaves and aminoacetonitrile, an analog of glycine, which is a competitive inhibitor of both glycine decarboxylase and serine hydroxymethyl transferase (8, 27). We wanted to know whether or not, at least in short-term experiments, some regulatory mechanism would come into effect to prevent or overcome the shortage of carbon due to the continuous drain in Calvin cycle intermediates caused by the accumulation of carbon in glycine. We studied the effects of the inhibitor AAN on net photosynthesis and RuBP levels at different O_2 and two different CO_2 concentrations and compared these with the untreated leaves. We also measured the levels of glycine, glyoxylate, and glycolate to check whether or not intermediates of the glycolate pathway could be involved in the regulation of RuBP carboxylase.

MATERIALS AND METHODS

Plant Material. Soybean plants (*Glycine max* [L] Merr. cv Pioneer 1677) were grown in 20-cm diameter pots (two plants per pot) with sterilized soil, in controlled environment chambers with 16 photoperiod at approximately 1000 $\mu E\ m^{-2}s^{-1}$ PAR of mixed daylight fluorescent and incandescent lights and 27°C day, 21°C night. They were watered every other day with modified double-strength Hoagland solution supplemented with iron chelate Sequestrene 138 Fe (Ciba-Geigy) at 16.6 mg/l. For each experiment, 12 2-cm diameter discs were punched with a corkborer from the fourth or fifth trifoliates of 1-month-old plants.

Gas Exchange. Net CO_2 exchange rates were measured in an open gas exchange system. Gas mixtures were made from pressurized gas cylinders of O_2 and N_2 and 2% CO_2 (v/v) in N_2. O_2 content of the inlet gas mixture was measured with an O_2 electrode (Yellow Springs Instrument, Yellow Springs, OH) immersed in the inlet gas stream humidifier flask and CO_2 content with a

[1] Supported by Department of Energy Contract No. DE-ACO2-80ER10865.

[2] Abbreviations: RuBP, ribulose-1,5-bisphosphate; BHB, butyl-2-hydroxy-3-butynoate; INH, isonicotinic acid hydrazide; AAN, aminoacetonitrile.

nondispersive IR gas analyzer (AR500, Anarad, Santa Barbara, CA).

For CO_2 gas exchange measurements the same IR gas analyzer was used in a differential mode and the gas stream was split so that half of it would serve as the reference. The flow rate was 2.2 l/min and the incident quantum flux 1200 $\mu E\ m^{-2}s^{-1}$ PAR. The light, provided by two 150-w projector floods, was filtered through an OCLI (Optical Coatings Laboratory Incorporated, Santa Rosa, CA) wide band hot mirror and 4 cm of water. The experiments were conducted at 23 ± 2°C.

Twelve leaf discs were placed on a wire screen in a thermostated Plexiglass chamber (25 ml) equipped with 20 gas inlets and outlets. The chamber was made airtight by a vacuum between two concentric O rings and a Plexiglass cover. At the end of the experiment, vacuum release allowed a rapid chamber opening and removing of the samples (≤2 s). The discs were allowed to photosynthesize for approximately 10 min, then were placed in the dark for 4 min to measure the postillumination burst and the dark respiration rate. The lights were turned on again and the discs were allowed to reach steady rates of photosynthesis. After 10 to 15 min they were quickly removed and immediately plunged into liquid N_2, then freeze dried.

Extraction and Separation of Metabolites. The dried discs were ground in a mortar and pestle with ground glass. The resulting powder was extracted at 0°C with 4 ml 0.5 N HCl and sonicated for 30 s. The extract was centrifuged and filtered.

RuBP was measured on crude extracts, glycine, glyoxylate, and glycolate after partial purification on Dowex-50-H+. The column was rinsed with water and the eluate containing glyoxylate and glycolate was made basic with NH_4OH and lyophilized. Glycine was eluted from the column with 2 N NH_4OH and air dried.

RuBP Measurements. RuBP was purchased as a tetrasodium salt and standardized according to Racker (22).

RuBP carboxylase was partially purified from spinach by the method of Racker (22) with slight modifications (11).

