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Ammonium assimilation in the blue-green alga *Spirulina platensis*

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A filamentous, non nitrogen-fixing blue-green alga (cyanobacterium), *Spirulina platensis* assimilated ammonium mainly via the glutamine synthetase–glutamate synthase (GS–GOGAT) pathway as reported in other blue-green algae. Though glutamine synthetase activity of this alga was very high, glutamine was not accumulated in the cells when 1 mM ammonium was added to the cells. The activity of glutamate dehydrogenase was not detected significantly. Ammonium was also assimilated via alanine dehydrogenase.

Key Index Word: Alanine dehydrogenase—ammonium assimilation—Anabaena cylindrica—*blue-green algae—glutamate dehydrogenase—glutamine synthetase*—Spirulina platensis.

Ammonium assimilation and amino acid synthesis are the essential steps in protein and nucleic acid production in plants. Two major enzyme systems have been known in ammonium assimilation: one is glutamate dehydrogenase and the other is glutamine synthetase (GS)–glutamate synthase (GOGAT) pathway. MIFLIN and LEA (1976) have reported the importance of the GS-GOGAT pathway in ammonium assimilation in plants instead of the glutamate dehydrogenase which had been considered as a major ammonium assimilating enzyme. Because of the high Km of glutamate dehydrogenase to ammonium, this enzyme has been thought to contribute mainly to ammonium production from glutamate under low ammonium conditions.

Other metabolic pathways to form amino acids from ammonium have long been assumed to be less important. However, it has been reported that alanine dehydrogenase is active in *Anabaena cylindrica*, a nitrogen-fixing blue-green algae (ROWELL & STEWART 1976). This enzyme assimilates NH_4^+ directly into pyruvate to form alanine. There should be varieties in the way of ammonium assimilation in blue-green algae.

Spirulina, a halophilic blue-green algae, is utilized as food in Lake Chad area and as a healthy aliment in U.S.A. and Japan. This algae seems to have high adaptability to extreme environmental conditions such as very high alkalinity (CIFERRI and TIBONI 1985), and might utilize particular pathways of ammonium assimilation and amino acid synthesis according to its environmental conditions.

We describe here, using *Spirulina platensis* cells, the importance of alanine dehydrogenase in ammonium assimilation in addition to the well known GS-GOGAT pathway when high amounts of ammonium was added.

Materials and Methods

Culture

The cells of *Spirulina platensis* were ob-

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tained from the Culture Collection of the Institute of Applied Microbiology, the University of Tokyo. They were grown for one week in the culture medium of OGAWA and TERUI (1970) containing per liter of deionized water, 1.68 g $NaHCO_3$, 0.25 g $NaNO_3$, 0.1 g K_2SO_4, 0.1 g NaCl, 0.05 g K_2HPO_4, 0.02 g $MgSO_4 \cdot 7H_2O$, 0.004 g $CaCl_2 \cdot 2H_2O$, 0.001 g $FeSO_4 \cdot 7H_2O$, 0.008 g $Na_2EDTA \cdot 2H_2O$ and 0.1 ml of A_5 microelement solution of KRATZ and MYERS (1955). Light was supplied continuously by fluorescent lamps specially designed for plant growth (National FL-10PG) at an intensity of 2,000 lux, and the culture was continuously bubbled with air at 25°C. Cells were harvested by centrifugation at 3,000 rpm for 10 min, washed twice with the fresh culture medium by centrifugation and resuspended in the same medium.

Amino acid analysis

The cells were incubated for 20 min in the light (2,000 lux) or dark and then centrifuged at 3,000 rpm for 10 min. The amino acids in the sedimented cells were extracted with 5% trichloroacetic acid and then the extract was washed with diethylether four times to remove the trichloroacetic acid. The water phase was evaporated and the amino acids in the dried sample were analyzed using a Hitach automatic amino acid analyzer.

