Korean J. Ecol. 18(1) : 17~30, 1995

Effects of Methylglyoxal on the Growth Dynamics of *Scenedesmus quadricauda*

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Methylglyoxal 이 *Scenedesmus quadricauda* 의 성장 역학에 미치는 영향

이 기 태

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ABSTRACT

The growth of *Scenedesmus quadricauda* (Turp.) Breb. is enhanced by methylglyoxal (MG), a general inhibitor of cell division, at threshold concentration in conjunction with treatment timing relative to growth stage. The stimulatory effect of MG on algal cell growth was most significant with 2.27-fold of untreated algal culture in cell number when 0.5 mM of MG was added to the algal culture at the beginning of logarithmic phase with an initial MG concentration of 0.535 mg MG /10^6cell. A Specific growth rates (SGRs) of MG-treated cultures were rapidly increased at the beginning of logarithmic phase with 1.89-fold of untreated algal culture. Cultures inoculated with high cell numbers of 2.4 to 4.8 × 10^4 cells /ml were less sensitive to 0.5 mM of MG treatment. The algal cell division was enhanced significantly when the concentration of MG in the medium at time of treatment was ranged from 0.392 to 0.924 mg MG /10^6cell. If the cell number of an algal culture at the time of inoculation was low (0.6 × 10^4 cells /ml) and MG was added before logarithmic phase, the cell number of 0.5 mM of MG-treated cultures were lower than those of controls. In algal cultures treated with high concentrations of MG (1.0 mM and 2.0 mM), the algal growth was inhibited. Photosynthetic rate of growth-enhanced algae by 0.5 mM of MG was significantly higher than that of untreated or 1.0 mM of MG-treated algal cell, while there was no significant difference among those groups in respiratory rate. Pyruvate concentration in 0.5 mM of MG-treated culture was increased after methylglyoxal treatment.

Key words: Growth dynamics, Methylglyoxal, *Scenedesmus quadricauda*, Stimulation

INTRODUCTION

Since 1965, the functions of methylglyoxal (MG) have been studied in relation to cell

본 연구는 1994년도 경희대학교 연구비지원에 의하여 수행되었음.

division, wound healing, and cancer. The growth-inhibiting effects of MG on a number of organisms and tissues have been shown and these studies suggested that MG strongly inhibited *in vivo* nucleic acid and protein synthesis (Egyud 1965, Egyud and Szent-Gyorgy 1966a and 1966b, Szent-Gyorgy *et al.* 1967, Morris 1969, Krymkiewicz *et al.* 1971). The inhibitory mechanism of MG on cell division is not clear, but MG or some 2-ketoaldehydes is reported to inhibit cell division by inhibiting protein synthesis at the translational level, probably react with the 7-methylguanosin 'cap' residue in mRNA (Kozarich *et al.* 1979, Carrington and Douglas 1986, Ranganatham and Tew 1993).

In spite of these toxic effects, there is still discussion of the identity and variety of 2-ketoaldehyde sources, some indigenous, some dietary or environmental, have been suggested (Carrington and Douglas 1986, Grosjean *et al.* 1993, Sayato *et al.* 1993). Formation of MG in animal tissue, some microorganisms, and yeast was reported from aminoacetone and dihydroxyacetone phosphate by amine oxidase or methylglyoxal synthase, respectively. As the bypath of glycolysis, methylglyoxal synthase which convert dihydroxyacetone phosphate to MG was isolated in goat liver (Ray and Ray 1981), *Escherichia coli* (Hopper and Cooper 1972), and *Pseudomonas saccharophila* (Cooper 1974). Also, MG formation by degradation of amino acid, threonine or glycine, in animal tissue and yeast was reported. Aminoacetone from threonine by threonine dehydrogenase or glycine by aminoacetone synthase was reported to produce MG by amine oxidase in goat liver (Ray and Ray 1987) or in yeast (Murata *et al.* 1986). MG transformation into lactate and pyruvate is related to energy metabolism, catabolic and anabolic dissociation processes in carbohydrates and proteins, and, probably, to maintenance of asymmetrical entropy *in vivo* on the constant level (Alekseev 1987). In biochemical studies, reported metabolic products of MG included lactate, pyruvate, and glucose (*via* gluconeogenesis) in animal tissues (Saez *et al.* 1985), in yeast (Murata *et al.* 1986), in *E. coli* (Saikusa *et al.* 1987), and in mold (Inoue *et al.* 1988).

In spite of its early discovery, little is known about the basic biological function of MG as the intermediate of glycolytic bypath. Biotransformation of MG to pyruvate and a stimulatory relationship to microtubule assembly by metabolic intermediates of MG metabolism indicate a reconsideration of MG effects on cell growth and cell division, especially in unicellular or coenobial organisms, is needed. The only reported study of the effects of MG on algae (Morris 1969) showed that MG, at less than 1 mM, inhibited growth and delayed the onset of cell division of *Chlamydomonas reinhardii*.

Most of all studies on MG were done with enzymes as a catalyzer of MG metabolism in animal tissues and yeast. There was no study about MG effects related to physiological responses especially growth dynamics and its effective concentrations. This study is one approach to understanding the effect of MG on the growth and cell division of the coenobial planktonic green alga, *Scenedesmus quadricauda* with the following objectives; physiological responses of green algae, the effectiveness of MG to the cell division or growth, the threshold concentration of MG to inhibit cell division.

