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Polyamine Biosynthetic Enzymes in the Cell Cycle of *Chlorella*[1]

CORRELATION BETWEEN ORNITHINE DECARBOXYLASE AND DNA SYNTHESIS AT DIFFERENT LIGHT INTENSITIES

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ABSTRACT

During the life cycle of *Chlorella vulgaris* Beijerinck var *vulgaris* fa. *vulgaris* growing synchronously, the specific activity of ornithine decarboxylase peaked at the 2nd hour of the cycle, whereas that of arginine decarboxylase changed only slightly, increasing towards the end of the cycle. The endogenous level of putrescine and spermidine on a per cell basis increased gradually up to the 8th hour of the cycle, and declined thereafter. Thus, the peak of ornithine decarboxylase activity and the polyamine increase preceded both DNA replication (which took place between the 6th and 8th hours of the cycle) and autospore release (which started at the 8th hour). A 2-fold increase in the light intensity caused doubling of the DNA content, resulting in doubling of the number of autospores per mother cell. It also brought about a 2-fold increase in the specific activity of ornithine decarboxylase and polyamine content, the peaks being at the same hour of the cycle under high and low light intensities. The increase in cell number and polyamine content in a *Chlorella* culture grown under high light intensity was inhibited by α-difluoromethyl ornithine, a specific inhibitor of ornithine decarboxylase, this inhibition being partially reversed by putrescine.

It is suggested that in *C. vulgaris* the sequence of events which relates polyamine biosynthesis to cell division is as follows: increased ornithine decarboxylase activity, accumulation of polyamines, DNA replication, and autospore release.

It is generally accepted that the diamine putrescine is synthesized in plants from arginine by ADC[2] via the intermediate agmatine (20, 26, 29). However, it has recently been shown (5–7, 13, 16) that in many dividing plant tissues putrescine is synthesized from ornithine by ODC, as found in microorganisms (1, 22, 30) and animal cells (4, 11, 23). After synthesis, putrescine may be converted into the other two major polyamines, spermidine and spermine.

Many roles have been suggested for polyamines including enhancement of RNA synthesis (2, 8), activation of RNA polymerase (10), and stabilization of DNA and RNA during replication (17, 24, 25), but their precise function is still not fully understood.

The technique of synchronizing cell cultures has opened an important avenue for studying the biochemical and physiological control of cell division. The photosynthetic unicellular alga *Chlorella* can be readily synchronized and may thus be used as a model system for studies on the cell cycle of plants. The course of the life cycle of *Chlorella* is influenced by light and temperature, *i.e.* high light intensity increases the synthesis of DNA (14) and the number of daughter cells produced from a mother cell, while high temperature shortens the duration of the cell cycle (21).

Thus, a synchronous culture of *Chlorella* was used to study the relationship between polyamine biosynthesis (ODC and ADC activities) and cell division. To investigate the correlation between increasing DNA content and polyamine biosynthesis, cell division was enhanced by increasing the light intensity.

MATERIALS AND METHODS

Culture Technique. *Chlorella vulgaris* Beijerinck var *vulgaris* fa *vulgaris*, isolated in Beer-Sheva, Israel, was cultured in an N-8 basal medium, according to Soeder *et al.* (28). The cultures were grown in 800-ml conical tubes with a 70-mm diameter or in 70-ml conical tubes with a 25-mm diameter. The tubes were plugged with cotton wool and maintained at 28°C. Illumination was supplied by a series of fluorescent lamps. The intensity of the light passing through tubes containing 1×10^7 cells/ml was 13.0 w/m^2 for the 800-ml tubes and 21.5 w/m^2 for the 70-ml tubes. CO_2 was supplied to the cultures at a final concentration of 3% in air.

Synchronization. Cell synchronization was achieved by a method similar to that described by Lorenzen and Hesse (18). The cultures were exposed to alternating periods of 8 h of light and 16 h of dark. Before the onset of the light period, the cultures were diluted to 1×10^7 cells/ml. After three or four such cycles, the cultures were considered to be synchronized. Synchronization was verified by monitoring cell size, which is smallest at the beginning of the cycle. For the high light intensity treatment, cultures were synchronized in all cycles in the 25-mm tubes and for low light intensity in the 70-mm tubes.