Enzyme assay

The cells were disrupted by sonication at 9 KHz and 200 W for 10 min at 2°C and then centrifuged at 30,000 xg for 30 min. The supernatant was subjected to ammoniumsulfate precipitation and the protein fraction obtained between 35 and 70% ammoniumsulfate was used for the enzyme assay after dialyzing overnight. The activities of glutamine synthetase, glutamate dehydrogenase and alanine dehydrogenase were determined acccrding to the methods of SHAPIRO & STADTMAN (1970), DOHERTY (1970) and YOSHIDA & FREESE (1970), respectively.

Results and Discussion

The free amino acid compositions in the blue-green alga *Spirulina platensis* incubated in the dark or light without ammonium were basically the same (Table 1). Glutamate occupied about 82% and 85% of the total amino acids in the light and dark, respectively. The next major amino acid was alanine but the content was less than 4% of the total amino acid. When NH_4Cl was added to the cell suspension at a concentration of 1 mM, total amino acid concentration increased in both light and dark.

Table 1. Effect of ammonium on amino acid composition of *Spirulina platensis* in the light and dark.

Amino acids	Start[1]	Light[2]		Dark[2]	
		Control	$+NH_4^+$	Control	$+NH_4^+$
				(nmoles mg^{-1} dry weight)	
Aspartate	0.28	0.22	0.32	0.09	0.21
Glutamate	13.90	16.24	26.57	16.71	20.45
Glycine	0.28	0.27	0.60	0.22	0.42
Alanine	0.88	0.88	2.39	0.77	2.05
Arginine	0.16	0.22	0.11	0.10	0.40
Others	2.06	1.99	3.16	1.75	2.29
Total	17.56	19.82	33.15	19.64	25.82

[1] Amino acids were extracted at the start of incubation.
[2] Amino acids were extracted after 20 min incubation.

Table 2. Effect of azaserine on amino acid composition in *Spirulina platensis* in the light.

Amino acids	Start[1]	Control[2]		+Azaserine[2]	
		$-NH_4^+$	$+NH_4^+$	$-NH_4^+$	$+NH_4^+$
				(nmoles mg^{-1} dry weight)	
Aspartate	0.56	1.42	3.70	0.23	0.23
Glutamate	25.83	27.43	28.80	15.13	n.d.
Glutamine	n.d.	n.d.	n.d.	n.d.	22.24
Glycine	0.17	0.17	0.55	0.43	0.66
Alanine	0.60	0.73	1.77	0.79	1.69

[1] Amino acids were extracted at the start of incubation.
[2] Amino acids were extracted after 20 min incubation.
n.d.: not detected.

However, the substantial change in the concentration was found only in glutamate and alanine. The alanine level in the cells incubated with ammonium was double in comparison with that without ammonium.

It has been reported that the addition of ammonium to the cells of *Anabaena cylindrica*, which contains GS-GOGAT pathway (MIFLIN & LEA 1977), resulted in the dramatic increase in cellular glutamine concentration (OHMORI and HATTORI 1974, OHMORI 1981, 1983). In *Spirulina* cells, glutamine was not detected in the presence of 1 mM ammonium (Table 2). This result seems to show that the activity of glutamine synthesis in *Spirulina platensis* is low and that this alga has an ammonium assimilating mechanism different from *Anabaena cylindrica*. To determine this possibility, the cells were incubated with azaserine which blocks GS-GOGAT pathway by inhibiting glutamine-amide transfer. Table 2 shows that glutamine accumulated in high amounts when both ammonium and 1 mM azaserine were present in the incubation medium. It was also noted that the addition of azaserine to ammonium-incubating cells, resulted in the disappearance of the glutamate pool. These results clearly show that glutamine and glutamate synthesis by their respective enzymes are operating in *Spirulina* cells. Once formed gultamine would rapidly be transformed to glutamate by the very active glutamate synthase and thus no glutamine pool could be formed in the cells. The glutamate pool would be maintained by the steady operation of GS-GOGAT system.

Table 3. Activities of the ammonium assimilating enzymes in *Spirulina* and *Anabaena* cells.