MATERIALS AND METHODS

Plants and Cultivation

The alga, *Scenedesmus quadricauda* (Turp.) Breb. (UTEX 614), was cultured in Bristol's medium as modified by Bold (Starr 1978). The medium (50 ml in 250 ml side armed flasks) was sterilized at 121℃ and 1.1 kg /cm^2 for 10 min. The alga was cultured at 25℃ and agitated on a reciprocal shaker at 80 cpm. Cultures were grown in growth chambers under continuous light (cool-white fluorescent tubes) at 45 μE m^{-2} sec^{-1}.

The algal cultures was inoculated with different initial concentration of cells, and the cell concentrations after inoculation was 0.5 to 4.8 $\times$ 10^4 cells /ml. In each experiment, MG (Sigma No. M 252) was added directly to the treatment flask to achieve the desired final concentration (0.25, 0.5, 0.75, 1.0, or 2.0 mM) at the desired time. At the time of treatment, the concentration of the cell in the cultures ranged from 2.4 $\times$ 10^4 cells /ml to 2.67 $\times$ 10^5 cells /ml. Each set of cultures was replicated at least in triplicate.

Growth Dynamics

Algal growth was measured by enumerating the cell number each day with the aid of an AO Spenser Bright-line Hemacytometer. To compare the rate of cell division of MG-treated cultures with controls, a specific growth rate (SGR) was calculated by dividing the difference of natural logarithmic cell numbers between two successive measurements by days on which two measurements were made (Toerien *et al.* 1971).

Photosynthetic and Respiratory Rate

The photosynthetic and respiratory rates of MG-treated and untreated cultures were determined at different stages in the growth cycles of the algae with oxygen meter (YSI, Model 53). A vial in the water bath (25℃) contained 3 ml of algal cultures from controls or MG-treated cultures was monitored for 30 min.

Methylglyoxal Assay

MG in the algal cultures was measured by modification of the method of Cooper (1974). A 0.10 ml of cell-free culture solution was mixed with 0.33 ml of 0.1 % 2,4-dinitrophenylhydrazine in 2 M HCl with 0.90 ml of DI water. After incubation at 30℃ for 15 min., 1.67 ml of 10% NaOH was added. The absorbance of this preparation was then measured at 555 nm after 15 min in spectrophotometer (Hitachi-Coleman, Model 124).

Product Assay

The determination of pyruvate was carried out by the spectrophotometric method described in Sigma Chemical Co., Standard Procedure No. 726 V.

Data Analysis

Data were analyzed with parametric ANOVA (α=0.05) and Duncan's multiple range test (α=0.05) with SAS.

RESULTS

Growth Dynamics

Methylglyoxal (MG) at the concentration of 2.0 mM inhibited growth of *Scenedesmus quadricauda* in the cultures inoculated with relatively high cell concentration (2.4 to 4.8 × 10^4 cells/ml). The mean cell number of the cultures inoculated with 2.4 × 10^4 cells/ml and treated with 2.0 mM of MG on day 2 from inoculation when the cell concentration was 0.694 0.013 × 10^5 cells/ml were significantly lower than those of controls throughout all stages of the growth (Fig. 1. A). Even when the algal cultures were inoculated with 4.8 × 10^4 cells/ml, and treated with 2.0 mM of MG on day 2 when the cell concentration was 1.146±0.010 × 10^5 cells/ml, the cell number of MG-treated cultures were significantly lower than those of controls (Fig. 1. B).

The specific growth rates (SGRs) of those cultures treated with 2.0 mM of MG were significantly decreased. The mean SGR of cultures treated with 1.0 mM of MG on day 1 from inoculation when the cell concentration was 0.500±0.013 × 10^5 cells/ml was lower than those of controls until day 5 after treatment (Table 1).

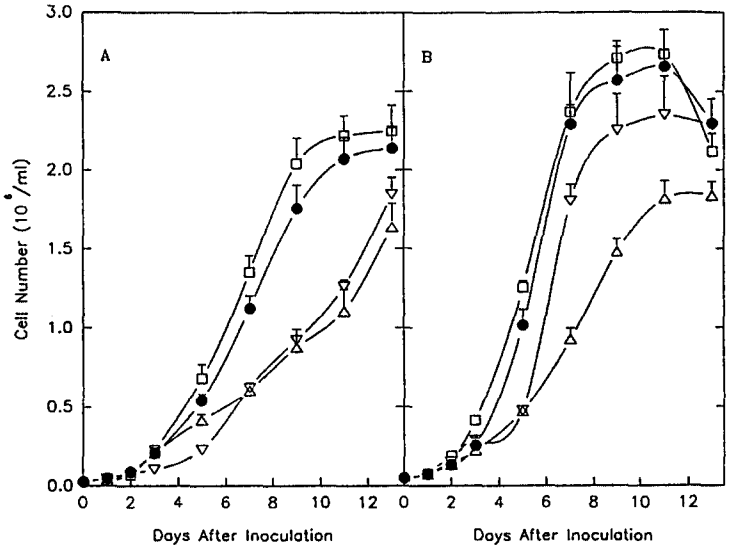

Fig. 1. Growth curves of *Scenedesmus quadricauda* in the culture of 2.4 × 10^4 (A) and 4.8 × 10^4 (B) cells/ml inoculation treated with methylglyoxal (MG) of 0.5 mM on day 0 (□), 1.0 mM on day 1 (▽), or 2.0 mM on day 2 (△) and without MG (●). The symbol and bar indicate mean and one positive standard deviation.

