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Effect of Inorganic Nitrogen on Photorespiration of Pea Leaves

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완두잎의 光呼吸에 미치는 無機窒素의 影響

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ABSTRACT

Leaf discs isolated from the pea seedling grown in nutrient solution containing 5 mM ammonia or nitrate exhibited a half level of photorespiration as compared with the nitrogen free control. The manifestation of the ammonia effect appeared somewhat earlier than that of nitrate effect, but this difference subsided as the culture periods was extended. The total amount of CO_2 fixed by leaves from nitrogen-supplemented seedlings showed approximately 1.5 fold increase over the control with the ammonia effect being manifested earlier than the nitrate effect. The activities of peroxisomal serine: glyoxylate aminotransferase were always higher with ammonia than nitrate, the two types of nitrogen source, however, had similar effect on conversion rate of glyoxylate into glycine. These results indicate that exogenous ammonia does not act directly as an effector of this aminotransferase in vivo. But changes in the level of the pool size of glycine and serine, both of which are the intermediates of photorespiratory process, suggest that exogenous ammonia inhibit the transformation of serine from glycine metabolically.

INTRODUCTION

In spite of the numerous studies on the metabolism of photorespiration, the physiological significance of photorespiration is still obscure.

In C_3-plant, it has been obvious that a large part of CO_2 fixed through photosynthesis is released by photorespiration. Many investigators have pointed out that relatively lower photosynthetic CO_2 fixation of C_3-plant is chiefly due to the photorespiration comparing with that of C_4-plant (Zelitch, 1966; Chollet and Ogren, 1975; Zelitch, 1975). Several chemicals including isonicotinic acid hydrazide, α-hydroxypyridine methanesulfonate and

glicidate were intensively studied as inhibitors of photorespiration (Miflin *et al.*, 1966; Servaites, 1977; Zelitch, 1978; Wildner and Larsson, 1979) to increase net photosynthetic CO_2 fixation, and some of those, especially glutamate and glyoxylate, were reported as effective inhibitors in tobacco leaf discs or isolated mesophyll cells (Oliver and Zelitch, 1977 a, b; Oliver, 1980). Whereas glyoxlate has been shown to be a specific inhibitor of glycine decarboxylase or L-serine hydroxymethyl transferase (Peterson, 1982), any report on regulatory mechanism of glutamate has not published yet. Oliver and Zelitch (1977b) suggested that increasing the pool size of glutamate caused decrease in glycolate synthesis and that glutamate inhibited photorespiration metabolically. Since glutamate is one of the key amino acid which is synthesized by incorporating exogenous nitrogen source into α-ketoglutarate, it has seemed to be possible of regulating the photorespiration by the change of the pool size of glutamate. In fact, Woo and Canvin (1980 a, b) reported that treatment of ammonia on spinach leaf cells or isolated mesophyll cells caused increasing of CO_2 fixation, and several reports have shown the possible mechanism of ammonia in regulating the photosynthetic carbon flow in alfalfa (Platt, 1977), papaver (Paul *et al.*, 1978) and bean (Mohamed and Gnanam, 1979). But all of the above studies were carried out with partially isolated plant organs or short term treatment of ammonia.

Therefore, present study was undertaken to elucidate the effect of inorganic nitrogen supplied as nutrients on photorespiration in whole plant.

MATERIALS AND METHODS

Plant growth condition. Pea (*Pisum sativum* L.) seeds were sterilized by 5% sodium hypochlorite solution, germinated in vermiculite and grew in green house for 15 days. When fourth to fifth nodes were appeared, removed cotyledons, peas were transplanted carefully to the modified Hoagland solution containing 5 mM ammonia [as $(NH_4)_2SO_4$], nitrate (as KNO_3) or no nitrogen source.

After transplantation peas were grown in a controlled environment chamber under 300 μ einstein $m^{-2} \cdot sec^{-1}$(continuous irradiation) at 28 C. Leaves were obtained from 3rd nodes in every experiment.

