Acta Physiol Plant (2007) 29:495–502
DOI 10.1007/s11738-007-0099-1

ORIGINAL PAPER

Polyamine metabolism and *S*-adenosylmethionine decarboxylase gene expression during the cytokinin-stimulated greening process

Ewa Sobieszczuk-Nowicka · Tadeusz Rorat · Jolanta Legocka

Received: 19 December 2006 / Revised: 5 September 2007 / Accepted: 7 September 2007 / Published online: 28 September 2007

Abstract This report deals with the effect of kinetin on the greening process, in relation to endogenous free polyamine levels and their metabolism in cucumber cotyledons. The kinetin response on free polyamine levels was found to be accompanied by an increase in free putrescine throughout the greening process. There was no significant difference in spermidine and spermine levels between control (water-treated) and kinetin-treated cotyledons; however, a slight increase in spermidine level, which was higher in control was observed at 4 h. In order to examine the action of kinetin on polyamine metabolism, particularly spermidine synthesis, the effect of kinetin on the level of *S*-adenosylmethionine decarboxylase mRNA and its enzyme activity were studied. First, an increase in the *S*-adenosylmethionine decarboxylase transcript level was observed at 30 min of illumination in water and kinetin-treated cotyledons, and next, the transcript decreased and was restored again at 2 h in kinetin-treated cotyledons and at 4 h in the control. This is the first report that demonstrates the light and kinetin regulation of *S*-adenosylmethionine decarboxylase transcript level. The highest *S*-adenosylmethionine decarboxylase activity was observed at 2 h of illumination, and it was higher in control when compared to kinetin-treated cotyledons. Spermidine and spermine levels observed in kinetin-treated cotyledons at 4 h of illumination may partly be a result of: lower *S*-adenosylmethionine decarboxylase activity inhibited by kinetin and/or higher by about 35% on kinetin polyamine oxidase activity. Experiments with methylglyoxal-bis (guanylhydrazone) and dicyclohexylamine showed that both spermidine synthesis inhibitors depressed chlorophyll accumulation in the greening cucumber cotyledons. Additionally, these results, indirectly confirm that polyamines may play some role in the greening process stimulated by kinetin.

Keywords Chlorophyll · Gene expression · Inhibitors of spermidine synthesis · Kinetin · Polyamines · *S*-adenosylmethionine decarboxylase

Abbreviations

DAO	Diamine oxidase
DCHA	Dicyclohexylamine
MGBG	Methylglyoxal-bis (guanylhydrazone)
PA(s)	Polyamine(s)
PAO	Polyamine oxidase
Put	Putrescine
SAMDC	*S*-adenosylmethionine decarboxylase
Spd	Spermidine
Spm	Spermine

Introduction

The main polyamines (PAs: Put, Spd, Spm) are ubiquitous components in the animal and plant kingdom. The physiological function of polyamines has been investigated in

Communicated by H. Gabrys.

E. Sobieszczuk-Nowicka · J. Legocka (✉)
Faculty of Biology, Department of Plant Physiology,
Adam Mickiewicz University, ul. Umultowska 89,
61-614 Poznan, Poland
e-mail: legocka@amu.edu.pl

T. Rorat
Laboratory of Biochemistry and Genetics,
Institute of Plant Genetics, Polish Academy of Sciences,
61-681 Poznan, Poland

various organs and in different developmental stages using a wide variety of plants. Accumulating evidence has shown that these polycationic compounds are intimately involved in cell proliferation and differentiation processes (Galston and Kaur-Sawhney 1995; Walden et al. 1997). Studies on transgenic plants with modified polyamine levels (Kumar et al. 1997; Masgran et al. 1997; Walden et al. 1997 reviewed in Kakkar and Sawhney 2002; Franceschetti et al. 2004) and mutants defective in polyamine synthesis (Kumar et al. 1996 reviewed in Kakkar and Sawhney 2002) revealed that changes in polyamine levels can affect leaf morphology, stem elongation, root growth, flower formation, etc.

