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A Comparative Study on the Nitrogen Metabolism of Symbiotic *Chlorella* from *Paramecium bursaria* with *Chlorella ellipsoidea*

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*Paramecium bursaria*와 共生하는 *Chlorella*와 *Chlorella ellipsoidea*의 窒素代謝에 관한 比較硏究

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ABSTRACT

The excretion of ammonia and glutamine synthetase activities were measured in aposymbiotic *Paramecium* and symbiotic *Paramecium*. The uptake of nitrate and ammonia, and specific enzyme activities of nitrate reductase, glutamate dehydrogenase and glutamine synthetase were investigated in symbiotic *Chlorella* from *Paramecium bursaria* and *Chlorella ellipsoidea*.

The ammonia concentration in the culture media of aposymbiotic *Paramecium* was increased according to the growth of the *Paramecium* but it was not changed in symbiotic *Paramecium*. Nitrate, the major nitrogen source, was taken up at a rate of 0.635 nmol/10^6 *Chlorella*/hr in *Chlorella ellipsoidea*. Most of ammonia was assimilated to glutamine by glutamine synthetase, of which activity was 1,467 μmol/mg protein/min in *Chlorella ellipsoidea*.

Contrary to *Chlorella ellipsoidea*, ammonia and glutamine transported from the *Paramecium* were the nitrogen source of symbiotic *Chlorella* and ammonia was taken up at a rate of 3.854 nmol/10^6 *Chlorella*/hr into symbiotic *Chlorella*. Most of ammonia were assimilated to glutamate by glutamate dehydrogenase in symbiotic *Chlorella*. The glutamate dehydrogenase (GDH/NADH) activity was 0.851 μmol/mg protein/min.

INTRODUCTION

Paramecium bursaria is a ciliated protozoan which usually lives in mutualistic relationship with *Chlorella*. These algae grow and divide within the cytoplasm of the host cell at the rate compatible with that of the host's growth. The symbiotic relationship between the organisms is hereditary (Karakashian, 1975).

It was noted that strains of *Chlorella* that had been endosymbionts released 86% of their total photosynthate into external environment and 95% of the photosynthate of the endosymbiotic algae was maltose(Muscatine *et al.*, 1967). Brown and Nielson(1974) reported that symbiotic *Paramecium bursaria* took up $Na^{14}CO_3$, and carbohydrate products of photosynthesis of the symbionts were transferred to the host *Paramecium*. Photosynthate and oxygen produced by symbiotic *Chlorella* were transferred to the *Paramecium*(Chang *et al.*, 1984).

Contrary to our knowledge of carbon metabolism of the symbiotic relationship, only a few studies about its nitrogen metabolism were reported. The *Paramecium* supplied the symbiotic *Chlorella* with some essential, yet unidentified nitrogen compounds(Reisser, 1976). Albers *et al.* (1982) noted that ammonia was excreted by aposymbiotic *Paramecium*, but it was taken up from the culture media by symbiotic one. Thus, the nitrogen sources of the symbiotic *Chlorella* within the endosymbiotic unit were not nitrate but probably ammonia and glutamine.

In addition to *Paramecium bursaria*, there were many studies about ammonia utilization of symbionts. In the marine platyhelminth, *Convoluta roscoffensis*, it was suggested that ammonia produced by uric acid catabolism in algae was assimilated into glutamine, which may be the main amino acid released back to the *Conboluta*(Boyle and Smith, 1975). Taylor (1978) reported that the photosynthetic rate of symbionts and the excretion rate of its photosynthate were increased by ammonia produced by the staghorn coral, *Acropora cervicornis*. In the tropical sea anemone, *Aiptasia pulchella*, it was noted that symbiotic zooxanthella took up ammonia according to diffusion kinetics(Wilkerson and Muscatine, 1984).