Table 1. Mean specific growth rate (SGR) of *Scenedesmus quadricauda* in the culture of 2.4×10^4 cells/ml inoculation treated with 0.5, 1.0, or 2.0 mM of methylglyoxal (MG)

Day / MG	0~1	1~2	2~3	3~5	5~7	7~9	9~11	11~13
	a[1]		b	a,b	b		a,b	b
0.0 mM	0.635	0.633	0.875	0.483	0.366	0.226	0.079	0.010
	(0.068)[3]	(0.034)	(0.003)	(0.032)	(0.018)	(0.018)	(0.012)	(0.002)
	b		a	a	b		b	b
0.5 mM	0.248[2]	0.781	1.212	0.549	0.340	0.205	0.047	0.006
	(0.230)	(0.304)	(0.039)	(0.018)	(0.018)	(0.026)	(0.003)	(0.004)
	a		c	b,c	a		a	a
1.0 mM	0.605	0.485[2]	0.329	0.411	0.490	0.212	0.155	0.190
	(0.046)	(0.015)	(0.155)	(0.110)	(0.073)	(0.046)	(0.027)	(0.014)
	a		b	c	c		a	a
2.0 mM	0.620	0.648	0.855[2]	0.352	0.195	0.166	0.138	0.019
	(0.068)	(0.037)	(0.193)	(0.031)	(0.072)	(0.103)	(0.074)	(0.031)

[1] Means with the same letter in column are not significantly different (Duncan's multiple range test, $\alpha=0.05$)

[2] One day after MG treatment

[3] One standard deviation

Also, the cell numbers of the cultures were lower than those of controls throughout all stages of the growth (Fig. 1. A). Cell division of MG-treated cultures with high inoculation (4.8×10^4 cells/ml) and treated with 1.0 mM MG on day 1 when algal concentration was $0.800 \pm 0.020 \times 10^5$ cells/ml was inhibited until day 7 (Fig. 1. B). Also, SGRs were lower than those of controls until day 5 after treatment except the culture with 1.0 mM-treatment which shows recovery of growth under decreased concentration of MG (Table 2).

When 0.5 mM MG was added to the algal cultures on day 0 when the cultures were inoculated with 4.8×10^4 cells/ml, the logarithmic phase began sooner than for controls (Fig. 1. B). The SGRs and cell numbers of MG-treated cultures were significantly higher than those of controls until day 5 (Table 2). After day 5, there was no significant difference between controls and MG-treated cultures in SGR. The cell numbers and SGRs of cultures treated with 0.5 mM MG on day 0 when algal concentration was 2.4×10^4 cells/ml were significantly lower than those of controls for one day after treatment (Fig. 1. A and Table 1). After day 2, the cell numbers and SGRs of MG-treated cultures increased to the level of controls. Furthermore, the cell numbers of MG-treated cultures were significantly higher than those of controls (up to 1.26-fold of control) at the middle of logarithmic phase (day 5).

The mean cell number of algal cultures inoculated with 0.6×10^4 cells/ml, and treated

Table 2. Mean specific growth rate (SGR) of *Scenedesmus quadricauda* in the culture of 4.8×10^4 cells/ml inoculation treated with 0.5, 1.0, or 2.0 mM of methylglyoxal (MG)

Day / MG	0~1	1~2	2~3	3~5	5~7	7~9	9~11	11~13
	b[1]	c		a	b	b		a,b
0.0 mM	0.367	0.623	0.657	0.696	0.414	0.051	0.021	−0.072
	(0.044)[3]	(0.049)	(0.076)	(0.010)	(0.016)	(0.019)	(0.030)	(0.033)
	a	a		b	b	b		b
0.5 mM	0.510[2]	0.830	0.796	0.563	0.322	0.060	0.004	−0.125
	(0.050)	(0.029)	(0.096)	(0.011)	(0.028)	(0.036)	(0.025)	(0.013)
	b	b		d	a	b		a
1.0 mM	0.377	0.747[2]	0.691	0.260	0.680	0.103	0.026	−0.012
	(0.049)	(0.060)	(0.173)	(0.028)	(0.104)	(0.058)	(0.013)	(0.094)
	b	c		c	b	a		a
2.0 mM	0.367	0.629	0.532[2]	0.377	0.334	0.223	0.116	0.017
	(0.033)	(0.021)	(0.024)	(0.057)	(0.022)	(0.096)	(0.090)	(0.026)

[1] Means with the same letter in column are not significantly different (Duncan's multiple range test, α=0.05)

[2] One day after MG treatment

[3] One standard deviation

with 0.5 mM of MG on day 2 when the cell concentration was $2.620 \pm 0.150 \times 10^4$ cells/ml, was not significantly different from that of controls until day 5. However the SGR of MG-treated cultures recovered and increased (up to 1.41-fold of controls) at the end of logarithmic phase (day 7) (Fig. 2. A).

