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Influence of polyamines on growth and metabolism of *Dunaliella primolecta*

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SUMMARY

Addition of natural polyamines (putrescine, spermidine or spermine) to the culture medium stimulates growth of *Dunaliella primolecta* and results in an increased photosynthesis (+200%), chl *a* content (+300%) and ATP content (+182%). The results are compared with those induced by synthetic polyamines, such as N,N′-bis (3-aminopropyl) diaminoethane (BAPDE) or 1,4,8,11,tetra-azacyclotetradecane (CYCLAM). BAPDE and CYCLAM increase photosynthesis, ATP level and chl *a* content to the same extent, but have a negative effect on the growth of the alga. The synthetic polyamines increase oxygen uptake, whereas the natural polyamines have no effect. These results are discussed in relation to the hypothesis of polyamines acting as growth regulators or as nitrogen source for the alga.

Key-words: Dunaliella primolecta, growth substances, metabolism, polyamines.

INTRODUCTION

In marine algae, the role of plant hormones in growth or morphogenesis is still not clear although previous studies have contributed somewhat towards an understanding in this field (Bradley 1991; Evans & Trewavas 1991).

Natural or synthetic auxins, gibberellins (GA) and cytokinins (CK), all stimulate growth of *Acetabularia* but their effects are to some extent limited and responses are rather irregular (see Puiseux-Dao 1970). The same applies to phytoplankton (McLachlan 1973; Borowitzka & Borowitzka 1989).

The absence of a clear relationship between growth and hormones does not necessarily imply that algae do not control their growth nor their development by means of hormones analogous or alike those found in higher plants. Various studies have shown the presence of auxins (Abe *et al.* 1972), cytokinins (Mooney & Van Staden 1986; Tay *et al.* 1987) and abscisic acid (ABA) in algae (Kingman & Moore 1982; Hirsch *et al.* 1989). On the other hand, algae might control their growth and development through

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Abbreviations: ATP, adenosine triphosphate; BAPDE, N,N′-bis (3-aminopropyl) diaminoethane; chl *a*, chlorophyll *a*; CYCLAM, 1,4,8,11,tetraazacyclotetradecane; PAs, polyamines; PUT, putrescine; SPD, spermidine; SPM, spermine.

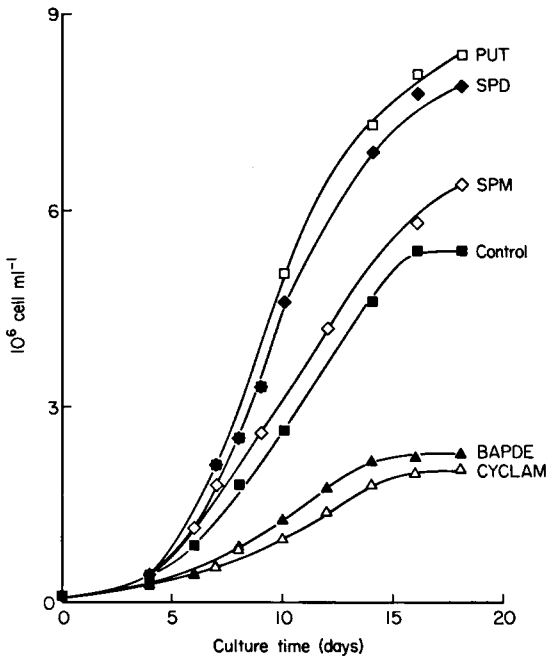

Fig. 1. Effect of polyamines (0·5 mM) on the growth of *D. primolecta*. PUT, putrescine; SPD, spermidine; SPM, spermine (natural polyamines); BAPDE and CYCLAM (synthetic polyamines).

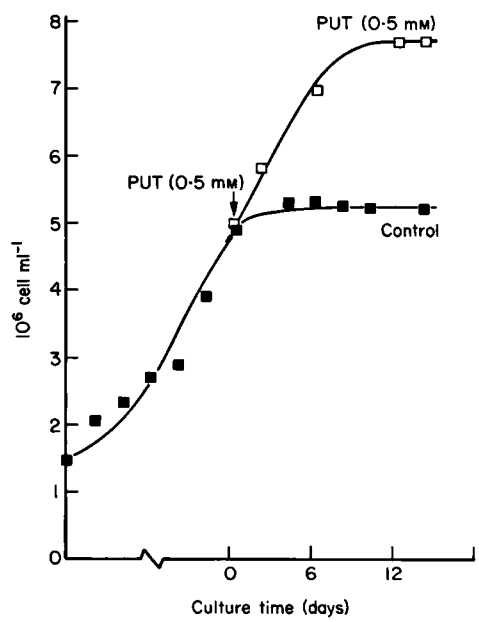

Fig. 2. Effect of putrescine (PUT: 0·5 mM) on the growth of *D. primolecta*. The polyamine was added (arrow) 15 days after the beginning of the experiment during the stationary phase.