RuBP was measured using a radiochemical assay (16) modified as follows: 0.5 ml of the HCl extract kept at 0°C was brought to pH 8 by the addition of 75 µl of Tris base 2 M and 44 µl KOH 4 N. The serum stoppered vials were degassed under N_2 and 0.5 ml of the following enzyme mixture which had been previously reactivated by incubation at 50°C for 25 min was added: 100 mM Tris-HCl (pH 8.6), 20 mM $MgCl_2$, 10 mM $NaH^{14}CO_3$ (11 kBq/µmol, 1 kBq = 27 nCi), and 1.4 mg/ml RuBP carboxylase. After 2 h at room temperature the reaction was terminated by the addition of 200 µl 6 N CH_3COOH and the mixture evaporated under an air stream with this operation repeated once to ensure the complete removal of $^{14}CO_2$. The dried residue was suspended in 2 ml water and acid stable ^{14}C content of 1-ml aliquots determined with a Beckman DPM 100 liquid scintillation spectrometer, after the addition of 10 ml of scintillation liquid (toluene, Triton, PPO 1000/500/4, v/v/w). A standard curve with 5 to 50 nmol RuBP per assay was run with each set of experiments in the same conditions.

The extraction of RuBP by $HClO_4$ instead of HCl did not give significantly different results and the latter method avoiding the precipitation of $KClO_4$ and centrifugation was more convenient.

Glycine Determination. Glycine was measured by a specific colorimetric assay according to Ohmori (17). It was benzoylated into hippuric acid which was then extracted with ethyl acetate. After evaporation of ethyl acetate, hippuric acid was reacted with acetic anhydride, *p*-dimethylaminobenzaldehyde in pyridine for color development and the A_{458} measured. A calibration curve with 1 to 8 µg of glycine was run with the experiment.

Glycolate and Glyoxylate Measurements. Glycolic acid oxidase and catalase were purchased from Sigma. Glycolate was converted to glyoxylate as in Laing (15) with glycolic acid oxidase in presence of catalase, and glyoxylate transformed to glyoxylate hydrazone with phenylhydrazine, then oxidized by ferricyanide to give 1,5-diphenylformazan (28). The method has been modified as follows: the lyophilized eluate containing glycolate and glyoxylate was dissolved in 50 mM Hepes K buffer (pH 8.3). To 0.1 ml aliquot containing no more than 50 nmol glycolate were added 0.25 ml Hepes K buffer, 0.1 ml catalase (20 U), and 0.05 ml glycolate oxidase (0.18 U). The mixture was incubated 1 h at 30°C. For glyoxylate measurements glycolate oxidase was omitted. Then 0.2 ml phenylhydrazine (25 mM in 0.5 N HCl) was added and the reaction allowed to take place at room temperature for 15 min. The tubes were chilled to 0°C on ice and 0.5 ml concentrated, cold HCl added followed by 0.3 ml potassium ferricyanide 50 mM. After vigorous mixing the color was developed at room temperature for 30 min and A_{550} determined. Standards containing 10 nmol glycolate or glyoxylate were run simultaneously.

Chl. For each experiment two leaf discs were set aside and Chl extracted with methanol and A_{650} and A_{665} measured.

Inhibitor. Aminoacetonitrile hydrochloride was purchased from Sigma. The leaf discs were floated on AAN (25 mM brought to pH 7 with KOH) for 50 min in the light and air in the growth chamber before CO_2 gas exchange rates were measured.

RESULTS

CO_2 Gas Exchange Analysis. Figure 1 shows a representative recording of CO_2 gas exchange analysis by soybean leaf discs. At 200 µl/l CO_2 and 2% O_2 (Fig. 1a), the rates of net photosynthesis and dark respiration were identical in AAN-treated samples and control samples. When the O_2 concentration was increased to 21% (Fig. 1b), the initial rate of CO_2 uptake was unaffected by AAN but in less than 30 s the rate in AAN-treated discs decreased for 6 to 10 min to a steady-state which was approximately 50% of the control levels. On transition to darkness, we did not observe as rapid CO_2 postillumination burst in the presence of AAN as in the control samples, but a slower release of CO_2 which after 3 min was faster than the dark respiration rate. However, the rate of dark CO_2 release decreased slowly with time and after approximately 15 min reached the same value as in the control samples