To date, there is limited data on a putative correlation between phytohormones and PAs. It is thought that several of the developmental processes modulated by cytokinins are also controlled by polyamines. A relationship between the physiological effects of PAs and cytokinins has been reported (Suresh et al. 1978; Cho 1983; Galston 1983; Walker et al. 1988; Altman 1989; Sergiev et al. 1995; Brault and Maldiney 1999) but little is known about direct interactions between these plant regulators and hormones.

The pronounced effect of cytokinins on the increase of Put and chlorophyll levels has been reported during light-induced greening of the etiolated cucumber cotyledons (Suresh et al. 1978; Walker et al. 1988). However, inhibition of Put biosynthesis in the presence of D-arginine and difluoromethyl-arginine did not affect chlorophyll production. The authors suggested that the large increase in Put content in cytokinin-treated cotyledons is not essential for the cytokinin-induced greening response.

As suggested by Legocka and Żarnowska (1999, 2000, 2002) polyamines might be involved in cytokinin-mediated mechanisms of chloroplast differentiation in tissue culture of carnation, expansion of cucumber cotyledons or gametophore bud formation of moss. It has been shown that in green tissue culture of *Dianthus caryophyllus* L. (cv. William Sim), which required N^6-benzyladenine for greening, a significant increase in the level of Put, Spd and Spm bound to plastid membranes was observed in comparison to the control (chlorophyll-less callus) (Legocka and Żarnowska 1999). Kotzabasis et al. (1993a, b) and Del Duca et al. (1994) reported that the main polyamines: Put, Spd, Spm are associated with the light-harvesting complex (LHC) and the photosystem II (PSII) of spinach and *Helianthus tuberosus*.

Following these reports we decided to investigate the metabolism of free polyamines during the early stage of kinetin-stimulated greening of cucumber cotyledons. In order to answer the question of whether free polyamines are involved in kinetin action and whether kinetin does play a role in expression of *S*-adenosylmethionine decarboxylase (SAMDC), a key enzyme in polyamine biosynthesis.

We have analysed the level of free PAs and diamine oxidase (DAO) and polyamine oxidase (PAO) activity. Based on the conclusion of Walker et al. (1988) that Put content in cytokinin-treated cotyledons is not essential for the cytokinin-induced greening response, it was decided to analyse the expression of SAMDC and its enzyme activity. Additionally, we used inhibitors of the enzymes of spermidine synthesis—methylglyoxal-bis(guanylhydrazone) (MGBG) and DCHA, which allowed us to examine the involvement of Spd in chlorophyll accumulation, and thereby the greening process.

Materials and methods

Plant materials

Cucumber seeds (*Cucumis sativus* L. cv. Racibór) were germinated on several layers of moistened tissue paper in the dark at 25°C for 6 days. The etiolated cotyledons were then excised under dim green light and next subjected to pretreatment: placed onto Petri dishes on filter paper wetted with water (control) or 100 μM aqueous kinetin solution at 25°C in the dark for 24 h. After pretreatment, the Petri dishes with cotyledons were exposed to white light (146 μmol m^{-2} s^{-1}) for 0.5, 1, 2, 4 or 6 h at 25°C and then used for analysis.

For the experiment with Spd synthesis inhibitors, the etiolated cotyledons were preincubated for 24 h in the dark with treatment shown in Table 1 and exposed to light for 6 h.

Polyamine analysis

Polyamines were extracted from 1 g of cotyledons with 5% cold perchloric acid (100 mg/1 ml of 5% PCA). Free PAs were measured in supernatant by direct dansylation according to Smith and Best (1977). The dansylated amines were separated by silica gel thin layer chromatography (TLC plates, Whatman silica gel 60A LK 6D) in cyclohexane/ethyl acetate (3/2, v/v). Spots were visualised under a UV lamp and identified by the RF values of PA standards. Next, spots were scraped into test tubes, eluted in ethyl acetate and quantified by spectrophotofluorometer (L-2000 Hitachi) with excitation at 350 nm and emission at 495 nm.