Table 1. Oxygen measurements during growth of *D. primolecta* with or without polyamines (0·5 mM) in the culture medium. Results expressed in $\mu l\ O_2\ h^{-1}\ 10^6$ cells^{-1} ± SE and in percentage of the control (in parentheses). Light energy=863 $\mu E\ m^{-2}\ s^{-1}$; temperature=25°C

Age of the culture (days)*		Respiration		Apparent photosynthesis	Gross photosynthesis	
3 (ep)	Control	0·22 ± 0·01		1·89 ± 0·61	2·11	
	PUT	0·27 ± 0·01	(123%)	1·99 ± 0·21	2·26	(107%)
15 (ep)	Control	1·06 ± 0·03		1·23 ± 0·15	2·29	
	PUT	1·30 ± 0·01	(122%)	4·23 ± 0·72	5·53	(241%)
15 (ep)	Control	1·18 ± 0·05		6·06 ± 0·23	7·24	
	SPD	1·09 ± 0·02	(92%)	8·69 ± 0·20	9·78	(135%)
36 (sp)	Control	0·73 ± 0·05		1·91 ± 0·18	2·64	
	SPD	0·63 ± 0·07	(86%)	3·92 ± 0·10	4·55	(172%)
22 (ep)	Control	1·14 ± 0·17		6·11 ± 0·30	7·25	
	SPM	1·25 ± 0·10	(110%)	8·97 ± 0·43	10·22	(141%)
36 (sp)	Control	1·03 ± 0·11		1·74 ± 0·20	2·77	
	SPM	0·88 ± 0·06	(85%)	7·44 ± 0·39	8·32	(300%)
17 (sp)	Control	0·39 ± 0·05		2·43 ± 0·31	2·82	
	BAPDE	0·60 ± 0·05	(154%)	4·37 ± 0·25	4·97	(176%)
36 (sp)	Control	0·73 ± 0·08		2·02 ± 0·15	2·75	
	BAPDE	2·03 ± 0·26	(278%)	9·24 ± 0·33	11·27	(410%)
8 (ep)	Control	0·68 ± 0·12		4·45 ± 0·21	5·13	
	Cyclam	0·72 ± 0·24	(105%)	5·44 ± 0·60	6·16	(120%)
36 (sp)	Control	0·69 ± 0·07		2·24 ± 0·26	2·93	
	Cyclam	1·84 ± 0·22	(267%)	5·17 ± 0·15	7·01	(239%)

*ep, exponential phase; sp, stationary phase.

Table 2. Effect of polyamines (0·5 mM) on the chlorophyll *a* content of *D. primolecta*. Results expressed in pg cell^{-1} ± SE and in percentage of the control C (without polyamines). 13 days=exponential phase; 21 days=beginning of stationary phase; and 36 days=stationary phase

	Polyamine					
Days	C	PUT	SPD	SPM	CYCLAM	BAPDE
13	0·63 ± 0·01	1·74 ± 0·19 276%	2·46 ± 0·08 390%	2·17 ± 0·26 345%	1·54 ± 0·16 259%	1·45 ± 0·43 230%
21	1·33 ± 0·31	1·86 ± 0·36 140%	1·75 ± 0·17 132%	2·51 ± 0·15 189%	1·66 ± 0·23 125%	2·19 ± 0·27 165%
36	1·54 ± 0·08	1·45 ± 0·07 94%	1·51 ± 0·27 98%	2·41 ± 0·35 156%	1·65 ± 0·15 107%	2·50 ± 0·50 162%

other substances as is suggested by Fries (1977). Derivatives of phenylacetic acid have a positive effect on *Fucus spiralis* (Fries 1977) and *Enteromorpha* sp. (Fries & Åberg 1978).

It is in this context that we studied the effects of polyamines (PAs) on the growth and metabolism of a unicellular alga, *Dunaliella primolecta*. Three reasons led us to this work.

Table 3. Effect of polyamines (0·5 mM) in the culture medium of *Dunaliella primolecta* on the amounts of ATP measured during the stationary phase. Results given in pmol of ATP 10^6 cells^{-1} ± SE (n=3) and in percentage of the control C

PA	C	PUT	SPD	SPM	BAPDE	CYCLAM
ATP	22·8 ± 0·27	28·7 ± 0·55	36·6 ± 3·13	64·3 ± 4·36	65·9 ± 5·99	41·9 ± 3·21
%	100	126	152	282	289	184