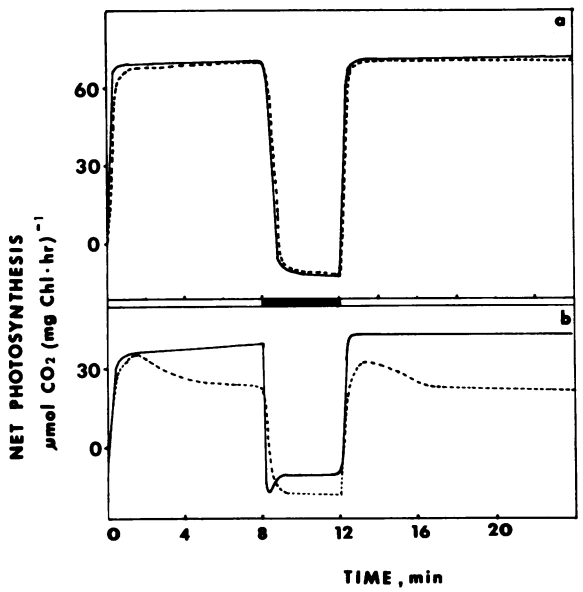

FIG. 1. Effect of AAN on net CO_2 exchange rates by leaf discs of soybean. a, 2% O_2, 200 µl/l CO_2, balance N_2. b, 21% O_2, 200 µl/l CO_2, balance N_2.——, Response of untreated discs; – – –, response of AAN-treated discs. Discs had been floated on water or AAN (25 mM, pH 7) in light and air for 50 min, preceeding experiment. Open or closed bars between graphs represent light (1200 $\mu E\ m^{-2}s^{-1}$ approximately 80% saturation) or dark conditions, respectively.

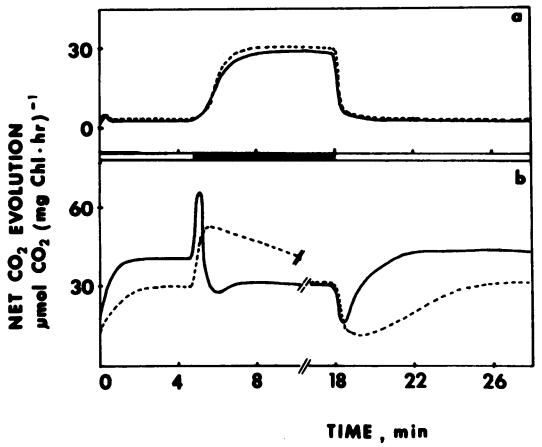

FIG. 2. Effect of AAN on net CO_2 release into CO_2-free gas by leaf discs of soybean. ——, response of untreated discs; – – –, response of AAN-treated discs (same conditions as in Fig. 1). a, Leaf discs were flushed with 2% O_2, 98% N_2; b, Leaf discs were flushed with 60% O_2, 40% N_2. Open and closed bars represent light and dark conditions, respectively.

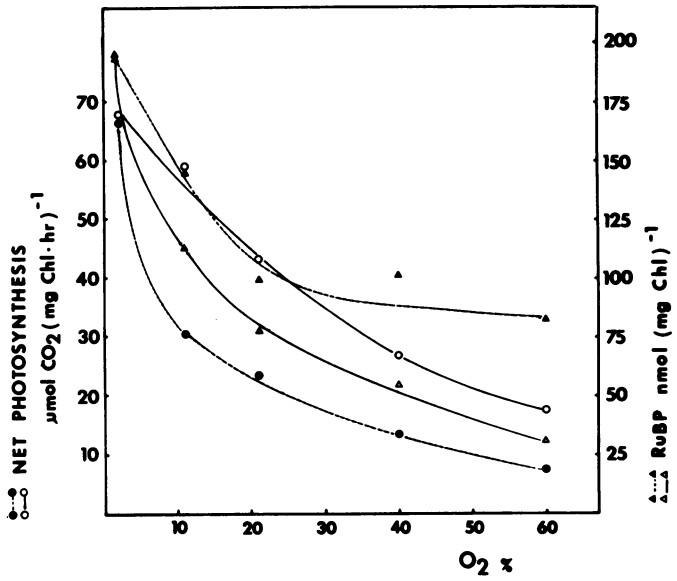

FIG. 3. Effect of O_2 concentration on steady net rate of CO_2 exchange (○, ●) and RuBP levels (△, ▲) at 200 μl/l CO_2 in control (——) and AAN-treated samples (– – –). Light intensity and AAN treatment conditions were same as in Figure 1. For RuBP measurements, samples were taken after they had reached steady-state rates of photosynthesis during second illumination period (see Fig. 1). Each point represents mean value of four to twelve replicates. SE of the mean values for RuBP ranged from 5.2% to 23% and for net photosynthesis from 4.3% to 19.2%.