Determination of photorespiration and total photosynthetic CO_2 fixation. Method of determination of photorespiration described previously was employed by Zelitch (1968).

Leaf discs were cut out from the same position of leaves with cork borer and 6 discs (approximately 120 mg) were floated on 2 ml water in 25 cm^3 Warburg vessel. $^{14}CO_2$ was liberated by injection of lactic acid to $NaH^{14}CO_3$ (20 μCi, 10 μmol) in side arm and assimilated into leaf discs during 50 min under 2,000 ft.C. And then excess $^{14}CO_2$ was removed and 100 ml of monoethanolamine: ethoxyethanol=2 : 1(v/v) solution was used to trap $^{14}CO_2$ released from leaf discs by photorespiration. At interval of 10 min, 1 ml of solution was sampled to determine the radioactivity by scintilating counting. Photorespiration rate was

calculated from the amount of $^{14}CO_2$ released between 10 and 30 min.

Immediately after determination of photorespiration rate, the leaf discs were treated with 80% boiling ethanol and distilled water for 10 min respectively. The extracts were combined and measured the radioactivity. The residues were collected on filter paper, ground with sea sand in motar thoroughly, immersed in the cocktail solution during overnight and measured the radioactivity. Total photosynthetic $^{14}CO_2$ fixation was calculated from the amount of ^{14}C of extracts, residues and released from leaf discs during photorespiration.

Assay of peroxisomal aminotransferases. Partially purified peroxisome fraction was obtained by differential and sucrose density gradient centrifugation by method of Lawyer and Zelitch (1978).

The serine: glyoxylate aminotransferase (E.C. 2.6.1.45) was assayed as the production of ^{14}C-glycine from 20 μmol (1-^{14}C) glyoxylate and 25 μmol L-serine (Kisaki and Tolbert, 1969). The glutamate: glyoxylate aminotransferase (E.C. 2.6.1.4) was also determined from the production of ^{14}C-glycine from (1-^{14}C) glyoxylate and L-glutamate as amino group donor in stead of serine.

Protein determination followed a method of Lowry *et al.* (1951).

Amino acid analysis. To determine the pool sizes of amino acids in each experimental group, following method was employed.

2-3 g of pea leaves were extracted by 100 ml boiling ethanol (80%) and concentrated the extracts by rotary evaporator below 40 C. Concentrated samples were melted with 10 ml distilled water and applied on Dowex 50W cation exchange resin column (10×100 mm). Eluting the column with water, amino acids were eluted with 2N ammonia water. Analysis of the amino acids was carried out by two dimensional paper chromatography; chromatogram was first developed with water saturated phenol solution containing 1% NH_4OH for 24 hrs and then developed with n-butanol:acetic acid:water=9:1:3(v/v) for 18 hrs in second dimension. The paper was dried thoroughly and sprayed 0.2% ninhydrin (w/w in ethanol) solution. Each amino acids was identified by comparing the Rf with the standards and decolored with 3 ml 50% ethanol to read at spectrophotometer 540.

RESULTS AND DISCUSSION

Effect of exogenous nitrogen sources on photorespiration. Fig. 1 showed that leaf discs obtained from which grew in 5 mM ammonia (ammonia group) exhibited lower photorespiratory rate than those from 5 mM nitrate (nitrate group) or nitrogen-free nutrient solution (N-free group). At 48 hrs after transplantation to water culture, photorespiration of ammonia group, approximately 5.4×10^4 cpm $^{14}CO_2$ released g.fr.wt.$^{-1}$, was only 65% of N-free group and 80% of the nitrate group. The rate of photorespiration of ammonia group and nitrate group, however, became a similar level with prolonging the culture periods to 96 hrs. In N-free group, the rate of photorespiration increased to