Although most of the studies demonstrated the uptake and utilization of ammonia, little was known about the assimilatory pathways of ammonia in symbiotic algae. The purpose of this study is to investigate not only the uptake and assimilation of ammonia, but also the change of the pathway of nitrogen assimilation using both symbiotic *Chlorella* and *Chlorella ellipsoidea*.

MATERIALS AND METHODS

Isolation and cultivation of *Paramecium bursaria*. *Paramecium bursaria* was isolated from Zaha-Yeon on the campus of Seoul National University in 1983. It was grown in Hay Infusion (Sonneborn, 1970). Aposymbiotic cultures of *Paramecium* were obtained initially by prolonged starvation in darkness (Karakashian and Karakashian, 1973). Cultures were grown at 25°C under diurnal illumination (12 hrs light, 12 hrs darkness) of white fluorescent tubes. The light intensity was 2,000 lux.

Isolation and cultivation of *Chlorella*. *Paramecium bursaria* was harvested by centrifugation at 1,000~1,500 rpm (Sorvall GLC-2B). The cell suspension was disrupted in a French Pressure Cell (Cat. No. 4-3398 French Pressure Cell, American Instrument Co. LTD.) at

5,000 psi. Symbiotic *Chlorella* in the extracts of *Paramecium bursaria* bursted in death and *Chlorella* survived was isolated by centrifugation at 2,000 rpm. Symbiotic *Chlorella* isolated were cultured in the modified Kessler's media (Weis, 1979). The modified Kessler's media had the composition, in g/liter: 0.8 g KNO_3, 0.47 g $NaH_2PO_4 \cdot 2H_2O$, 0.36 g $Na_2HPO_4 \cdot 12H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.013 g $CaCl_2 \cdot 2H_2O$, 0.406 g $FeCl_3 \cdot 6H_2O$, 0.017 g $MnCl_2 \cdot 4H_2O$, 0.021 g $ZnSO_4 \cdot 7H_2O$, and 0.1% (w/v) proteose peptone, 0.02% (w/v) yeast extract. 6 μg/liter penicillin was also added; the final pH was adjusted to 6.3.

Chlorella ellipsoidea, reference strain, was grown in the medium containing, in g/liter: 1.25 g KNO_3, 1.25 g $MgSO_4 \cdot 7H_2O$, 1.25 g glucose, and supplemented with 1 ml of Fe-solution and 1 ml of trace element solution. The final pH was adjusted to 6.5. Fe-solution contained 0.02% (w/v) $FeSO_4 \cdot 7H_2O$ and supplemented with 2 drops of sulfuric acid. Trace element solution had the composition, in mg/100 ml: 286 mg H_3BO_3, 250 mg $MnSO_4 \cdot 7H_2O$, 22.2 mg $ZnSO_4 \cdot 7H_2O$, 7.9 mg $CuSO_4 \cdot 7H_2O$, and 2.1 mg Na_2MoO_4.

Symbiotic *Chlorella* and *Chlorella ellipsoidea* were cultured at 25°C in the Mini-fermentater (Model No. M-1000, New Brunswick Sci. Co.). The light intensity was 5,000 lux.

Determination of nitrate. For the measurement of nitrate taken up by *Chlorella*, the cultures of *Chlorella* were transferred into the nitrate medium for 7 hrs and centrifuged (Beckman Model J-21 B) at 10,000 *g* for 10 min. Nitrate was determined quantitatively by rapid colorimetric method (Cataldo *et al.*, 1975).

Determination of ammonia. For the measurement of ammonia excreted by *Paramecium bursaria*, the cultures of *Paramecium* were filtered with filter paper (Toyo Filter Paper No. 2). And for the measurement of ammonia taken up by *Chlorella*, the cultures of *Chlorella* were transferred into ammonia medium (Albers *et al.*, 1982) for 7 hrs and centrifuged at 10,000g for 10 min. The ammonia concentrations of the prepared solutions were measured by the phenyl hypochlorite method of Solorzano (1969).