(data not shown). When switching on the lights again, there was a partial recovery of the photosynthetic capacity, but after 1 min the rate of CO_2 uptake decreased again. These gas exchange patterns are similar to those observed with the mutant of *Arabidopsis thaliana* deficient in serine hydroxymethyl transferase (26).

Figure 2 is an example of CO_2 release in CO_2-free gas. At 2% O_2 (Fig. 2a), there was no significant difference in the rate of CO_2 evolution either in the light or dark between the AAN-treated sample and the control sample. At 60% O_2 (Fig. 2b), the rate of CO_2 release in the light by the AAN-treated sample was approximately 75% of the control values. On darkening, the control leaves showed a rapid postillumination burst which is thought to be the result of the decarboxylation of some glycolate pathway intermediates (6). With the inhibitor however, CO_2 was released

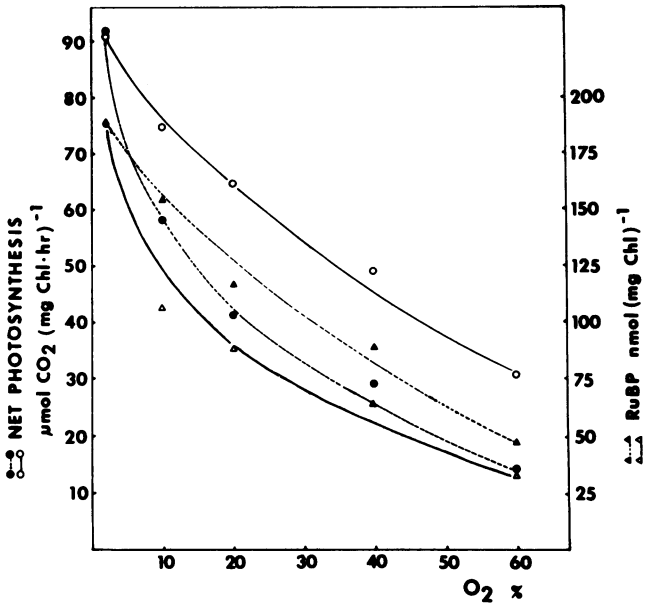

FIG. 4. Effect of O_2 concentration on the steady-state net rate of CO_2 uptake (○, ●) and RuBP levels (△, ▲) at 330 μl/l CO_2 in control (——) and AAN-treated samples (– – –). Except for CO_2 concentration, other conditions were same as in Figure 3. Each point represents mean value of eight replicates and SE of the mean values for RuBP ranged from 5.8% to 18.9% and for net photosynthesis from 13.6% to 20.9%.

at a slower rate but for a much longer time before the same dark respiration steady rate as in the control sample was reached. One can calculate that AAN-treated leaves evolved approximately 10 times more CO_2 than the control plants. When switching on the lights again a steady rate of CO_2 evolution was attained much more slowly than with the control plants.

From the linear portion of the response of net photosynthesis to CO_2 concentration (data not shown), we have determined the CO_2 compensation points at 21% O_2. They were 47.6 ± 5.2 μl/l in the control discs and 57.3 ± 15.8 μl/l in the AAN-treated discs, so they were not significantly different. The carboxylation efficiency which is the slope of the curve relating net photosynthesis to CO_2 concentration was reduced from 0.27 in the control samples to 0.17 in the AAN-treated samples. The light-dependent CO_2 release which is the extrapolation of that curve to CO_2 concentration equal 0 represented again 75% of the control values as it was in Figure 2b.

Figures 3 and 4 show the effect of O_2 and AAN on net photosynthesis at two different CO_2 concentrations. The control samples showed a typical inhibition of net photosynthesis by O_2. The percentage of inhibition by O_2 ranged from 29% to 36% at 21% O_2 and 66% to 75% at 60% O_2 at 330 and 200 μl/l CO_2, respectively. At 11% O_2 and greater, AAN inhibited net photosynthesis in addition to the O_2 effect. When expressed as a percentage of the control values at the same O_2 concentration (Fig. 5), the degree of inhibition caused by AAN was not significantly dependent on the O_2 concentration beyond 11%, at 200 μl/l CO_2. Net photosynthesis was inhibited by approximately 50% compared with the control samples. At 330 μl/l CO_2 however, the inhibition of net photosynthesis caused by AAN seemed to be more dependent on the O_2 concentration varying from 23% at 11% O_2 to 54% at 60% O_2 (Fig. 5).