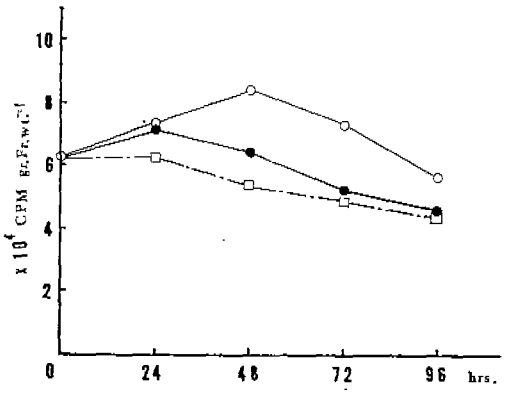

Fig. 1. $^{14}CO_2$ evolved from leaf discs under light condition. Leaf discs were obtained from water cultured pea with NH_4^+(□—·—□), NO_3^-(●——●) or no nitrogen source (○——○) added.

maximum level (8.4×10^4 cpm $^{14}CO_2$ released gr.fr.wt.$^{-1}$) at 48 hrs after transplantation, but decreased to 5.7×10^4 cpm $^{14}CO_2$ released gr. fr.wt.$^{-1}$ up to 96 hrs. Oliver and Zelitch (1977) discussed that photorespiratory CO_2 evolution could be affected by the concentration of glutamate and aspartate *in vivo*. The glycidate, a compound which also inhibited photorespiration, increased the pool size of glutamate in tabacco leaf discs (Zelitch, 1974).

Those reports indicated the photorespiration rate being closely related with the level of glutamate, and therefore, in our experiment, the lower photorespiration of nitrogen supplied groups might be caused by the higher level of glutamate than nitrogn free control.

Effect on total photosynthetic CO_2 fixation. In spite of the highest photorespiratory level, N-free group had the lowest total photosynthetic CO_2 fixation ability in three groups, which meant incorporation of CO_2 into soluble components and residues being inhibibited under nitrogen deficient condition (data not shown). Total photosynthetic CO_2 fixation also increased more rapidly by the treatment of ammonia than by the nitrate treatment. In ammonia group, maximal level of CO_2 fixation (1.5×10^7 cpm $^{14}CO_2$ incorporation gr.fr.wt.$^{-1}$) was reached after 48 hrs to water culture, whereas nitrate group required 96 hrs to reach the similar level of ammonia group. Woo and Canvin (1980 a, b) reported that ammonia increased the CO_2 fixation in spinach leaf cells or isolated mesophyll cells. The treatment of ammonia stimulated the rapid degradation of sugar into organic acids via TCA cycle for the mass aminating of organic acids to amino acids in bean (Mohamed and Gnanam, 1979) and alfalfa leaves (Platt, 1977). Therefore treament of ammonia to young plant, which may have not sufficient sugar, causes the rapid decrease the levels of sugar-phosphates and consequently activates the CO_2 fixation. This suggestion is supported by the report

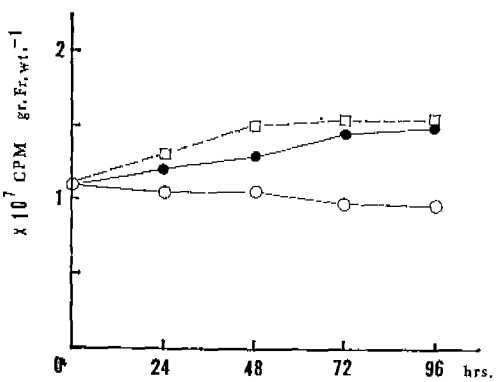

Fig. 2. Total amounts of ^{14}C incroporated into leaf discs cultured with NH_4^+ (□---□), NO_3^-(●——●) or no nitrogen source (○——○).

of Buchanan and Schurmann (1973) that RuBP carboxylase was deactivated by the increase of the fructose-bisphosphate level. Our experiment, however, showed the two types of inorganic nitrogen source as ammonia and nitrate having little different effects on photosynthetic CO_2 fixation with extending the culture periods and indicated the photorespiration and photosynthetic CO_2 fixation being more greatly affected by the exogenous nitrogen level supplied to the plants rather than the types of nitrogen source.

