FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 43, No. 1, 2005
pp. 65-67

GA_3 content in young and mature antheridia of *Chara tomentosa* estimated by capillary electrophoresis

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Abstract: The content of gibberellic acid (GA_3) in male sex organs of *Chara tomentosa* L. was estimated using capillary electrophoresis. Young antheridia contained 0.25 μg GA_3 while mature ones 0.48 μg per antheridium. Although there are significant differences in GA_3 content in antheridia between *C. vulgaris* and *C. tomentosa*, these values calculated per one spermatid are 2.4 and 3.3 pg, respectively. The present results compiled with the previous knowledge about regulation of GA_3-dependent development of *Characeae* species allow an implication that the mechanisms controlling antheridia differentiation in both species can be similar.

Key words: Antheridia - Capillary electrophoresis - *Chara tomentosa* - Gibberellic acid

Introduction

Studies on generatively mature thallus of algae *C. vulgaris* revealed the involvement of gibberellic acid in the regulation of its development and spermatogenesis. Exogenous gibberellic acid hastens antheridium development and increases the number of spermatozoids in antheridial filaments [2, 16]. Simultaneously, lengthening of thallus internodes and pleuridium axes becomes restricted [11]. The above data were supported by tests with AMO1618 - an inhibitor of gibberellin synthesis - showing an opposite effect [11]. During spermatogenesis, GA_3 increases endopolyploidy levels in some non-generative cells in antheridium, *e.g.* DNA content in manubria rises by approximately 20% [16].

It has been repeatedly shown that plasmodesmata, which form a system of intercellular communication, play an important role in differentiation of multicellular plant organisms [10]. Spontaneous or induced break of intercellular connections stops symplasmic communication between *C. vulgaris* antheridia and thallus [7, 17]. During first stages of antheridium development, plasmodesmata are open. Later, due to spontaneous symplasmic isolation, plasmatic connections between shield cells as well as between shield cell, basal cell and manubrial cell break [10]. During antheridium proliferative stage, plasmodesmata between antheridial filament cells become selectively plugged with an osmophilic substance. This leads to the formation of antheridial filament domains characterized by shifted synchrony of dividing cells.

Spermiogenesis (sperm differentiation) is also preceded by the break of plasmodesmal connections due to spontaneous symplasmic isolation between basal and capitular cells as well as between basal and subbasal cells. Two final mitotic divisions of antheridial filament cells occur in complete symplasmic isolation between antheridium and thallus [7]. Simultaneously, endoreplication in manubria and capitular cells stops [7]. This may be due to lower gibberellin activity inside antheridium, as suggested by the decrease in radioactive GA_3 penetration into antheridium [8]. This result seems to be supported by capillary electrophoresis measurements of GA_3 content [5, 3]. It was observed that *C. vulgaris* antheridia in the proliferative phase (I and II thallus nodes) contained approx. 5.3 times more GA_3/antheridium than those during spermiogenesis [5].

The aim of the present study was to establish whether there was a difference in GA_3 content between young and mature antheridia of another *Characeae* species, *i.e. C. tomentosa*. In this dioecious algae, the number of chromosomes in haploid antheridial filament cells is 14 and DNA content for 1C - 7.4 pg. In *C. vulgaris*, which is a monoecious plant, the number of chromosomes is 28 (1n) and content of 1C DNA is 13.5 pg [19]. During proliferative phase, subsequent mitotic cycles (5-6) yield 64 and 46 cells in antheridial filaments of *C. vulgaris* and *C. tomentosa*, respectively. In *C. vulgaris*,

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Table 1. Comparison of GA_3 content in young and mature antheridia of *Chara vulgaris* and *Chara tomentosa*

GA_3 content per	*C. tomentosa*		*C. vulgaris*	
	Young	Mature	Young	Mature
Gram fresh weight (μg)	137 ± 20	86 ± 9	512 ± 95*	32.5 ± 5*
Antheridium (μg)	0.25 ± 0.056	0.48 ± 0.051	0.09 ± 0.003*	0.017 ± 0.001*
Antheridial filament (ng)	0.08 ± 0.017	0.15 ± 0.016	0.81 ± 0.099	0.15 ± 0.039
Spermatozoid (pg)	–	3.3 ± 0.4	–	2.4 ± 0.014

Means ± SD of three independent experiments. *Data derived from [5].

S+G2+M cell cycle for two-cell antheridial filaments lasts 42 h and decreases to 26 h at 32-cell stage, while in *C. tomentosa* these values are 41.5 h and 21 h, respectively. Budding capitular cells produce about 111 antheridial filaments in *C. vulgaris* and as many as 3217 in *C. tomentosa*. The average number of spermatids in mature antheridia is 7000 and 145700 for *C. vulgaris* and *C. tomentosa*, respectively [18].

Materials and methods

Antheridia from apical part of *Chara tomentosa* L. were investigated. The plants were collected from the pond in Łódź Botanical Garden. The thalli were rinsed with distilled water and then antheridia from proliferative stage (I-II nodes) and spermiogenesis (III-IV nodes) were examined separately.