RuBP Levels. At both CO_2 concentrations (Figs. 3 and 4), the control samples showed a decrease in RuBP levels correlated with the decrease in net photosynthesis when the O_2 concentration was increased from 2% to 60%. The RuBP levels were not significantly different at these two CO_2 concentrations; they were approximately 190 nmol/mg Chl at 2% O_2 and decreased to a value of 30

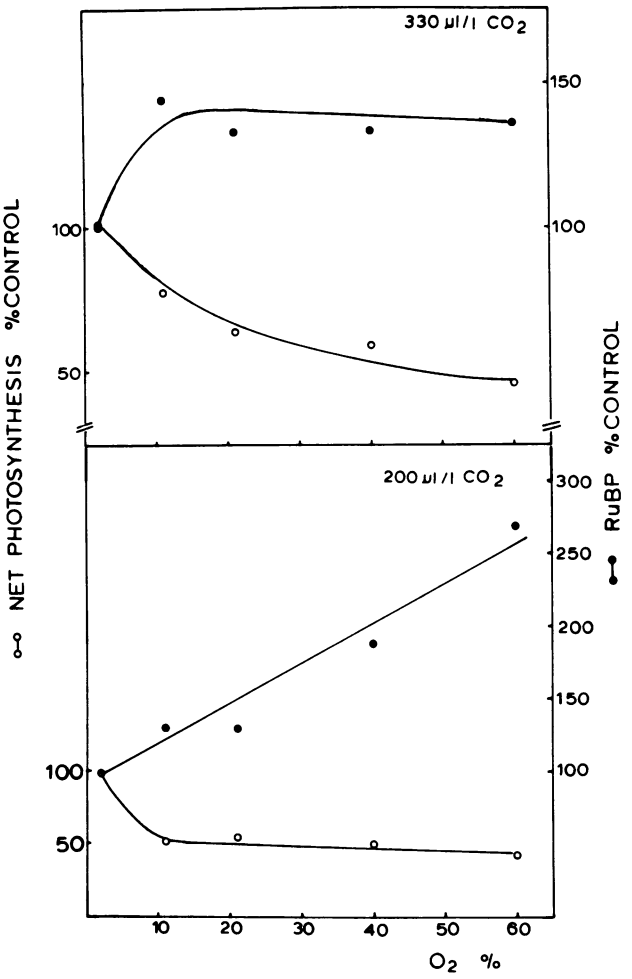

FIG. 5. Effect of AAN on net photosynthesis (O——O) and RuBP (●——●) at different O_2 concentrations and 200 or 300 µl/l CO_2. Results in Figures 3 and 4 are expressed as a percentage of control values at corresponding O_2 concentration.

nmol at 60% O_2. With the addition of the inhibitor, the RuBP levels were unchanged at 2% O_2 but were higher than in the untreated discs at the same O_2 concentration when the latter was increased. At 200 µl/l CO_2 (Fig. 5), they were 1.3 times higher at 11% O_2 and 2.6 times higher at 60% O_2. At 330 µl/l CO_2 (Fig. 5), they were approximately 1.4 times higher than in the control samples whatever the O_2 concentration from 11% to 60%.

Glycolate Pathway Intermediates Levels. As expected, since AAN is an inhibitor of glycine decarboxylase, glycine accumulated in AAN-treated discs (Table I) and its accumulation was partly dependent on the O_2 concentration. However, the values presented are the results of glycine accumulation during the 50-min incubation period in air plus the 30 min of the treatment with the O_2 and CO_2 concentrations indicated, so it is impossible to calculate a rate of synthesis in a given condition, moreover, the degree of inhibition of glycine decarboxylase is not known. Glyoxylate levels were measured at 200 µl/l CO_2 and 2% and 40% O_2. They were very low: 7 to 9 nmol/mg Chl at 2% O_2 and at 40% O_2 11 nmol/mg Chl in the control samples and 27 nmol/mg Chl with the inhibitor. Thus, AAN caused approximately a 2-fold increase in glyoxylate levels under photorespiratory conditions. At 40% O_2 the levels of glycolate decreased with the inhibitor from approximately 90 nmol/mg Chl in the control disc to 34 nmol/mg Chl in treated discs.