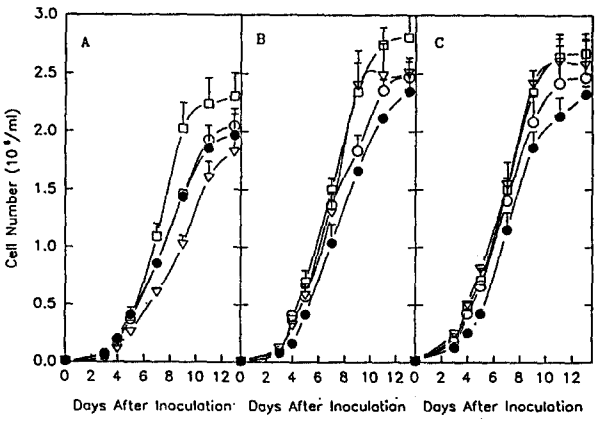

Fig. 2. Growth curves of *Scenedesmus quadricauda* in the culture of 0.6×10^4 (A), 1.2×10^4 (B), and 1.8×10^4 (C) cells/ml inoculation treated with methylglyoxal (MG) of 0.25 mM (○), 0.5 mM (□), or 1.0 mM (▽) and without MG (●). The symbol and bar indicate mean and one positive standard deviation.

The mean cell number and SGR of cultures treated with 1.0 mM MG were significantly lower than those of controls after treatment. But the growth of MG-treated algal cell was recovered from two days after treatment, where the mean SGR of treated cultures was significantly higher than that of controls (Table 3). There was no difference in cell number or SGR between controls and cultures treated with 0.25 mM MG. The mean cell number of cultures inoculated with 1.2×10^4 cells/ml, and treated with MG on day 2 when the cell concentration was $3.90 \pm 0.15 \times 10^4$ cells/ml, was significantly higher than that of controls throughout all stages of growth cycle (Fig. 2. B). Cell numbers of MG-treated cultures were increased up to 2.27 or 1.94-fold of controls one day after MG treatment with 0.5 or 1.0 mM, respectively. Also, SGRs of MG-treated cultures were significantly higher than those of controls at one day after MG treatment (Table 3).

Cultures inoculated with 1.8×10^4 cells/ml, and treated with MG on day 2 produced significantly higher cell numbers than those of controls (Fig. 2. C). Cell numbers of MG-treated cultures with 1.0 mM were increased (up to 1.95-fold of controls) at 2 days after treatment. Also, SGRs of MG-treated cultures were significantly higher than those of controls (up to 1.92-fold of controls) at one day after treatment (Table 3). After day 3, SGRs of MG-treated cultures were not significantly different from those of controls.

Table 3. Mean specific growth rate (SGR) of *Scenedesmus quadricauda* in the culture of 0.6, 0.2 or 1.8 $\times 10^4$ cells/ml inoculation treated with 0.25, 0.5, or 1.0 mM of methylglyoxal (MG) on day 2

Inoculation	0.6×10^4 cells/ml			1.2×10^4 cells/ml			1.8×10^4 cells/ml		
Day / MG	2~3	3~4	4~5	2~3	3~4	4~5	2~3	3~4	4~5
0.0 mM	a[1] 0.989 (0.155)[2]	b 1.109 (0.032)	0.716 (0.076)	c 0.713 (0.157)	c 0.730 (0.109)	a 0.895 (0.197)	c 0.699 (0.061)	0.692 (0.097)	0.485 (0.166)
0.25 mM	a 1.071 (0.071)	b 0.989 (0.055)	0.651 (0.120)	b 0.873 (0.012)	a 1.478 (0.099)	b 0.347 (0.168)	b 1.043 (0.086)	0.833 (0.083)	0.454 (0.118)
0.5 mM	a 0.756 (0.234)	b 1.134 (0.191)	0.882 (0.134)	a 1.345 (0.025)	b 1.116 (0.163)	a,b 0.608 (0.119)	a,b 1.212 (0.147)	0.816 (0.049)	0.376 (0.117)
1.0 mM	b 0.103 (0.085)	a 1.395 (0.047)	0.852 (0.028)	a 1.345 (0.039)	b 0.973 (0.020)	a,b 0.587 (0.175)	a 1.334 (0.017)	0.700 (0.089)	0.480 (0.042)

[1] Means with the same letter in column are not significantly different (Duncan's multiple range test, α=0.05)

[2] One standard deviation

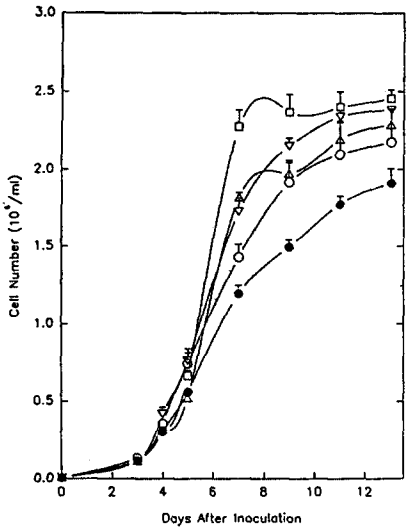

Fig. 3. Growth curves of *Scenedesmus quadricauda* in the culture of 0.8 × 10^4 cells/ml inoculation treated 0.5 mM of methylglyoxal (MG) on day 2 (○), day 3 (▽), day 4 (□), or day 5 (△) and without MG (●). The symbol and bar indicate mean and one positive standard deviation.