Isolation and purification of GA_3 were carried out according to [1]. The identification of GA_3 was done using the Capillary Electrophoresis System - BioFocus 3000 (BioRad) using the CZE (capillary zone electrophoresis): in 30 mmol·dm^{-3} sodium borate buffer, pH 8.5; capillary: 24×25 μm, silica coated; detection: 258 nm; injection: high pressure, 20 psi; temperature: ambient; voltage: 10 kV. Electropherograms were recorded and analysed using BioFocus Integration software 5.0, based on Microsoft Windows, at a detector rise time setting of 1 s and data sampling rate of 5 Hz. The UV spectrum of GA_3 was measured by BioFocus 3000 UV scanning detector. The retention times of standard and measured GA_3 are shown in Figure 1.

Results and discussion

Gibberellins, due to their involvement in induction and regulation of sex organs in algae [13], homosporous ferns [21] and higher plants [20, 22], are called plant sex hormones. High content of GA_3 in young nodes and several-fold less content of GA_3 in older nodes in male *C. tomentosa* thalli was revealed by capillary electrophoresis and biotest. The average GA_3 content in the male thallus was 4-fold higher than in the female one [6]. High GA_3 content was also observed in young nodes of *C. vulgaris* thalli [4].

Electrophoretic measurements showed that 1 g fresh weight (FW) of *C. tomentosa* young antheridia contained about 137 μg GA_3 while mature ones 87 μg which gives 0.48 μg and 0.25 μg of GA_3 per antheridium, respectively (Tab.1). Comparing the present results with data from Maszewski's paper [18] it was estimated that the amount of GA_3 per one mature antheridial filament of both *C. vulgaris* and *C. tomentosa* was 0.15 μg while in young antheridia GA_3 content was 10-fold higher in *C. vulgaris* than in *C. tomentosa.* Finally, however, the amount of GA_3 per one spermatid in mature antheridium of *C. vulgaris* and *C. tomentosa* was similar: 2.4 and 3.3 pg, respectively.

The present findings indicate that in *C. tomentosa*, young antheridia contain 60% more GA_3 per 1 g FW than mature ones. Similar changes in GA_3 content related to the developmental stage were observed in *C. vulgaris* [5]. It is worth noticing that a similar amount of GA_3 corresponds to one spermatid in mature antheridia of both species (Tab. 1).

The results obtained on the basis of endogenous and exogenous GA_3 measurements clearly indicate that proliferation phase of antheridial filament development requires a high level of GA_3 both for division of antheridial filament cells and for endoreplication of nongenerative antheridial cells. Low concentration of GA_3 in mature antheridia may reflect no need for GA_3 during spermiogenesis, when its level is limited due to spontaneous symplasmic isolation observed during development of *C. vulgaris* antheridia [17]. Low GA_3 level may be required for normal spermiogenesis, when spermatid growth is blocked as high chromatin condensation and starch accumulation in plastids occur [14]. Low GA_3 level facilitates starch accumulation in mature nodes of thallus [4]. Stimulation of internode elongation and maintenance of thallus apical domination also requires lower GA_3 level [2].

The presented data suggest that regardless of differences between *C. vulgaris* and *C. tomentosa* developmental cycles, GA_3 may play a similar role in the regulation of sex organ development. In both species, high and low GA_3 levels are associated with antheridial filament cell proliferation and spermatid differentiation, respectively. This similarity is especially visible when GA_3 content is expressed per one spermatid. It may mean that differentiation mechanisms of *C. tomentosa* antheridia are similar to those of *C. vulgaris* where intercellular communication plays a crucial role.

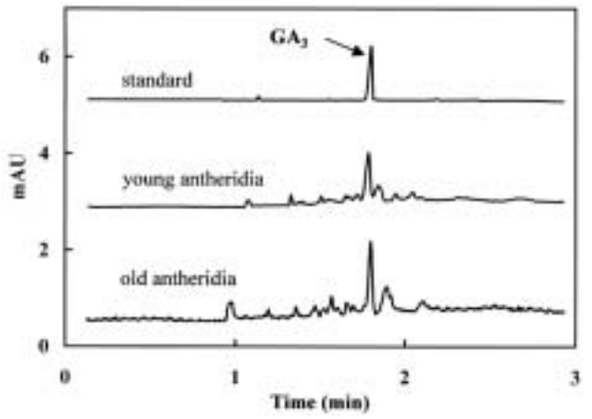

Fig. 1. Capillary zone electrophoresis electropherogram of GA_3 isolated from young and mature antheridia of *Chara tomentosa* and electropherogram of standard gibberellic acid (GA_3).

Acknowledgements: We are grateful to Prof. Maria Kwiatkowska for her valuable advice and comments during the experimental part and preparation of the manuscript.This study was supported by University of Łódź, grant no. 506/820.

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Received May 17, 2004
Accepted after revision September 3, 2004