Measurement of the number of *Paramecium* and *Chlorella*. The number of *Paremecium* was counted on a $\phi 36 \times 10$ mm Falcon dish (Chang *et al.*, 1984). The number of *Chlorella* was counted on a haemacytometer (Bürker Haemacytometer).

Determination of protein. The protein content of crude extracts was determined according to Lowry *et al.* (1951).

Preparation of crude extracts. *Chlorella* was harvested by centrifugation at 2,000 rpm (Sorvall GLC-2B) and resuspended in the suitable buffer (see below). The cell suspension was disrupted in a French Pressure Cell (Cat. No. 4-3398 French Pressure Cell, American Instrument Co. LTD.) at 20,000 psi. The extracts were centrifuged at 27,000 *g* for 20 min. The supernatant fraction was then tested for enzyme activity and isoenzyme.

Enzyme assay. Nitrate reductase (NR). Nitrate reductase activity was determined by measuring the rate of nitrite formation as described by Pistorius *et al.* (1976). Reaction mixture contained 200 μmol potassium-phosphate buffer (pH 7.6), 20 μmol KNO_3, and 0.2 μmol NADH. The reaction was triggered by the addition of 400 μl of algal extracts. Final

volume was 1.6 ml. After incubation at 20°C for 30 min, the reaction was stopped by adding 200 μl of 0.5M Zn-acetate and 200 μl of 46 mg/l phenazine methosulfate (PMS). 1ml of sulfanilamide (1% in 1.5N HCl) and 1 ml of N-(1-naphthyl) ethylenediamine dihydrochloride (0.02%) were added to 1 ml of supernatant of reaction mixture. The developed color was measured at 540 nm. The specific enzyme activity was expressed in μmol NO_2^-/mg protein/hr (unit).

Glutamate dehydrogenase (GDH). GDH/NADH activity was measured according to Schmidt (1970). Reaction mixture contained 300 μmol potassium phosphate buffer (pH 8.5), 900 μmol ammonium sulfate, 18 μmol α-ketoglutarate, 3.9 μmol EDTA, and 0.45 μmol NADH. The reaction was started by adding of 20μl of algal crude extracts. Final volume was 3ml. It was incubated at 30°C for 5 min.

GDH/NAD activity was measured according to Cammaerts and Jacobs (1985). The assay mixture contained 123 μmol tris (Sigma T-1378)-HCl (pH 8.8), 150 μmol monosodium glutamates, and 1.5μmol NAD. The reaction was started with the addition of 20 μl of crude extracts.

These assays were based on the method of utilizing the NADH producing the reduction of the dye, p-iodonitrotetrazolium violet (Hinmen and Blass, 1981). We used PMS as the intermediate electron carrier.

After incubation, 0.34 ml of p-iodonitrotetrazolium violet (6 mM) and 0.034ml of PMS (0.65 mM) were added to the reaction mixture. The developed color was measured at 500 nm. The specific enzyme activity was expressed in μmol NADH/mg protein/min.

Glutamine synthetase (GS). Glutamine synthetase was assayed by r-glutamyl transferase activity. The transferase activity was determined according to Ahmad and Hellebust (1984). Assay mixture contained 100μmol Imidazole-HCl buffer(pH 7.0), 0.4 μmol ADP, 3.0 μmol $MnCl_2$, and 30 μmol sodium arsenate. It was incubated at 37°C for 15 min.

The r-glutamylhydroxamate content was measured by the addition of 2ml of stop solution. Stop solution contained 0.37 M $FeCl_3$ and 0.2M trichloroacetic acid (TCA) in 0.67 N HCl (Shapiro & Stadmen, 1970). Blank was run without the addtion of hydroxylamine. The specific enzyme activity was expressed in μmol γ-glutamylhydroxamate/mg protein/min (unit).