DAO and PAO activity

Activity of DAO [EC 1.5.3.11] and PAO [1.4.3.3] was estimated as described by Roy and Gosh (1996) using a Clark electrode. Cucumber cotyledons were homogenised in 0.1 M KH_2PO_4 (2 cm^3 g^{-1} of tissue) and centrifuged at

Table 1 Influence of kinetin and two inhibitors of spermidine synthesis (MGBG and DCHA) on the level of total chlorophyll (a + b) in cucumber cotyledons

Treatment	Chlorophyll (a + b) [μg/g fw]	% Control
Control (H_2O)	536 ± 43	100
+0.1 mM MGBG	492 ± 51	91
+1.0 mM MGBG	274 ± 22	51
+2.5 mM MGBG	205 ± 23	38
+5.0 mM MGBG	183 ± 19	34
+10.0 mM MGBG	103 ± 20	19
Kinetin	987 ± 100	100
+0.1 mM MGBG	780 ± 76	80
+1.0 mM MGBG	468 ± 40	47
+2.5 mM MGBG	386 ± 32	39
+5.0 mM MGBG	208 ± 20	21
+10.0 mM MGBG	97 ± 15	9
Control (H_2O)	594 ± 38	100
+0.1 mM DCHA	511 ± 44	86
+1.0 mM DCHA	504 ± 12	84
+2.5 mM DCHA	503 ± 23	84
+5.0 mM DCHA	384 ± 30	64
+10.0 mM DCHA	330 ± 26	55
Kinetin	1,010 ± 102	100
+0.1 mM DCHA	968 ± 36	95
+1.0 mM DCHA	987 ± 40	97
+2.5 mM DCHA	836 ± 27	82
+5.0 mM DCHA	759 ± 20	75
+10.0 mM DCHA	761 ± 37	75

The 6-day-old, etiolated cotyledons were preincubated for 24 h in the dark with the treatment shown in the table and then exposed to light for 6 h. Data points represent means ± SE of three replicates

20,000*g* for 30 min at 4°C. The assay mixture contained 0.1 M potassium phosphate buffer, pH 7.0, protein extract (190 μg protein per 1.5 ml assay mixture) and 0.2 mM Put or Spd as a substrate for DAO and PAO, respectively. The samples were preincubated without substrates for 10 min at 37°C. The reaction was started by the addition of Put and Spd at a concentration of 0.2 mM. The reaction was carried out at 30°C under air gaseous phase.

SAMDC enzyme activity

S-adenosylmethionine decarboxylase [EC 4.1.1.50] activity was determined by radiochemical method as described by Suresh and Adiga (1977) and modified by Bagni et al. (1983). Plant material was homogenised in five volumes of 100 mM Tris–HCl (pH 7.6), containing 25 μM pyridoxal phosphate and 50 μM EDTA. Samples were centrifuged at 20,000*g* for 30 min at 4°C. The supernatant was used to assay SAMDC activity by measuring count per minute (CPM) of [$^{14}CO_2$] evolution from 3.7 kBq [1-^{14}C]-*S*-adenosylmethionine (2.04 GBq mmol^{-1}, Amersham, UK) per mg protein.

Protein determination

Protein content in the crude extract was quantified by the Bradford method (1976) with bovine serum albumin as standard.

RNA isolation and Northern-blot analysis

Total RNA was extracted, separated on a 1% (w/v) agarose-formaldehyde gel and transferred on to a GeneScreen hybridisation membrane (NEN, NEF 983) as described earlier by Rorat et al. (1998). Equal loading of RNA in each line was verified using ethidium bromide. The [^{32}P]-dCTP labelled 360 pz fragment of cucumber *SAMDC* cDNA was used as a probe for Northern hybridisation. The *SAMDC* fragment was isolated from total cellular cucumber RNA by RT-PCR using primers designed from *Solanum sogarandinum SAMDC* cDNA, *SAMDC* (direct)—TTC AGA TCT GCT GCA TAC TC—Tm 57°C and *SAMDC* (reverse)—ACA CGA TGG TGC ACT CAG C—Tm 57.7°C.