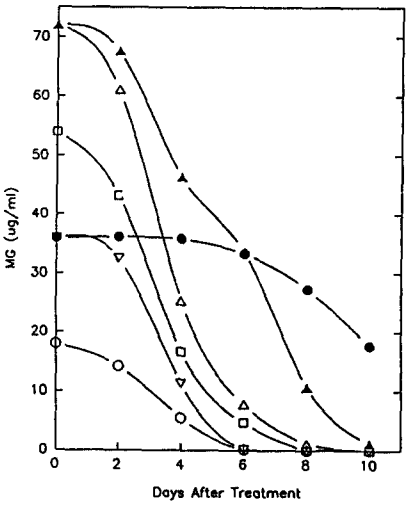

Fig. 4. Degradation of methylglyoxal (MG; g/ml) in the cultures of *Scenedesmus quadricauda* treated with various concentrations of methylglyoxal (○ - 0.25 mM, ▽ - 0.5 mM, □ - 0.75 mM, and △ - 1.0 mM at 1.6 × 10^4 cells/ml inoculation; ▲ - 1.0 mM at 0.6 × 10^4 cells/ml inoculation; ● - 0.5 mM without algae).

When the algal cultures inoculated with 0.8 × 10^4 cells/ml were treated with 0.5 mM of MG, which was the most effective concentration of MG to stimulate algal growth, on day 2, 3, 4, or 5, the cell numbers and SGRs of all MG-treated cultures were significantly higher than those of controls (Fig. 3 and Table 4).

The mean cell number and SGR of MG-treated cultures were significantly increased whenever the MG was treated to the culture. The most effective timing for MG treatment was observed at the culture of day 4 treatment (up to 1.91-fold of controls) one day after MG treatment.

Biodegradation of Methylglyoxal

MG in the algal cultures was degradaded by the algae. Generally, MG in algal culture was not detected after day 6 at 0.25 or 0.5 mM of MG-treated cultures and day 8 at 0.75 mM of MG-treated cultures (Fig.4).

The pattern of MG degradation in 1.0 mM of MG-treated culture was different, depending on the inoculation concentration of the cultures. At day 4 after MG-treatment with 1.0 mM, MG concentration in the algal cultures inoculated with 1.2 × 10^4 cells/ml

Table 4. Mean specific growth rate (SGR) of *Scenedesmus quadricauda* in the culture of 0.8 × 10^4 cells /ml inoculation treated with 0.5 mM of methylglyoxal (MG) on day 2, 3, 4 or 5

Day / MG	2~3	3~4	4~5	5~7	7~9	9~11	11~13
	b 1)	b	b	b,c	a		
0.0 mM	0.621 (0.023)2)	0.971 (0.126)	0.608 (0.092)	0.379 (0.014)	0.112 (0.015)	0.086 (0.005)	0.040 (0.008)
	a	b	a	c	a		
0.5 mM on day 2	0.774 (0.070)	0.964 (0.030)	0.737 (0.041)	0.332 (0.026)	0.146 (0.023)	0.042 (0.046)	0.019 (0.017)
	b	a	b	b	a		
0.5 mM on day 3	0.615 (0.020)	1.289 (0.153)	0.599 (0.069)	0.412 (0.027)	0.106 (0.043)	0.045 (0.058)	0.010 (0.002)
	b	b	a	a	b		
0.5 mM on day 4	0.591 (0.048)	1.005 (0.061)	0.762 (0.069)	0.621 (0.039)	0.020 (0.013)	0.023 (0.016)	0.004 (0.018)
	b	b	b	a	b		
0.5 mM on day 5	0.651 (0.020)	1.004 (0.065)	0.504 (0.067)	0.629 (0.044)	0.038 (0.048)	0.054 (0.028)	0.021 (0.028)

1) Means with the same letter in column are not significantly different (Duncan's multiple range test, α=0.05)

2) One standard deviation

was 26 μg /ml which is the lower level than that of 0.5 mM of MG-treatment. While MG concentration in the algal cultures treated 1.0 mM of MG and inoculated with 0.6 × 10^4 cells /ml at day 4 after treatment was 47 μg /ml which is the higher level than that of 0.5 mM MG-treatment.

Photosynthetic and Respiratory Rate

The evolution of oxygen molecule as a result of photosynthesis for 30 min. of 0.5 mM MG-treated cultures were greater than those of controls (up to 1.60-fold of controls) which corresponded to the data for cell numbers (Fig. 5 A), while there was no significant difference among the cultures on respiratory rate (Fig. 5 B).