Isoenzyme. Electrophoresis. Isoenzyme was detected with polyacrylamide gel electrophoresis. The gel was prepared from 6% acrylamide and 0.16% bisacrylamide. The gel was polymerized with 0.05% tetramethylethylenediamine (TEMED) and 0.1% ammonium persulfate (Harris and Hopkinson, 1978). The gel buffer contained 50 mM tris and 25 mM boric acid. The pH was adjusted with boric acid to 8..8 (McLellan, 1982). The gel was run at 100 V for 20 min and followed by running at 150 V for 2 hrs approximately.

Enzyme detection. GDH/NAD was detected after the gel was incubated in a solution consisting of 4.4 mmol tris-HCl(pH 8.8), 5.4 mmol monosodium glutamate, 0.05 mmol NAD. Final volume was 50 ml. After the gel was incubated at 30°C for 15 min, 1 ml of 5 mg/ml

PMS and 1 ml of 10mg/ml nitro blue tetrazolium (Sigma N-6876) were added.

The transferase was detected after the gel was incubated in a solution consisting of 5.0 mmol Imidazole HCl(pH 7.0), 3.5 mmol glutamine, 1.75 mmol hydroxylamine HCl(pH 7.0), 0.02 mmol ADP, 0.15 mmol $MnCl_2$, and 1.5 mmol sodium arsenate. Final volume was 50ml. After the gel was incubated at 37°C for 20 min, the reaction was terminated by adding 44 ml of stop solution(see above). The bands appeared as purple zones against a yellow background and were not permanent unless the gel was stored at 4°C (Sharon *et al.*, 1984).

RESULTS

Uptake of nitrate and ammonia. When isolated symbiotic *Chlorella* was incubated in nitrate medium, the change of nitrate concentration was not detected. But the nitrate in the culture medium decreased at a rate of 0.635 nmol/10^5 *Chlorella*/hr in *Chlorella ellipsoidea* (Table 1).

Table 1. Uptake of nitrate and ammonia by symbiotic *Chlorella* and *Chlorella ellipsoidea*

Nitrogen sources	Isolated symbiotic *Chlorella*	*Chlorella ellipsoidea*
	nmol/10^6 *Chlorella*/hr	
Nitrate	N.D.*	0.635±0.213
Ammonia	3.854±0.182	N.D.*

*N.D.: Not-detected.

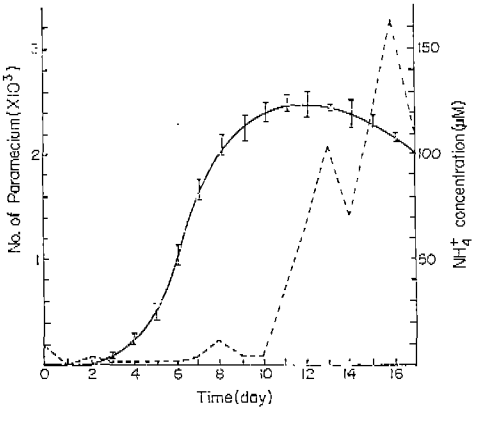

Fig. 1. The change of ammonium concentration (···) in the culture media according to the growth of aposymbiotic *Paramecium bursaria*(—). Each point of the growth curve was the mean of five experiments.

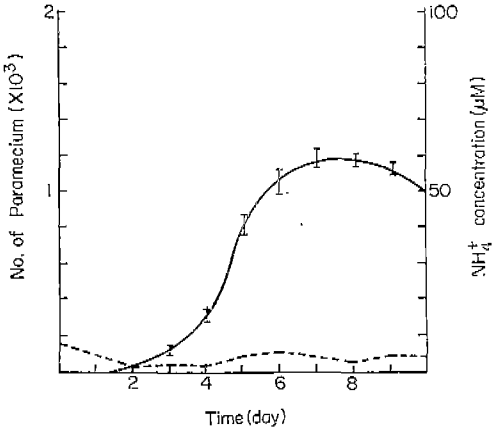

Fig. 2. The change of ammonium concentration (···) in the culture media according to the growth of symbiotic *Paramecium bursaria*(—). Each point of the growth curve was the mean of five experiments.