Prehybridisation, hybridisation and washing were performed as described by Rorat et al. (1998).

Chlorophyll analysis

The extraction of chlorophyll was carried out according to Hiscox and Israelstam (1979) using dimethylosulfoxide (DMSO). Fresh material (100 mg) was incubated with 5 ml of DMSO at 65°C for 3 h. The content of chlorophyll was quantified by spectrophotometer (Shimadzu UVVis-160) with emission at 645 and 663 nm.

Statistical analyses

Each data point is the mean of three replicates obtained from three independent experiments. The statistical analysis was performed using Standard Method STATISTICA Stat Soft Inc.

Results

Changes in the level of free PAs during the greening process

Changes in the level of free PAs were analysed during cytokinin-stimulated differentiation of chloroplasts of

cucumber cotyledons. Cucumber cotyledons contained Put, Spd, Spm. The analysis revealed that Put was the most abundant free form of PAs in the cucumber cotyledons (Fig. 1). As shown in Fig. 1 the level of Put was about 4- to 8-fold higher than the level of Spd and about 40- to 60-fold higher than the level of Spm. The level of free Put increased slightly during greening in cotyledons incubated with water. The amount of Put in the cotyledons treated with kinetin increased during greening and was higher by about 59% at 6 h of light exposure in comparison to the control (Fig. 1). The level of free Spd in control cotyledons increased till 4 h of light exposure by about 50% in comparison to 0 time and then decreased at 6 h. In kinetin-treated cotyledons the level of Spd fluctuated without any spectacular changes but was insignificantly lower than in the control. The Spm level was changing slightly during greening and was similar in cotyledons incubated with water and kinetin (Fig. 1).