DISCUSSION

The inhibition of net photosynthesis by AAN in photorespiratory conditions confirms many of the studies mentioned before and clearly shows that inhibiting the glycolate pathway is not a means of increasing photosynthesis. AAN has been shown to be an effective inhibitor of glycine decarboxylase in mitochondria at far lower concentrations than INH, to have no side effects on dark respiration, and no effect on CO_2 fixation in chloroplasts (27). Indeed our experiments conducted at 2% O_2 with the inhibitor did not show any difference in the rates of net photosynthesis or CO_2 evolution in CO_2-free gas as compared with the control samples. One would have expected, however, to measure no more CO_2 release at 60% O_2 than at 2% O_2 in CO_2-free gas when the inhibitor was present; but it was much higher at 60% than at 2%, and the observed rates were approximately 75% of the control at the same O_2 concentration. There may be two possibilities to explain that CO_2 release in the light: (a) the inhibition of the glycine decarboxylase was not complete; as the inhibitor is competitive with respect to glycine, an accumulation of glycine may have overcome the inhibition. This would explain the observation that during the second exposure to light, the rate of CO_2 release gradually increased as the glycine pool built up. (b) The second possibility could be that, due to continuous accumulation of glycine, there was a shortage of NH_2 group donors and as suggested in Ref. 26, glycine being no longer synthesized, glyoxylate instead would have been decarboxylated. This hypothesis could also explain the decrease in net photosynthesis observed in photorespiratory conditions (Fig. 1b) since the decarboxylation of glyoxylate would have released twice as much CO_2 as glycine; but had it been the case in these experiments, one would have observed even more CO_2 release in the light in CO_2-free gas. Moreover, this hypothesis cannot account for the amount of CO_2 released in the dark which at 60% O_2 (Fig. 2b) represented approximately 2 µmol/mg Chl; the glyoxylate pool was small (approximately 30 nmol/mg Chl), and in the dark there should be no more glyoxylate synthesis. We would thus favor the first explanation which is supported by the fact that the amount of glycine accumulated in the light (approximately 5 µmol/mg Chl) can account for the amount of CO_2 released in the dark.

The control leaves showed a decrease in RuBP levels correlated with the decrease in net photosynthesis when the O_2 concentration was increased. It could follow that lower levels of RuBP when increasing O_2 concentration may in part account for the decreased rate of photosynthesis, the other factors intervening in the inhibition by O_2 being an increased release of CO_2 due to an increased production of glycolate and a competitive inhibition of RuBP carboxylase by O_2 with respect to CO_2 (4). RuBP levels measured in air were approximately 90 nmol/mg Chl. If we consider a chloroplast volume of 25 µl/mg Chl (10), that corresponds to a concentration of 3.6 mM in the chloroplasts. In a recent study (21) with wheat seedlings, this concentration of RuBP appeared to limit CO_2 fixation, considering the concentration of binding sites of the RuBP carboxylase. However, it is possible that RuBP carboxylase concentration was lower in soybean; thus RuBP may still have been saturating.

Given these results and the possible limitation of CO_2 uptake by low concentrations of RuBP at high O_2, one would have expected that preventing the recycling of the carbon to the Calvin cycle and storing it in glycine would have further depleted the Calvin cycle intermediates and among them RuBP. Our results do not apparently support this view because, at O_2 levels above 2%, the RuBP levels were higher in the presence of the inhibitor. As we observed a decrease in net photosynthesis, higher RuBP levels would mean that RuBP carboxylase was inhibited. AAN itself did not inhibit RuBP carboxylase since we did not observe any decrease in net photosynthesis at 2% O_2. The inhibition may have been the result of an accumulation of an intermediate of the glycolate pathway in photorespiratory conditions which could then have had a regulatory effect on RuBP carboxylase. Indeed the glycine levels were 7 to 10 times higher than in the control

Table I. *Effect of AAN on Glycine Levels at Various O_2 Concentrations*

The leaf discs were preincubated in the light and air for 50 min with 25 mM AAN or water as indicated. Samples were taken after they had reached steady-state rates of net photosynthesis in 200 μl/l CO_2 and O_2 as indicated. For details see Figure 3. Glycine was measured as described in "Material and Methods."