When symbiotic *Chlorella* was incubated in ammonia medium, the ammonia decreased at a rate of 3.854 nmol/10^6 *Chlorella*/hr. But the change of ammonia concentration was not detected in the medium of *Chlorella ellipsoidea* (Table 1).

The ammonia concentration in the culture medium of aposymbiotic *Paramecium* was increased according to the growth of the *Paramecium* (Fig. 1), but it was not changed in symbiotic *Paramecium* (Fig. 2).

Activities of NR, GDH and GS. To demonstrate the utilization of ammonia as the nitrogen source in symbiotic *Chlorella*, nitrate reductase and glutamate dehydrogenase were assayed in symbiotic *Chlorella* and *Chlorella ellipsoidea* (Table 2). The nitrate reductase activity in symbiotic *Chlorella* could not be detected, but it was 46 nmol/mg protein/hr in *Chlorella ellipsoidea*. The GDH/NADH activity was detected in both symbiotic *Chlorella* and *Chlorella ellipsoidea*. The GDH/NAD activity in symbiotic *Chlorella* could not be detected. The lack of the GDH/NAD activity in symbiotic *Chlorella* could be explained by the uptake of ammonia. But the GDH/NAD activity was measured to 0.277 umol/mg protein/min in *Chlorella ellipsoidea*.

To investigate the transfer of glutamine from the *Paramecium* to symbiotic *Chlorella*, the glutamine synthetase activity was measured in symbiotic *Chlorella* and *Chlorella ellipsoidea* (Table 2). And it was also measured in aposymbiotic *Paramecium* and the *Paramecium* and the *Paramecium* fraction of symbiotic *Paramecium* (Table 3). The glutamine synthetase activity of symbiotic *Chlorella* was very low, but it was much higher in *Chlorella ellipsoidea*. The glutamine synthetase activity was about twice higher in the *Paramecium*

Table 2. Specific enzyme activities of symbiotic *Chlorella* in *Paramecium bursaria* and *Chlorella ellipsoidea*

Enzymes	Symbiotic *Chlorella*	*Chlorella ellipsoidea*
	μmol/mg protein/hr	
NR	N.D.*	0.046±0.003
	μmol/mg protein/min	
GDH/NADH	0.851±0.055	0.377±0.134
GDH/NAD	N.D.*	0.277±0.098
GS	0.125±0.002	1.467±0.157

*N.D.: Not-detected.

Table 3. Specific enzyme activity of glutamine synthetase in aposymbiotic *Paramecium* and the *Paramecium* fraction of *Paramecium bursaria*

Enzyme	The *Paramecium* fraction of *Paramecium bursaria*	Aposymbiotic *Paramecium*
	μmol/mg protein/min	
r-Glutamyl transferase	0.617±0.060	0.333±0.014