Aminotransferase activities. Serine: glyoxylate aminotransferase (SGATase) was reported to be inhibited irreversibly by low concentration of ammonia (1 mM) *in vitro* (Rehfeld and Tolbert, 1972; Smith, 1973). Therefore, if ammonia act as a specific inhibitor of a enzyme involved in photorespiration, it would be expected for ammonia inhibiting SGATase formally. After 24 hrs to water culture, ammonia group showed 10,563 dpm ^{14}C-glycine produced mg protein^{-1} of SGATase activity which was the highest level in experimental group. Moreover, through all of the culture periods activities of SGATase of ammonia group were always higer than those of nitrate group (Table 1). Production of glycine from glyoxylate catalysed glutamate: glyoxylate aminotransferase (GGATase) was higher level in nitrate group than in ammonia group (Table 2), but conversion rate of glyoxylate to glycine was seemed to be almost same in two groups. For example, at 24 hrs after transplantation, ammonia group showed the production of $4,910\times10^3$ dpm ^{14}C-glycine gr. fr. wt.$^{-1}$ and $4,949\times10^3$ dpm in nitrate group. This result was coincided with the previous report (Walton and Butt, 1981) that, in peroxisome, SGATase and GGATase were in mutural assistant relation for amination of glyoxylate and showed that aminotransferase involved in photorespiration did not inhibited directly by the nitrogen sources supplied on root. While N-free group showed a

Table 1. Activities of serine: glyoxylate aminotransferase in peroxisomes

Period of water culture (h)	Nitrogen source	Protein (mg/g·fr·wt)	Total activity ($\times 10^3$ dpm/ g·fr·wt)	Specific activity ($\times 10^3$ dpm/ mg·protein)
0		0.29	1,134	3,831
24	—	0.28	2,154	7,204
	NH_4	0.30	3,169	10,563
	NO_3	0.28	2,833	10,082
48	—	0.29	2,208	7,434
	NH_4	0.33	1,247	3,745
	NO_3	0.28	1,023	3,577
72	—	0.27	815	3,041
	NH_4	0.42	466	1,099
	NO_3	0.39	322	815
96	—	0.25	636	2,544
	NH_4	0.46	337	733
	NO_3	0.37	236	638

Table 2. Activities of glutamate: glyoxylate aminotransferase in peroxisomes

Period of water culture (h)	Nitrogen source	Protein (mg/gr·fr·wt)	Total activity ($\times 10^3$ dpm/ gr·fr·wt)	Specific activity ($\times 10^2$ dpm/ mg·protein)
0		0.29	1,884	6,365
24	—	0.28	1,805	6,447
	NH_4	0.30	1,741	5,803
	NO_3	0.28	2,116	7,530
48	—	0.29	1,027	3,457
	NH_4	0.33	721	2,160
	NO_3	0.28	728	2,545
72	—	0.27	728	2,716
	NH_4	0.42	680	1,604
	NO_3	0.39	732	1,853
96	—	0.25	702	2,808
	NH_4	0.46	500	1,087
	NO_3	0.37	527	1,424

similar activity of GGATase with nitrogen supplemented group, SGATase of N-free group was two-three times higher than that of ammonia or nitrate group. It may be a caused of higher photorespiration in N-free group, since in spite of the synthetic rate of glycine being the highest among the three groups, the pool size of glycine of N-free group did not increase compared with other two groups, which meant a rapid conversion of glycine into serine.