Metabolic Products

Pyruvate concentration in algal cultures without MG-treatment was none or very low throughout all stages of growth cycle, while concentration of pyruvate in MG-treated cultures was increased gradually as the cell number increased after MG treatment (Fig. 6). The highest mean cell number was observed on day 10 in 0.5 mM MG-treated (on day 4) culture, and the concentration of pyruvate produced by the culture was 5 times higher

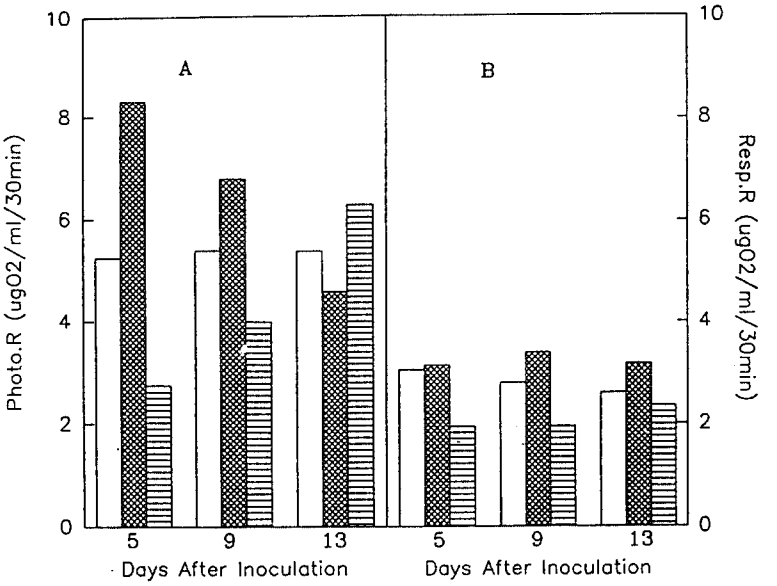

Fig. 5. Photosynthetic (A) and respiratory (B) rate of the cultures of *Scenedesmus quadricauda* treated with 0.5 (▩) and 1 mM (▤) of methylglyoxal (MG) or without MG treatment (□).

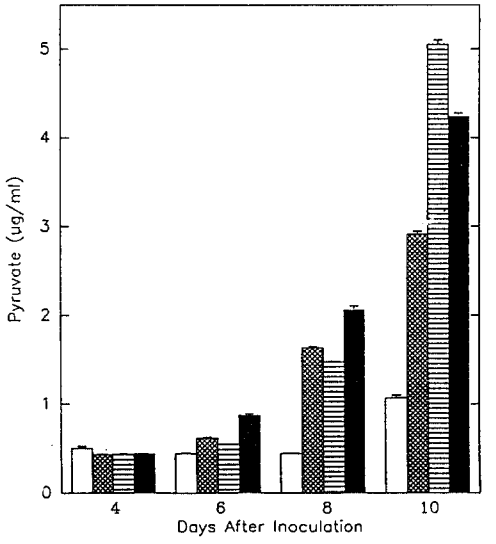

Fig. 6. Pyruvate (μg/ml) in the cultures of *Scenedesmus quadricauda* treated with 0.5 mM of methylglyoxal (MG) on day 3 (▩) or 5 (▤), and 1.0 mM of MG on day 7 (■), or without MG treatment (□).

than that of controls.

Cell Size

Microscopic observation revealed no difference in cell size of the algae between controls and 0.5 mM MG-treated cultures (width × length; 0.010 × 0.003 μm, approx.), while cells from 1.0 mM MG-treated cultures, which had inhibitory effect on cell growth, were smaller than those of controls.

Threshold Concentration

Methylglyoxal (MG), added to the algal cultures which were different in inoculation concentration, amount of MG, and the time of MG addition was recalculated to compare the effectiveness of MG on cell division each other. To generalize the MG concentration and to decide the threshold concentration of MG

whether stimulate or inhibit cell division of *Scenedesmus quadricauda* in the batch cultures, added MG was recalculated by the unit of mg MG per 10^6 cells.

A high concentration of MG (2.0 mM) inhibited cell division of the algae and reduced the specific growth rate (SGR) compared to those of controls or other MG-treated cultures with lower than 2.0 mM (0.25, 0.5, 0.75, or 1.0 mM). The initial MG concentrations in the treated cultures were 1.109 to 1.700 mg MG /10^6 cells. When the inoculum concentration was 0.6×10^4 cells /ml or, when MG was added within 24 hours from the inoculation, the growth of MG-treated algae was significantly inhibited. The initial MG concentration were 0.784 to 1.693 mg MG /10^6 cells. When the inoculum concentration was higher than 1.2×10^4 cells /ml and MG was added 2 days after inoculation, the cell number and SGR were significantly higher than those of controls (up to 1.95 and 1.91-fold of controls, respectively). The initial MG concentrations were 1.127 to 1.849 mg MG /10^6 cells. When MG was added to the cultures at 2 days after inoculation, there was no difference in cell number and SGR between controls and MG-treated cultures. The initial MG concentration was 0.267 mg MG /10^6 cells.

When 0.5 mM of MG was added to the algal cultures on day 0 with initial concentration of 1.502 mg MG /10^6 cells, cell division was inhibited for 2 days after treatment (Fig. 1). If the cell number of an algal culture was low (0.6×10^4 cells /ml) and MG was added before logarithmic phase (day 0~2), the cell number and SGR of 0.5 mM MG-treated cultures were lower than those of controls for one day after treatment. However, the SGR and cell number of treated cultures increased rapidly (3 days after MG treatment) and the cell number was increased up to 1.63-fold of controls and SGR was increased up to 1.33-fold of controls at the end of the logarithmic phase (day 7~11). When 0.25 or 0.75 mM of MG was added to the cultures after day 2, and the initial MG concentration was lower than 0.732 mg MG /10^6 cells, there was no differences in cell number and SGR between controls and MG-treated cultures.