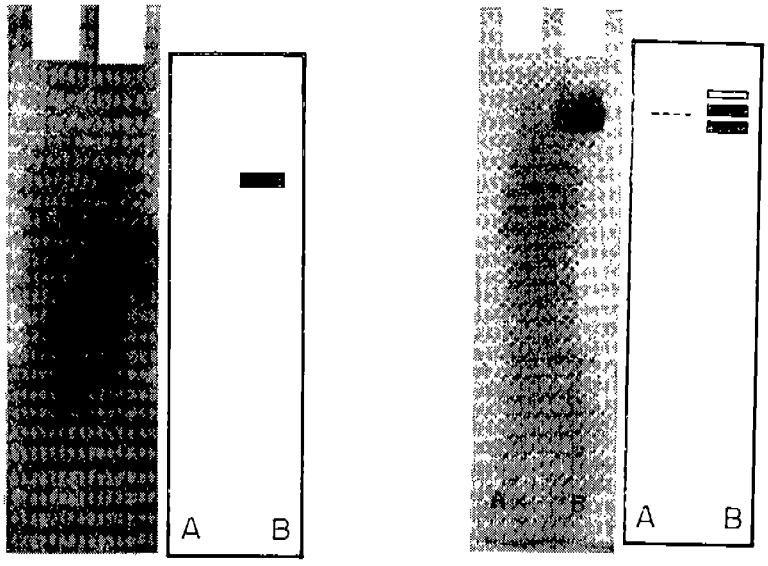

Fig. 3. The electrophoretic band patterns and zymograms for glutamate dehydrogenase(GDH/NAD) and glutamine synthetase(GS). A is symbiotic *Chlorella* and B is *Chlorella ellipsoidea.*

fraction of symbiotic *Paramecium* than in the aposymbiotic *Paramecium.* According to these results, the transfer of glutamine to *Chlorella ellipsoidea* could be supported.

Isoforms of GDH and GS. To demonstrate the lack of GDH/NAD and glutamine synthetase in symbiotic *Chlorella,* banding patterns of GDH/NAD and glutamine synthetase of symbiotic *Chlorella* were compared with those of *Chlorella ellipsoidea* by the polyacrylamide gel electrophoresis.

Isoenzyme patterns of GDH/NAD from symbiotic *Chlorella* and *Chlorella ellipsoidea* were compared in Fig. 3. *Chlorella ellipsoidea* contained only one GDH/NAD isoenzyme, but no band was detected in symbiotic *Chlorella.*

Isoenzyme patterns of glutamine synthetase were also compared in Fig. 3. *Chlorella ellipsoidea* contained three glutamine synthetase isoenzymes: one in thin band, the others in thick bands. But a very thin band was observed in symbiotic *Chlorella* only in the gel, but not in this photograph.

DISCUSSION

The uptake of ammonia was measured to investigate the utilization of ammonia by

symbiotic *Chlorella*. Ammonia was excreted into the culture medium according to the growth of aposymbiotic *Paramecium* (Fig. 1), but not in symbiotic *Paramecium* (Fig. 2). According to the result of Table 1, *Chlorella ellipsoidea* absorbed nitrate in the culture medium and symbiotic *Chlorella* which was isolated from *Paramecium bursaria* took up ammonia. A similar observation could be made with zooxanthella-bearing tropical sea anemone: zooxanthella took up ammonia by diffusion kinetics (Wilkerson and Muscatine, 1984). The utilization of ammonia in symbiotic *Chlorella* could be supported by the GDH/NAD assay. The GDH/NAD activity in *Chlorella ellipsoidea* was 0.277 μmol/mg protein/min, but it could not be measured in symbiotic *Chlorella*. And the isoenzyme of GDH/NAD in *Chlorella ellipsoidea* had one thick band (Fig. 3). A similar observation could be made with *Chlorella pyrenoidosa:* it had one isoenzyme band of GDH/NAD (Talley *et al.*, 1972). But it was not also detected in symbiotic *Chlorella*.

The uptake of nitrate was measured to investigate the nitrogen source of *Chlorella ellipsoidea*. Nitrate was taken up at a rate of 0.635 nmol/10^6 *Chlorella*/hr by *Chlorella ellipsoidea*. But it was not detected in symbiotic *Chlorella* (Table 1).

These results suggested that ammonia transported by the *Paramecium* be the nitrogen source in symbiotic *Chlorella* and nitrate be the major nitrogen source in *Chlorella ellipsoidea*.

As ammonia was utilized as the nitrogen source in symbiotic *Chlorella*, many variations were discovered in the enzymes involved in the nitrogen metabolism. Above all, nitrate reductase involved in the nitrate assimilation was not detected in symbiotic *Chlorella* (Table 2). It was noted that the nitrate uptake and nitrate reductase were inhibited by ammonia (Solomonson and Spehar, 1977). Essentially, it was thought that nitrate reductase was unnecessary because of the presence of ammonia in the *Paramecium*.