Changes of the pool size of amino acids. Supplement of ammonia increased the pool sizes of amino acids in almost all species of plants (Kanazawa *et al.*, 1972; Jessup and Fowler, 1977; Larson *et al.*, 1981). In our experiment, the pool sizes of glutamine,

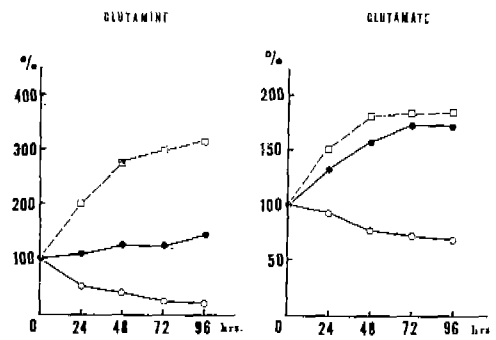

Fig. 3. Total amounts of glutamine and glutamate in pea leaves after various periods of water culture. NH_4^+(□---□), NO_3^-(●——●), N-def. (○—·—○)

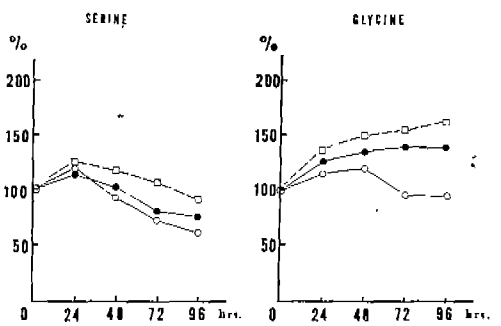

Fig. 4. Total amounts of serine and glycine in pea leaves after various periods of water culture. NH_4^+(□---□), NO_3^-(●——●), N-def(○——○).

glutamate and glycine also increased up to 300%, 180% and 160%, respectively, only after 48 hrs to water culture in ammonia group, while nitrate group required much longer periods to reach a similar level of amino acids with ammonia group (Figs. 3 and 4).

On the other hand, in N-free group, almost all amino acids decreased immediately after transplantation, that might be caused by nitrogen deficiency. But the pool size of serine decreased not only in N-free group but in ammonia and nitrate group (Fig. 4). In case of ammonia and nitrate group, these results could not be caused by the nitrogen deficiency.

Serine is understood to be synthesized from glycine by a combined action of glycine decarboxylase and serine hydroxymethyl transferase (Rabson *et al.*, 1962; Kisaki *et al.*, 1971; Bird *et al.*, 1972), releasing of NH_3 and CO_2 stoichiometrically (Kisaki *et al.*, 1971). Reassimilation of this photorespiratory evolved NH_3 had been a subject of recent reseach (Wallsgrove, 1979; Hartmann and Ehmke, 1980) and cytoplasmic glutamate dehydrogenase or glutamine synthetase were thought to be responded for the reassimilation of NH_3 (Wallsgrove *et al.*, 1980; Woo *et al.*, 1982).

Our experiments showed that exogenous nitrogen sources could affect those enzymes to assimilate the photorespiratory NH_3 and, therefore, increasing the level of available nitrogen in plant leaf cells might influence the transformation of serine from glycine and, consequently, decreased the photorespiratory CO_2 evolution.

摘 要

5 mM의 ammonia나 nitrate를 함유한 배양액에서 생장한 완두(*Pisum sativum* L.) 잎은 질소원이 결핍된 경우에 비해 50% 이상 광호흡이 억제되었다. 또한 이런 효과는 nitrate에 비해 ammonia가 빠르게 나타났으며 배양시간이 길어지면 이 두 질소원의 효과는 비슷하였다. Total CO_2 고정능에 있어서도 질소원을 공급한 경우 질소원이 결핍되었을 때보다 150% 정도 CO_2 고정능이 증가하였으며, 역시 nitrate 보다는 ammonia의 효과가 빠른 것으로 나타났다. Peroxisomal serine; glyoxylate aminotransferase의 활성은 ammonia를 처리한 경우에 가장 높았으나, glyoxylate에서 glycine으로 전환되는 율은 이 두 질소원 사이에 크게 차이가 나지 않았다.

그러나 glycine과 serine의 pool size 변화를 측정한 결과는 ammonia가 glycine이 serine으로 전환되는 대사과정에 영향을 미칠 수 있는 가능성을 제시하고 있었다.

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