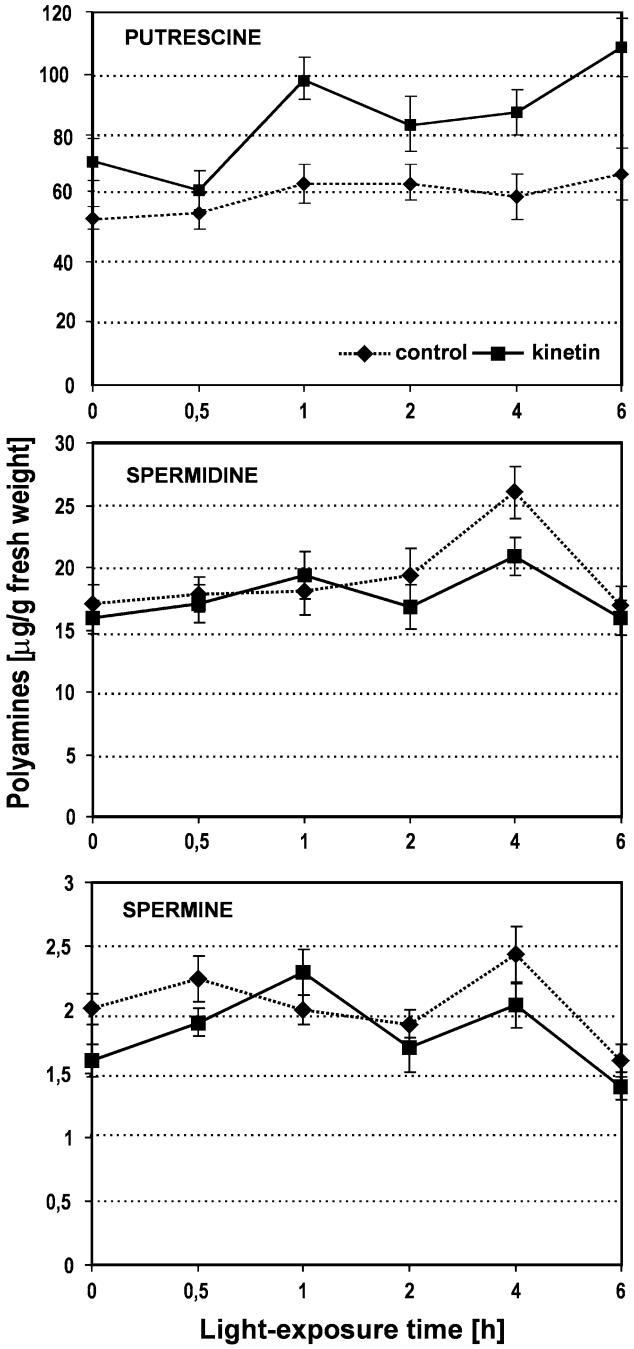

Fig. 1 Kinetic analysis of free polyamine levels: putrescine, spermidine and spermine in cucumber cotyledons during the greening process. The 6-day-old, etiolated cucumber cotyledons were preincubated for 24 h in the dark either with water, designed as control (0 h), or kinetin (0 h) and then exposed to the light for 0.5, 1, 2, 4 or 6 h, respectively. Data points represent means ± SE of three replicates

DAO and PAO activity

During the early stage of greening the activity of DAO increased in the first 2 h of light exposure and then gradually decreased, at 6 h returning to its initial level (Fig. 2). The activity of DAO in the kinetin-treated cotyledons was lower than that determined in the control at 2 and 4 h of illumination (Fig. 2). Conversely, the activity of PAO in the control cotyledons insignificantly decreased during the first hours of the greening, whereas in the kinetin-treated cotyledons it increased by about 25% at 4 h of light exposure compared to the enzyme activity level in the control (0 h) and then decreased (Fig. 2).

SAMDC transcript level and the activity

As shown in Fig. 3 the *SAMDC* transcript was already observed at 0 and 5 min. At 30 min of illumination its significant increase was noticed in both control and cytokinin-treated cotyledons. The transcript level decreased (at 1 h) and was restored again at 2 and 4 h in the kinetin and water incubated cotyledons, respectively (Fig. 3) The *SAMDC* transcript abundance was insignificantly higher in cotyledons incubated with kinetin than in those incubated with water.

SAMDC activity was analysed in protein extracts from both water- and kinetin-treated cotyledons. A large increase, seven times higher than at 0 h, was observed in SAMDC activity during greening with a maximum at 2 h of light exposure in the control cotyledons (Fig. 4). At the same time, in kinetin-treated cotyledons SAMDC activity was lower by 48% as compared to the control. Thereafter, at 4 and 6 h of light exposure in both water and kinetin-treated cotyledons the activity level significantly decreased (Fig. 4).