Generally, ammonia was assimilated by glutamate dehydrogenase and glutamine synthetase, as soon as nitrate was converted into ammonia. Most of ammonia was assimilated into glutamine by glutamine synthetase because the Km value for ammonia of glutamate dehydrogenase was much higher than that of glutamine synthetase (Salisbury and Ross, 1985). In *Chlorella ellipsoidea*, the glutamine synthetase activity was about five times higher than that of glutamate dehydrogenase (Table 2).

Contrary to this result, the glutamate dehydrogenase activity of symbiotic *Chlorella* was over twice higher than that of *Chlorella ellipsoidea*, and the glutamine synthetase activity of symbiotic *Chlorella* was very low. Also, *Chlorella ellipsoidea* contained three bands of glutamine synthetase isoenzyme but only a very thin band was observed in symbiotic *Chlorella*. *Chlorella sorokiniana* had two isoenzymes: one had a molecular weight of 398,000 daltons located in cytosol and the other had a molecular weight of 360,000 daltons located in chloroplast (Beudeker and Tabita, 1985), but *Chlorella ellipsoidea* had another isoenzyme.

These results noted that most of ammonia transported by the *Paramecium* was assimilated to glutamate by glutamate dehydrogenase in symbiotic *Chlorella* and that glutamine was

hardly synthesized in symbiotic *Chlorella*. And it was also implied that the glutamine produced by the *Paramecium* could be transported to symbiotic *Chlorella*.

Glutamine was not only required as a building block of most proteins, but it also provided a source of such important biological compound as the nucleotides (Meister, 1974). And the amide group of glutamine was utilized for the synthesis of such amino acids as arginine, tryptophan, histidine, and asparagine (Stryer, 1981). Therefore glutamine should be supplied from the *Paramecium*, the environment of symbiotic *Chlorella*, because it was hardly synthesized in itself.

To investigate the supply of glutamine from the *Paramecium* to symbiotic *Chlorella*, glutamine synthetase was assayed in the Paramecium fraction of symbiotic *Paramecium* and the aposymbiotic *Paramecium* (Table 3). The glutamine synthetase activity in the *Paramecium* fraction of symbiotic *Paramecium* was 0.617 μmol/mg protein/min. It was not only