Enzyme	Activity	
	Spirulina platensis	*Anabaena cylindrica*
	(μmoles hr^{-1} mg^{-1} protein)	
Glutamine synthetase	10.6	13.4
Glutamate dehydrogenase	n.d.	1.2
Alanine dehydrogenase	2.0	2.3

n.d.: not detected.

Table 3 shows the enzyme activities in the cells of *Spirulina* and *Anabaena*. Glutamine synthetase was found highly active in both algal species suggesting GS-GOGAT pathway is the major ammonium-assimilating mechanism. Alanine dehydrogenase activity was also found in both species but at about one fifth lower than glutamine synthetase activity. The activity of glutamate dehydrogenase was detected only in *Anabaena* cells and the level of the activity was ten times lower than that of glutamine synthetase. The low activity of glutamate dehydrogenase in *Anabaena* cells has been reported by HAYSTEAD *et al.* (1973) and BATT & BROWN (1974).

Alanine is formed from pyruvate and glutamate by the aminotransfer reaction or by direct ammonium incorporation into pyruvate. The former reaction is mediated by

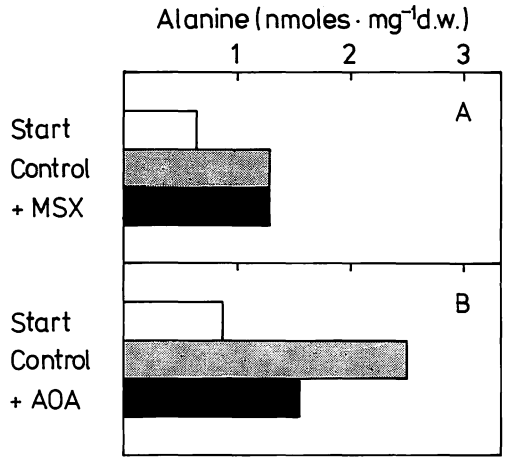

Fig. 1. Effects of methionine sulfoximine (MSX) (A) and aminooxyacetate (AOA) (B) on the increase in alanine concentration in *Spirulina platensis*. Amino acids were extracted from an aliquot of the cell suspension at the start of incubation (=start). Rest of the cells were incubated for 20 min in the presence of 1 mM NH_4Cl with inhibitors or without (=control) in the light.

glutamate-pyruvate aminotransferase and latter by alanine dehydrogenase. As shown in Table 2, the addition of ammonium increased cellular concentration of alanine independent of the presence or absence of azaserine. Fig. 1A shows that the increase in the cellular alanine pool by the addition of ammonium was not affected by 1 mM methionine sulfoximine (MSX), an inhibitor of glutamine synthesis. Fig. 1B shows that the addition of ammonium increased cellular alanine concentration in the presence of 1 mM aminooxyacetate (AOA), an inhibitor of aminotransferase, though the increase was less intense in comparison with that in the absence of AOA. These results reveal that alanine dehydrogenase is operating together with glutamate-pyruvate aminotransferase.

Though alanine dehydrogenase has not been appreciated in its role in amino acid synthesis, the data obtained in this experiment shows the importance of this enzyme in ammonium assimilation in *Spirulina*.

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大森和子*・大森正之：藍藻 *Spirulina platensis* におけるアンモニア同化**

藍藻 *Spirulina* は，たんぱく質含量が高く，食料としてまた健康食品として利用されている。この藻類のアンモニア同化は，グルタミン合成酵素—グルタミン酸合成酵素系により行われていることが明らかとなった。しかし，高濃度 (1 mM) のアンモニア存在下において，らん藻 *Anabaena* に見られるようなグルタミンの細胞内蓄積は見られなかった。アンモニア同化における他の重要な酵素であるグルタミン酸脱水素酵素の活性は検出されなかったので，*Spirulina* では，この酵素によるアンモニアの同化はないものと推定した。この藻類では，アラニン脱水素酵素によってもアンモニアの同化が行われていることが示唆された。(*154 東京都世田谷区太子堂 1－7－57 昭和女子大学生活科学科，**164 東京都中野区南台 1－15－1 東京大学海洋研究所 海洋生化学部門)