ODC Activity. Fifty-ml samples were centrifuged and frozen in liquid N_2. The frozen cells were thawed in 4 ml of phosphate buffer and sonicated as previously described (7). The reaction mixture contained 250 μl of the crude enzyme preparation and 58 μl of L-[1-^{14}C]ornithine (13 μM, 4.9 μCi/μmol) diluted with 42 μl unlabeled L-ornithine (368 μM) to a final concentration of 381 μM. The assay procedure was identical to that previously described.

ADC Activity. The procedure used for ADC was similar to that for ODC except that 58 μl of L-[U-^{14}C]arginine (8 μM, 128 μCi/μmol) were diluted with 42 μl of unlabeled L-arginine (368

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[2] Abbreviations: ADC, arginine decarboxylase; ODC, ornithine decarboxylase; α-DFMO, α-difluoromethyl ornithine.

μM) to a final concentration of 376 μM.

Assays. During the fourth cycle, samples were taken at 2-h intervals for analysis of enzyme activities and of the contents of protein, DNA, and polyamines.

Protein and DNA Contents. Ten-ml samples were centrifuged, frozen in liquid N_2, and stored until analyzed. For protein determination, the cells were hydrolyzed with NaOH, and protein content was determined by the method of Lowry *et al.* (19). DNA content was determined according to Giles and Myers (9).

Endogenous Levels of Polyamines. Cell samples were thawed in 4 ml of distilled H_2O and sonicated as previously described (7). $HClO_4$ was added to the crude extract to a final concentration of 3%. After centrifugation, 200 μl of the supernatant were dansylated overnight, and the dansyl derivatives were then separated by TLC as previously described (3) with putrescine, spermidine, and spermine as markers. The results are the average of three different experiments, two replications each.

RESULTS AND DISCUSSION

When *C. vulgaris* was grown synchronously at a light intensity of 13.0 w/m^2, there was a 4- to 5-fold increase in the number of daughter cells at the end of the light period (Fig. 1). DNA content started to increase at the 6th h of the cycle and reached its maximal level at the 8th h (Fig. 1). The increase of ODC activity preceded that of DNA synthesis and autospore release. The

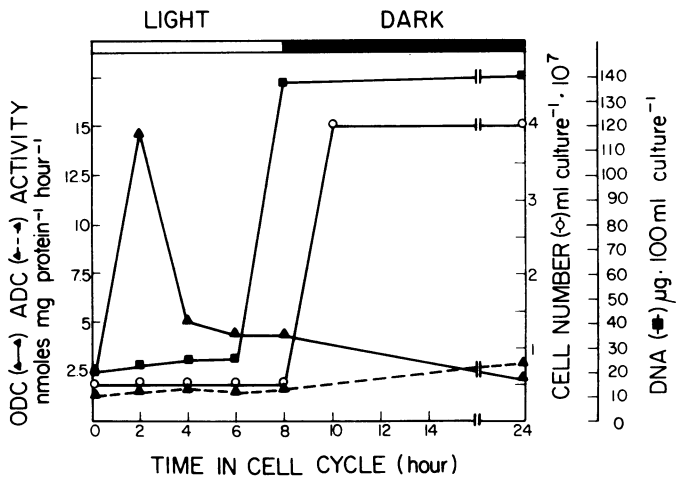

FIG. 1. DNA content (■——■), cell number (○——○), and the activity of ODC (▲——▲) and ADC (▲- -▲) during the cell cycle of *C. vulgaris*. The culture was synchronized in the 800-ml tubes provided with 13.0 w/m^2 light intensity.

Table I. *Effect of Light Intensity on the Endogenous Level of Polyamines during the Cell Cycle of C. vulgaris*

The cultures were synchronized in 800-ml tubes for the low light intensity (13.0 w/m^2) and in 70-ml tubes for the high light intensity (21.5 w/m^2).