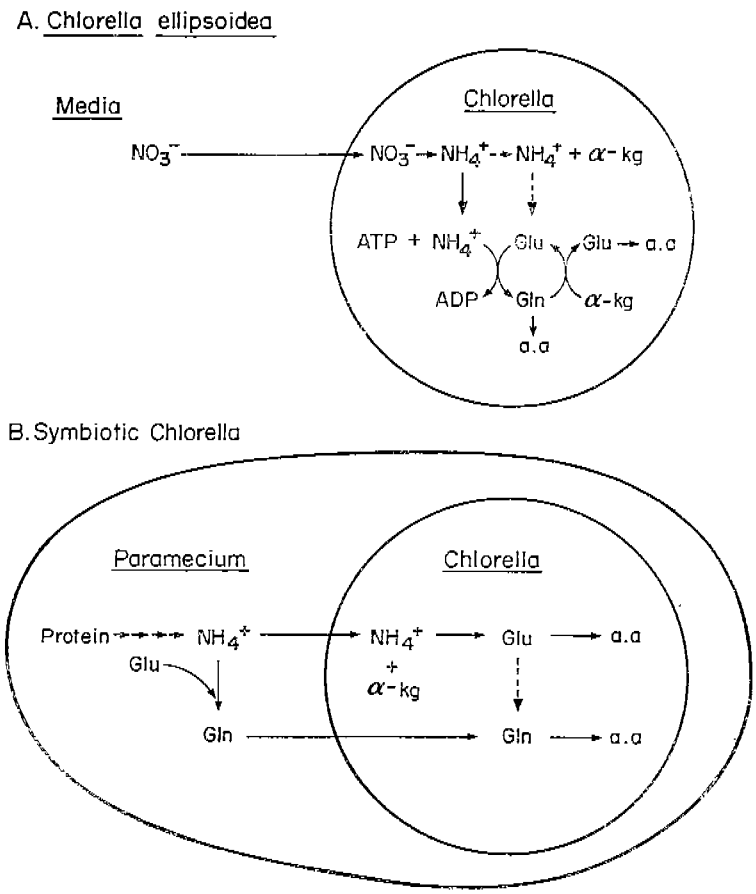

Fig. 4. Comparative diagrams of the pathway of the nitrogen metabolism between *Chlorella ellipsoidea* and symbiotic *Chlorella*: α-kg, α-ketoglutarate; Glu, Glutamate; Gln, Glutamine; a.a, amino acid.

much higher than that in symbiotic *Chlorella* but also about twice higher than that in aposymbiotic *Paramecium*. Albers *et al.* (1982) reported that symbiotic *Chlorella* did not grow with glutamate but grew very rapidly with glutamine. And they proposed the possible existence of a glutamine/glutamate shuttle between the *Paramecium* and the endosymbiotic *Chlorella*. Since it was so, glutamine synthesized in the *Paramecium* was transported to symbiotic *Chlorella*. But in the marine platyhelminth, *Convoluta roscoffensis*, glutamine synthesized by symbiotic algae was transported to the *Convoluta* (Boyle and Smith, 1975).

By the results of ammonia uptake, nitrate reductase, glutamate dehydrogenase, glutamine synthetase, and isoenzyme, the modified nitrogen metabolism of symbiotic *Chlorella* was compared with that of *Chlorella ellipsoidea* in Fig. 4. In *Chlorella ellipsoidea*, nitrate was the major nitrogen source and it was assimilated to glutamate and glutamine via ammonia. Most of ammonia were assimilated to glutamine by glutamine synthetase. But the nitrogen source in symbiotic *Chlorella* was ammonia and glutamine transported by the *Paramecium*. Most of ammonia was assimilated to glutamate by glutamate dehydrogenase and the glutamate was thought to be utilized for the sythesis of the other amino acids. Glutamine transported by the *Paramecium* was utilized for the synthesis of nucletides and amino acids.

摘 要

짚신벌레의 일종인 *Paramecium bursaria*와 공생하는 *Chlorella*의 변화된 질소대사를 알아보기 위해 짚신벌레와 공생하는 *Chlorella*와 *Chlorella ellipsoidea*의 질소대사에 관여하는 효소의 활성도 및 nitrate와 ammonia의 흡수량과 방출량을 조사한 결과는 다음과 같다. 공생하지 않는 짚신벌레는 ammonia를 배양액으로 방출하는데 비해 공생하는 짚신벌레는 전혀 방출하지 않았다. *Chlorella ellipsoidea*는 nitrate를 0.635 nomol/10^6 *Chlorella*. min의 속도로 흡수해서 ammonia로 전환한 다음 glutamate와 glutamine을 합성했다. 대부분의 ammonia는 glutamine synthetase를 통해 glutamine으로 합성되며 그 활성도는 1.467 umol/mg protein/min이었다. 이와는 대조적으로 짚신벌레와 공생하는 *Chlorella*는 숙주인 짚신벌레에서 공급된 ammonia와 glutamine을 질소원으로 이용하며 3.854nmol/10^6 *Chlorella*/min의 속도로 ammonia를 흡수했다. 짚신벌레로 부터 공급된 대부분의 ammonia는 glutamate dehydrogenase에 의해 glutamate로 합성되며 그 활성도는 0.851 μmol/10^6 *Chlorella*/min이었다. 그리고 *Chlorella ellipsoidea*에서는 하나의 GDH/NAD isoenzyme이 나타났으며 3개의 glutamine synthetase isoenzyme이 나타났다. 반면에 공생하는 *Chlorella*에서는 하나의 희미한 glutamine synthetase isoenzyme만을 볼 수 있었다.

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