	Oxygen				
	2%	11%	21%	40%	60%
	Glycine Content				
	μmol/mgChl				
Control	0.18 ± 0.03[a]	0.31 ± 0.06	0.44 ± 0.18	0.62 ± 0.11	0.73 ± 0.15
+ AAN	1.65 ± 0.2	2.68 ± 0.24	2.90 ± 0.26	3.47 ± 0.72	5.62 ± 0.56

[a] Mean values of four to nine experiments ±SD.

samples, but this was true also at 2% O_2 (since the incubation period had been conducted in air), in which case there was no inhibition of net photosynthesis and no change in RuBP levels; thus it is unlikely that in photorespiratory conditions high levels of glycine directly affected the RuBP carboxylase activity. Moreover, 4 min in the dark led to a partial recovery of the photosynthetic capacity and during that short period the glycine pool was not depleted considering the rate of CO_2 release in the dark.

Among the other glycolate pathway intermediates measured, glyoxylate increased 2 to 3 times in presence of AAN, but its concentration was still low (approximately 1 mM if it were all present in chloroplasts). A recent study (5) showed that 10 mM glyoxylate caused a reversible inhibition of the RuBP carboxylase-oxygenase; however the inhibition occurred only with the fully activated enzyme in the absence of effectors which may not be the actual state of the enzyme *in vivo* (21). Higher concentrations of glyoxylate have been reported to inhibit photosynthesis by chloroplasts (20), but to stimulate net photosynthesis in tobacco leaf discs (20) and isolated soybean mesophyll cells (18). In these experiments too, the order of addition of CO_2 and glyoxylate was critical. It is thus difficult to interpret these results *in vivo* and further experiments are needed to demonstrate that glyoxylate can be an inhibitor of the carboxylase *in vivo*.

There is some evidence that in severe photorespiratory conditions endogenous carbohydrates are used to provide carbon skeletons for the Calvin cycle and glycolate synthesis (3, 12). This may have happened in our experiments too, and we can imagine that a regulatory mechanism came into effect to cause a partial inhibition of RuBP carboxylase, slowing the wasteful process of using endogenous carbohydrates to synthesize glycine. How this regulatory mechanism may operate cannot be deduced from the present experiments. However, it seems clear that diverting much carbon from the Calvin cycle for the synthesis of glycine instead of mainly starch and sucrose has a negative effect on photosynthesis possibly because of a regulatory effect on RuBP carboxylase.