Time in Cell Cycle	Putrescine		Spermidine	
	High light intensity	Low light intensity	High light intensity	Low light intensity
h	*nmol/10^9 cells*			
0	19.7	18.3	31.6	27.7
2	30.4	24.9	47.6	40.3
4	45.0	25.2	139.7	80.1
6	90.0	44.8	165.9	89.8
8	104.3	64.8	192.0	94.4
24	12.9	15.2	17.9	24.0

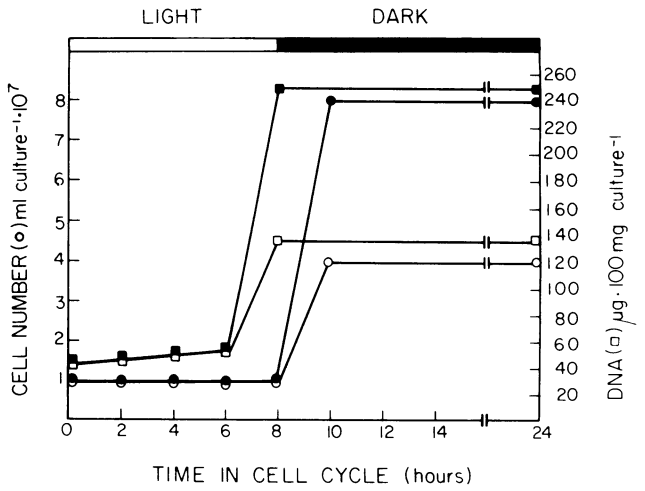

FIG. 2. Effect of light intensity on DNA content and cell number during the cell cycle of *C. vulgaris*. (□ and ■), DNA content under low and high light intensity, respectively; (○ and ●), cell number under low and high light intensity, respectively. The cultures were synchronized in 800-ml tubes for the low light intensity (13.0 w/m^2) and in 70-ml tubes for the high light intensity (21.5 w/m^2).

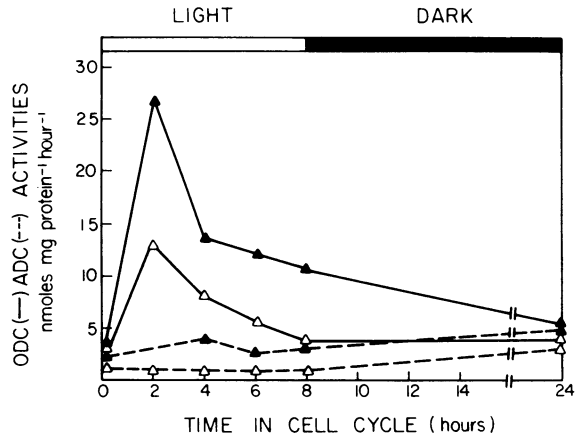

FIG. 3. Effect of light intensity on the activity of ODC and ADC. (△——△ and ▲——▲), ODC activity under low and high light intensity, respectively; (△- - -△ and ▲- - -▲), ADC activity under low and high light intensity, respectively.

Table II. *Effect of α-DFMO on Cell Number of C. vulgaris*

α-DFMO was added to culture medium at a final concentration of 25 mM. Putrescine was added to the culture medium at a final concentration of 1 mM. Cell number · ml^{-1} · 10^7

Treatment	1st Cycle	2nd Cycle
	cell number ± SE	
Control	8.08 ± 0.62	7.07 ± 0.35
α-DFMO	6.68 ± 0.69	3.58 ± 0.37
α-DFMO + putrescine	7.76 ± 0.69	5.60 ± 0.25
Putrescine	8.67 ± 0.39	8.25 ± 0.32

precise timing of these events seems to be significant to the cell cycle.

The only polyamines that could be detected in these experiments were putrescine and spermidine. Spermine, which is present in other plants (29), was not detected at all. Norspermine and norspermidine, which are found in other species of *Chlorella* (12), were also not found, probably due to the inability of the TLC system to separate them.

Table III. *Effect of α-DFMO on the Endogenous Level of Polyamines during the Cell Cycle of C. vulgaris*
Experimental details are given in the legend to Table II.