The stimulation of cell division of *S. quadricauda* was most significant when the cultures were treated with 0.5 mM MG, especially when MG was added at the beginning of logarithmic phase. The initial MG concentrations were 0.118 to 0.924 mg MG /10^6 cells. The highest cell number (up to 2.27-fold of controls) or SGR (up to 1.89-fold of controls) was observed when 0.5 mM MG was added to algal cultures with initial MG concentrations of 0.392 to 0.924 mg MG /10^6 cells.

DISCUSSION

The effectiveness of methylglyoxal (MG) on cell division of *Scenedesmus quadricauda* was dependent on the MG concentration in the culture, initial inoculum, cell number and growth rate at the time of MG-treatment. In algal cultures treated with high concentrations (2.0 mM) of MG, the inhibition of cell division was similar to that reported for *Escherichia coli* (Egyud and Szent-Gyorgy 1965) and *Chlamydomonas reinhardii* (Morris

1969). Morris also reported that treatment with MG at an early stage of growth prevented increase in cell size and inhibited cell division and exponential growth. In *S. quadricauda* however, 0.5 mM MG treatment at early logarithmic phase resulted in no difference in cell size. Rather, cell numbers increased exponentially and the high value of specific growth rate (SGR) was maintained until the end of logarithmic phase.

The stimulatory effect of MG on cell division of *S. quadricauda* was significant when MG was added to 0.5 mM. When MG was added to the algal cultures, MG was degradaded rapidly. The SGR and cell number of all MG-treated cultures were not significantly different from those of controls if MG concentration was decreased to the level of 0.25 mM.

The green algae, *S. quadricauda* detoxified MG through glyoxalase system. The glyoxalase system forms probably the main line of cellular defense against the cyto-toxic ketoaldehydes, which are formed endogenously in a variety of cell types, e.g., by glycerol metabolism, from dihydroxyacetone phosphate or amino acids degradation metabolism. If such materials were allowed to accumulate intracellularly the inevitable result would be cell death. The growth of algae decreased the initial amount of MG added to the cultures. The formation of pyruvate as the evidence of detoxification of MG was reported with an active metabolic process rate of 70% pyruvate to 30% L-lactate (Saez *et al.* 1985). Formation of pyruvate from MG was reported in yeast (Murata *et al.* 1986). MG metabolism in algae may be a bypath of glycolysis or gluconeogenesis for the efficient conversion of MG to glucose *via* pyruvate. The conversion of MG to pyruvate in the culture of *S. quadricauda* was positively correlated to the amount of treated MG, especially after growth recovery of treated cultures. The rate of biotransformation from MG to pyruvate was 10% in the MG treated cultures. Possibly, an active cell growth stimulated by proper concentration of MG needed more carbon source than that of controls.

A literature review did not indicate any reports on the growth dynamics of algae with MG as a growth stimulator. Also, studies to determine the threshold concentrations of MG to stimulate cell division in any system were not found. It is apparent that the stimulation of cell division in *S. quadricauda* by MG depends upon certain threshold concentrations of MG at the time of treatment (0.4 to 0.9 mg MG /10^6 cells). The function of MG and its metabolites or intermediates are not fully understood. The stimulatory effect of MG on algal cell division may also be due, in part at least, to the 'retine and promine' theory of the cell growth regulation (Szent-Gyorgyi 1968). The regulation of cell division is hypothesized by two mutually antagonistic substances; a growth retarding 'retine' and a growth promoting 'promine', and their regulatory activaton is dependent on their balance within the cell. 'Retine' was proposed to be MG or some 2-ketoaldehyde, and 'promine' to be the degradative enzymes (Carrington and Douglas, 1986). The threshold concentration of MG for enhencement of algal cell growth support 'retine and promine' hypothesis. However further studies on bio-products, degradative or synthetic enzymes on the physiological response to MG are needed.

적 요

生物의 성장을 抑制하는 중간대사산물로 알려진 methylglyoxal(MG)이 綠藻類인 *Scenedesmus quadricauda*의 세포분열에 미치는 影響을, MG의 處理濃度와 處理時期를 달리하여 *S. quadricauda*의 成長力學, 光合成率 및 MG의 흡수에 따른 생성물 등으로 考察하여, *S. quadricauda*의 細胞分裂에 따른 성장곡선 대한 MG의 影響과 有效濃度를 결정하였다.

對數期에 0.5mM의 MG가 處理되었을 때, *S. quadricauda*의 細胞分裂은 促進되어 最高 2.27倍로, 그리고 고유생장율(Specific Growth Rate, SGR)은 1.89 倍가 증가되었는데 이때 10^6 세포당 MG의 농도는 0.392 에서 0.924 mg이었다. 接種濃度가 1.2×10^4 에서 4.8×10^4cells/ml인 배지에 0.5 mM 의 MG가 初期 處理된 *S. quadricauda* 는 처리하지 않은 경우보다 對數期에 조기 進入하였으며 SGR은 급격히 增加하였다. MG는 0.25 mM 또는 0.75 mM로 처리될 경우 綠藻의 數는 處理되지 않은 綠藻의 數에 비교하여 유의한 차이를 보이지 않았다. MG 濃度 1.0 mM 以上으로 處理되어 綠藻세포에 대한 MG의 초기 농도가 1.127 mg MG/10^6cells 이상인 경우 細胞分裂은 抑制되었으나 배지 내의 MG 농도가 0.5 mM으로 처리한 수준으로 감소할 경우 성장은 회복되었다. 배지내 MG는 조류세포에 의하여 분해되어 피루빈산의 농도는 0.5 mM을 처리할 경우 증가하였다. MG에 의하여 성장이 촉진된 조류의 광합성율은 증가하였으나 암호흡율은 다른 세포와 비교하여 유의한 차이를 보이지 않았다.