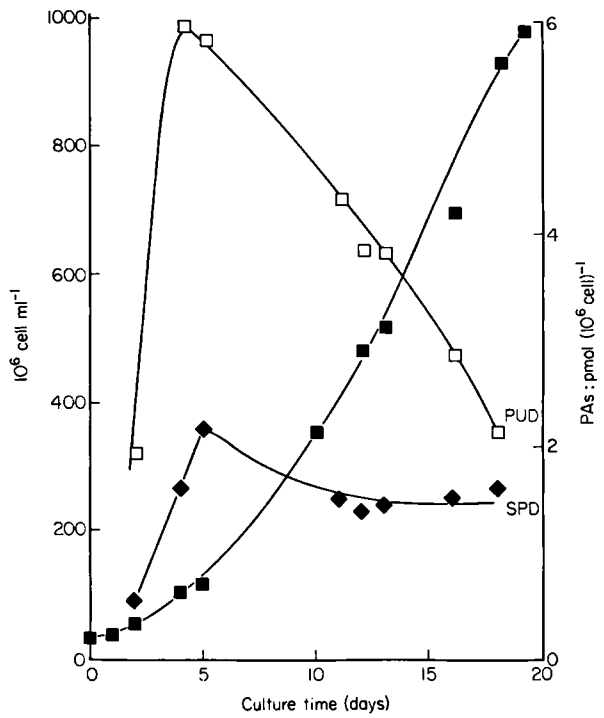

Fig. 3. Changes in polyamine contents during culturing (days) of *D. primolecta*. Results are expressed in pmol (10^6 cell)$^{-1}$: □, putrescine (PUT); ◆, spermidine (SPD). Growth of the culture (expressed as 10^6 cell ml^{-1}), is also presented.

(1) Natural PAs such as putrescine, spermidine and spermine have recently been considered as growth regulators in higher plants. Their polycationic, molecular structure enables them to bind to nucleic acids, membrane phospholipids and proteins. Thus, PAs can at the same time have an effect on the synthesis of these macromolecules, on membrane permeability and on cellular division (Galston & Kaur-Sawhney 1987). PAs are considered important in growth regulation (Smith 1985; Evans & Malmberg 1989) although it is not yet clear whether they act directly as a growth substance *sensu stricto* or as an intermediary messenger in plant hormone expression (Evans & Malmberg 1989).

(2) PAs have been found in many algae, especially Chlorophyceae (Hamana & Matsuzaki 1982). The effect of added PAs was studied in *Porphyridium* sp., a unicellular

red alga with a pectocellulosic cell wall (Scoccianti *et al.* 1989). This study showed that PAs are rapidly taken up by the cells which might be partially due to their binding to negative charges of the wall.

(3) *Dunaliella* can be considered as a 'natural protoplast' since it lacks a pectocellulosic cell wall (Marano 1976; Grizeau *et al.* 1985) which would usually prevent the passage of positively or negatively charged molecules.

The aim of this work was: (i) to test the effect of natural PAs on the growth and metabolism of *Dunaliella*; (ii) to test the effect of two synthetic PAs; (iii) to see whether any natural PAs are synthesized by *Dunaliella* during its growth cycle.

MATERIAL AND METHODS

Culture condition

Bacteria-free algae (*Dunaliella primolecta* Butcher) (from Dr Green's laboratory, Plymouth, UK) were grown in cotton-stoppered Erlenmeyer flasks containing 200 ml Conway medium described by Walne (see Videau *et al.* 1979 for more details). The algae were grown under alternate light–dark periods (12:12) (0·009–0·013 $\mu E\ cm^{-2}\ s^{-1}$). Light intensity was measured by means of a quantameter QSL 100 (Biospherical Instruments Inc., San Diego, CA) at the water level. The temperature was $25 \pm 1°C$ (Borowitzka & Borowitzka 1989).

Cell growth was monitored by counting the cells under a light microscope by means of Malassez haemocytometer.

Cell pigment concentration and ATP level

Pigment was extracted according to the method of Strickland & Parsons (1972). Pigment contents were estimated from equations given by SCOR UNESCO (1966) for chlorophylls.

ATP concentrations were determined by the firefly bioluminescent procedure (Pradet 1967) with a nucleotimeter 107, CLV, Interbio.

Oxygen exchange measurements

To measure oxygen, a pO_2 analyser (YSI 53 oxygen monitor) was connected to a chart recorder and kept in contact with a cell culture stirred with a magnetic rod. The cells were maintained at constant temperature (25°C) by water flowing through a cooling jacket with a circulating pump. Photosynthesis measurements were made under an illumination of 863 $\mu E\ m^{-2}\ s^{-1}$ (quantameter QSL 100).

Polyamine treatments

Putrescine (PUT), spermidine (SPD) and spermine (SPM) were used as natural polyamines. BAPDE (N,N'-bis (3 aminopropyldiaminoethane)) and CYCLAM (1,4,8,11,tetraazacyclotetradecane, a cyclic PA) were used as synthetic PAs. The PA solutions were adjusted to pH=8 and filter-sterilized before they were added to the medium at a final concentration of 0·5 mM.