Time in the Cell Cycle	Putrescine				Spermidine			
	1st cycle		2nd cycle		1st cycle		2nd cycle	
	Control	α-DFMO	Control	α-DFMO	Control	α-DFMO	Control	α-DFMO
h	*nmol/10⁹ cells*							
0	24.2	23.4	20.7	12.9	25.3	24.4	24.0	22.7
2	30.3	28.0	33.3	22.2	40.8	43.4	38.0	30.0
4	51.3	51.3	48.0	32.6	112.0	108.7	96.0	33.0
8	112.0	67.2	104.0	68.0	220.0	129.0	174.0	66.0
24	17.3	10.4	13.5	19.0	27.5	21.5	21.8	14.5

The levels of putrescine and spermidine, calculated on a per cell basis, increased gradually from the beginning of the light period to values 5- to 6-fold higher than those at zero time (Table I). By the end of the dark period (24th h), the concentrations of these two polyamines had fallen to, or dropped below, those at zero time. The results indicate an accumulation of both putrescine and spermidine towards the 8th h of the cycle. The significance of this pattern is not completely clear, particularly when viewed in the light of recently published data on polyamine conjugates (27). Another factor to be considered is the possible turnover of endogenous polyamines, which may affect their actual concentrations at any given moment.

When *C. vulgaris* cells were exposed to high light intensity (21.5 w/m^2), the number of autospores produced per mother cell at the end of the cycle was eight as compared with four under about half the light intensity (13.0 w/m^2) (Fig. 2). DNA replication occurred between the 6th and the 8th h of the cycle, as under low light intensity, but twice as much DNA was produced (Fig. 2).

The peak specific activity of ODC (at the 2nd h of the cycle) under high light intensity was twice that under low light intensity. Under both light intensities, ODC specific activity had fallen to its zero time level by the end of the cycle. ADC activity displayed the same patterns under low and high light intensities, although its absolute level was twice as high under the latter condition (Fig. 3).

The endogenous levels of cellular polyamines over the course of the cell cycle were compared under high and low light intensities (Table I). On a per cell basis, spermidine and putrescine content increased up to the stage of autospore release (8 h), the amount under high light intensity being double that under low light intensity.

If ODC activity is essential for the synthesis of polyamines, which in turn are essential for cell division, it is expected that inhibition of ODC activity would result in inhibition of cell division. This premise was verified by studying the effect of α-DFMO, an ODC-specific inhibitor (6, 15, 22) on the light-dependent enhancement of cell number and increase in polyamine content (Tables II and III). In these experiments, α-DFMO was added to the medium of cultures grown under high light intensity at the onset of the light period. α-DFMO inhibited cell division by 16.5 and 49% after the first and second cycles, respectively (Table II). This inhibition was partially reversed by putrescine. Putrescine added alone caused only a small increase in the cell number.

Parallel to the inhibition of the increase of cell number, α-DFMO also inhibited the rise in putrescine and spermidine levels during the cell cycle, starting from the 8th h of the cycle (Table III). In the second cycle, the inhibition of the increase of both polyamines was more pronounced, especially that of spermidine, the level of which reached only a third of its level in the control.

It is noteworthy that α-DFMO caused only a slight inhibition in both cell number and polyamine content in the first cycle as compared with the marked inhibition in the second cycle. Similar results have been previously obtained in other systems (22). It is possible that in the first cycle there is still a substantial endogenous pool of polyamines, which prevents the inhibition at this stage, but in the following cycle, this pool has already been exhausted.

In light of the findings presented above, we propose the following sequence of events in the cell cycle of *C. vulgaris* leading to cell division at the end of the cycle:

Increased ODC activity → accumulation of polyamines (putrescine and spermidine) → DNA replication → autospore release

This sequence is based on the increases in ODC activity and polyamine content before DNA replication, the doubling of the levels of these three substances under increased light intensity, and the inhibition of cell division by the ODC specific inhibitor α-DFMO. It seems that light intensity may control the number of autospores by first regulating the levels of ODC and the polyamines, which in turn affect the DNA content and the number of autospores. It is also possible that ODC activity and DNA synthesis are regulated correlatively but independently by other factors that are controlled by light intensity.

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