LITERATURE CITED

Alekseev, V.S. 1987. Methylglyoxal: metabolism and biochemical activity. Ukraina Biochemical Journal 59: 88-94.

Carrington, S.J. and K.T. Douglas. 1986. The glyoxalase enigma - the biological consequences of a ubiquitous enzyme. IRCS Medical Science 14: 763-768.

Cooper, A. 1974. Methylglyoxal formation during glucose catabolism by *Pseudomonas saccharophila*. Eur. J. Biochem. 44: 81-86.

Egyud, L.G. 1965. Studies on autobiotics: Chemical nature of retine. Proc. Natl. Acad. Sci. U.S.A. 54: 200-207.

Egyud, L.G. and A. Szent-Gyorgyi. 1966a. Cell division, SH, Ketoaldehydes, and Cancer. Proc. Natl. Acad. Sci. U.S.A. 55: 388-392.

Egyud, L.G. and A. Szent-Gyorgyi. 1966b. On the regulation of cell division. Proc. Natl. Acad. Sci. U.S.A. 56: 203-207.

Grosjean, D., E. Grosjean and E.L. Williams. 1993. Atmospheric chemistry of unsaturated alcohols. Environmental Science & Technology 27: 2478-2485.

Hopper, D.J. and A. Cooper. 1972. The purification and properties of *Escherichia coli* methylglyoxal synthase. Biochem. J. 128: 321-329.

Inoue, Y., H. Rhee, K. Watanabe, K. Murata and A. Kimura. 1988. Metabolism of 2 oxoaldehydes in mold: Purification and characterization of two methylglyoxal reductase from *Aspergillus niger*. Eur. J. Biochem. 171: 213-218.

Kozarich, J.W. and J.L. Deegan. 1979. 7-Methylguanosine-dependent inhibition of globin mRNA translation by MG. J. Biol. Chem. 254: 9345-9348.

Krymkiewicz, N., E. Dieguez, U.D. Rekarte and N. Zwaig. 1971. Properties and mode of action of a bactericidal compound (=methylglyoxal) produced by a mutant of *Escherichia coli*. J. Bact. 108: 1338-1347.

Morris, I. 1969. The effect of methyl glyoxal on growth and cell division of *Chlamydomonas reinhardii*. Physiol. Plant. 22: 1059-1068.

Murata, K., T. Saikusa, Y. Fukuda, K. Watanabe, Y. Inoue, M. Shimosaka and A. Kimura. 1986. Metabolism of 2 oxoaldehydes in yeasts: Possible role of glycolytic bypath as a detoxification system in L-threonine catabolism in *Saccharomyces cerevisiae*. Eur. J. Biochem. 157: 297-301.

Ranganathan, S. and K.D. Tew. 1993. Analysis of glyoxalase-I from normal and tumor tissue from human colon. Biochimica et Biophysica Acta 1182: 311-316.

Ray, M. and S. Ray. 1981. Isolation of methylglyoxal synthase from goat liver. J. Biol. Chem. 256: 6230-6233.

Ray, S. and M. Ray. 1987. Aminoacetone oxidase from goat liver formation of methylglyoxal from aminoacetone. J. Biol. Chem. 262: 5974-5977.

Saez, G.T., P. Blay, J.R. Vina and J. Vina. 1985. Glucose formation from methylglyoxal in rat hepatocytes. Biochem. Soc. Trans. 13: 945-946.

Saikusa, T., H. Rhee, K. Watanabe, K. Murata and A. Kimura. 1987. Metabolism of 2 oxoaldehydes in bacteria: purification and characterization of methylglyoxal reductase from *Escherichia coli*. Agric. Biol. Chem. 51: 1893-1899.

Sayato, Y., K. Nakamura and H. Ueno. 1993. Toxicological evaluation of products formed by ozonation of aqueous organics. Japanes J. of Toxicology and Environmental Health 39: 251-265.

Starr, R.C. 1978. The culture collection of algae at the University of Texas at Austin. J. Phycol. 14: 47-100.

Szent-Gyorgyi, A. 1977. The living state and cancer. Proc. Natl. Acad. Sci. U.S.A. 74: 2844-2847.

Szent-Gyorgyi, A., L.G. Egyud and J.A. McLaughlin. 1967. Ketoaldehydes and cell division. Science 155: 539-541.

Toerien, D.F., C.H. Huang, J. Radimsky, E.A. Pearson and J. Scherfig. 1971. Provisional algal assay procedures-final report. SERL Report No. 71-6. Sanitary Engineering Research Laboratory. Univ. of California, Berkeley. pp. 25-28.

(Received 12 January, 1995)