Polyamine analysis

After filtration of 150 ml of the algal suspension (millipore filter 0·45 μm) the PAs were extracted in 5% (v/v) cold $HClO_4$. Free PAs were dansylated and separated with thin

layer chromatography using a modification (Féray *et al.* 1992) of the procedure described by Flores & Galston (1982).

The results given here represent the means of at least three serial determinations.

RESULTS

Effects of natural and synthetic PAs on the growth of D. primolecta

The growth curves obtained after adding natural and synthetic PAs to the culture medium are quite classical in shape (Fig. 1) with a lag phase of 6 days after inoculation, an exponential phase lasting between 15 and 20 days and a stationary phase. The three natural PAs cause an increase in cellular density. On the contrary, the synthetic PAs BAPDE and CYCLAM inhibit cellular division from the beginning of the exponential phase until the end of the growth phase.

In order to confirm the positive effect of natural PAs on growth, another experiment was run in which the natural PA, PUT, was added at the beginning of the stationary phase. This caused growth to start off again (Fig. 2).

Effect of PAs on metabolism

The effects of PAs on respiration and photosynthesis and on chl *a* and ATP content were studied in order to try to establish a relationship between the specific effects (positive or negative) of PAs on growth and metabolic pathways.

Data were collected either during the exponential phase (ep) or during the stationary phase (sp). Results indicate that natural PAs have only a slight effect on oxygen consumption (Table 1). However, natural and synthetic PAs all stimulate photosynthetic oxygen production especially at the end of the growth period (sp). Synthetic PAs simultaneously stimulate respiration and photosynthesis.

PAs also increase the chl *a* content (Table 2); this stimulatory effect tends to decrease during culturing especially for the natural PAs.

The effect of natural or synthetic PAs on ATP production was studied in cultures maintained in the light during the stationary phase when PAs have the greatest effect on growth and photosynthesis. Results clearly show that all added PAs significantly increase the ATP content of the cells (Table 3).

Endogenous PA content during growth

The analysis of endogenous PAs shows an increase of PUT and SPD during the lag phase and a decrease in their content during the exponential phase (Fig. 3). It should be noted that only trace amounts of SPM could be detected during the growth cycle.

DISCUSSION

The present results show that natural PAs (PUT, SPD, SPM), well known to act on cell division and development, all stimulate growth of the microalga *D. primolecta* (Fig. 1). Also, when PUT was added at the beginning of the stationary phase a stimulation of growth was observed (Fig. 2). The biochemical mechanism(s) by which PAs influence plant growth is still unknown (Roux 1993), but two hypotheses can be put forward to explain the present results.

(1) Extra PAs may act as a source of N, thus alleviating nitrogen limitation. Nitrogen is often limiting in phytoplanktonic growth.
(2) PAs may act as growth regulators or modulators of the hormonal control (Rastogi & Davies 1991).

With regard to the first hypothesis, our results clearly show that all added PAs (natural or synthetic) stimulate photosynthesis of the algae (Table 1). Their chl *a* content (Table 2) and ATP content (Table 3) are also increased. This stimulatory action on energy metabolism might result from the presence of extra nitrogen due to degradation of the PAs. We then have to suppose that, as in legumes and cereals, PAs are degraded through the polyamine–oxidase pathway producing pyrroline and aminopyrroline (Smith 1985; Galston & Kaur-Sawhney 1987). However, it is not known whether such a pathway exists in algae. This hypothesis cannot explain the difference in effect of natural and synthetic PAs on growth: both types lead to increases in photosynthesis, chl *a* content and ATP content, while only natural PAs stimulate growth. Moreover, the synthetic PA: BAPDE has been reported to be only partially degraded in aminopropyldiaminoethane (APDE) in potato plants (Massé *et al.* 1989). This would tend to weaken the role of PAs as a mere source of nitrogen.

In higher plants, PAs inhibited photosynthesis (Pjon *et al.* 1990) while Phelps & McDonald (1990) reported that PAs inhibited the oxidation of NADH in mitochondria. The observation that natural and synthetic PAs have different effects on cell division supports the hypothesis that they act as growth regulators. Furthermore, high endogenous levels of PUT and SPD were detected during the life cycle of *D. primolecta* (Fig. 3). Although these results do not allow us to conclude unambiguously that PAs act as growth regulators, they might be involved in some way in the transduction of stimuli such as hormones or light. Recent reports in the literature also indicate that PAs modulate the second messenger systems or change the conformation and function of specific enzymes such as protein-kinases (Roux 1993; Tiburcio *et al.* 1993).

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