LITERATURE CITED

1. ANDREWS TJ, GH LORIMER, NE TOLBERT 1973 Ribulose diphosphate oxygenase. I. Synthesis of phosphoglycolate by fraction-1 protein of leaves. Biochemistry 12: 11–17
2. ANDREWS TJ, GH LORIMER 1978 Photorespiration still unavoidable? FEBS Lett 90: 1–9
3. BASSHAM JA, M KIRK 1973 Sequence of formation of phosphoglycolate and glycolate in photosynthesizing *Chlorella pyrenoidosa*. Plant Physiol 52: 407–411
4. CHOLLET R, WL OGREN 1975 Regulation of photorespiration in C_3 and C_4 species. Bot Rev 41: 137–179
5. COOK CM, NE TOLBERT 1982 Inhibition of spinach ribulose bisphosphate carboxylase/oxygenase by glyoxylate. Plant Physiol 69: S-52
6. D'AOUST AL, DT CANVIN 1972 The specific activity of CO_2 evolved in the light and darkness by sunflower leaves following periods of photosynthesis in $^{14}CO_2$. Photosynthetica 6: 150–157
7. DORIVARI S, DT CANVIN 1980 Effect of butyl 2-Hydroxy-3-Butynoate on sunflower leaf photosynthesis and photorespiration. Plant Physiol 66: 628–631
8. GARDESTRÖM P, A BERGMAN, I ERICSON 1981 Inhibition of the conversion of glycine to serine in spinach leaf mitochondria. Physiol Plant 53: 439–444
9. GUNTHER G, G BAUMANN, J KLOS, J BALFANZ 1979 Effects of inhibitors and herbicides on net photosynthesis and photorespiration of sugar beet (*Beta vulgaris*) and white lambsquarter (*Chenopodium album*). Biochem Physiol Pflanzen 174: 616–628
10. HELDT HW, F SAUER 1971 The inner membrane of the chloroplast envelope at the site of specific metabolite transport. Biochim Biophys Acta 234: 83–91
11. HITZ WD, CR STEWART 1980 Oxygen and carbon dioxide effects on the pool size of some photosynthetic and photorespiratory intermediates in soybean (*Glycine max* L Merr.). Plant Physiol 65: 442–446
12. KAMINSKA Z, S MALESZEWSKI 1980 The effect of O_2 and CO_2 concentration on photosynthesis glycolate accumulation in bean leaves treated with α-hydroxy-2 pyridinemethane-sulfonic acid (α-HPMS), the glycolate oxidase inhibitor. Photosynthesis Res 1: 45–51
13. KIRK MR, U HEBER 1976 Rates of synthesis and source of glycolate in chloroplasts. Planta 132: 131–141
14. KUMARASINGHE KS, AJ KEYS, CP WHITTINGHAM 1977 Effects of certain inhibitors on photorespiration by wheat leaf segments. J Exp Bot 28: 1163–1168
15. LAING WA 1974 Physiological and biochemical properties of soybean ribulose diphosphate carboxylase. PhD thesis. University of Illinois Urbana
16. LATZKO E, M GIBBS 1974 D-Ribulose-1, 5-diphosphate and pentose monophosphate. Radiochemical assay. *In* HU Bergmeyer, ed, Methods of Enzymatic Analysis, Ed 2, Vol 3. Academic Press, New York, pp 1385–1389
17. OHMORI S, M IKIDA, Y WATANABE, K HIROTA 1978 A simple and specific determination of glycine in biological samples. Anal Biochem 90: 662–670
18. OLIVER DJ 1980 The effects of glyoxylate on photosynthesis and photorespiration by isolated soybean mesophyll cells. Plant Physiol 65: 888–892
19. OLIVER DJ 1981 Role of glycine and glyoxylate decarboxylation in photorespiratory CO_2 release. Plant Physiol 68: 1031–1034
20. OLIVER DJ, I ZELITCH 1977 Increasing photosynthesis by inhibiting photorespiration with glyoxylate. Science 196: 1450–1451
21. PERCHOROWICZ JT, DA RAYNES, RG JENSEN 1981 Light limitation of photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Proc Natl Acad Sci USA 78: 2985–2989
22. RACKER, E 1962 Ribulose diphosphate carboxylase from spinach leaves. Methods Enzymol 5: 266–270
23. RATHMAN CKM, R CHOLLET 1980 Photosynthetic and photorespiratory carbon metabolism in mesophyll protoplasts and chloroplasts isolated from isogenic diploid and tetraploid cultivars of ryegrass (*Lolium perenne* L.). Plant Physiol 65: 489–494
24. SERVAITES JC, WL OGREN 1977 Chemical inhibition of the glycolate pathway in soybean leaf cells. Plant Physiol 60: 461–466
25. SOMERVILLE CR, WL OGREN 1980 Photorespiration mutants of *Arabidopsis thaliana* deficient in serime glyoxylate aminotransferase activity. Proc Natl Acad Sci USA 77: 2684–2687
26. SOMERVILLE CR, WL OGREN 1981 Photorespiration-deficient mutants of *Arabidopsis thaliana* lacking mitochondrial serine transhydroxymethylase activity. Plant Physiol 67: 666–671
27. USUDA H, GP ARRON, GE EDWARDS 1980 Inhibition of glycine decarboxylation by aminoacetonitrile and its effect on photosynthesis in wheat. J Exp Bot 31: 1477–1483
28. VOGEL GD, C VAN DER DRIFT 1970 Differential analysis of glyoxylate derivatives. Anal Biochem 33: 143–157
29. YUN SJ, R ISHII, SB HYEON, A SUZUKI, Y MURATA, S TAMURA 1979 Effects of some chemicals on photorespiration and photosynthesis in the excised rice leaves. Agric Biol Chem 43: 2207–2209
30. ZELITCH I 1966 Increased rate of net photosynthetic carbon dioxide uptake caused by the inhibition of glycolate oxidase. Plant Physiol 41: 1623–1631