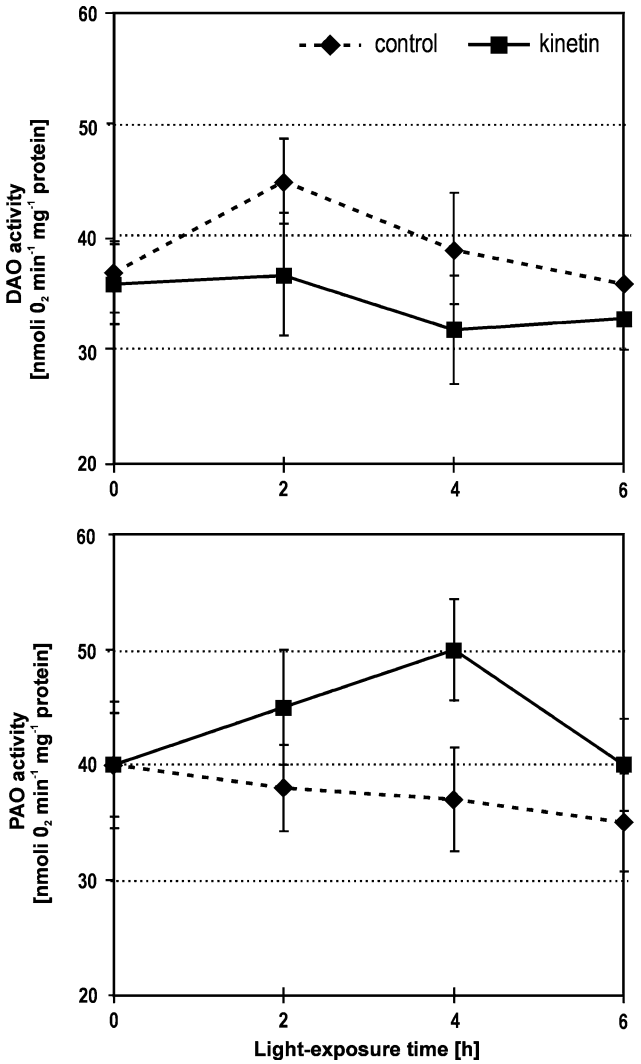

Fig. 2 Kinetic analysis of the activity of DAO and PAO in crude protein extract from cucumber cotyledons. The 6-day-old, etiolated cotyledons were preincubated for 24 h in the dark either with water, designed as control (0 h), or kinetin (0 h) and then exposed to the light for 2, 4 or 6 h, respectively. Data points represent means ± SE of three replicates

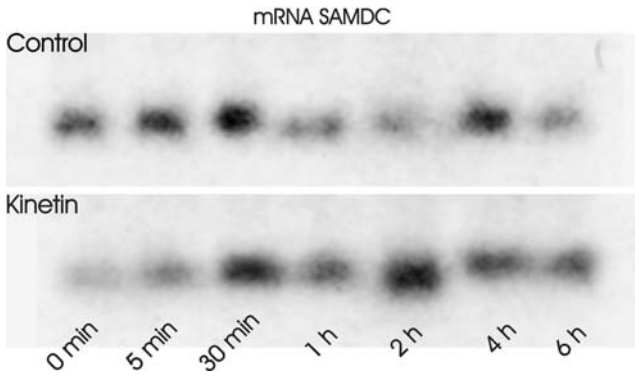

Fig. 3 Northern blot analysis of *SAMDC* mRNA level. Total RNA was isolated from the 6-day-old, etiolated cucumber cotyledons which were preincubated in the dark either with water, designed as control (0 h), or kinetin (0 h) and then exposed to the light for 5 min, 0.5, 1, 2, 4 or 6 h, respectively

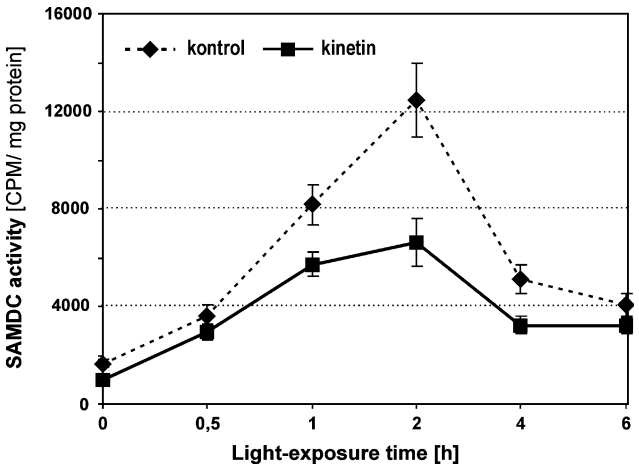

Fig. 4 Kinetic analysis of the SAMDC activity in crude protein extract from cucumber cotyledons. The 6-day-old, etiolated cotyledons were preincubated for 24 h in the dark either with water, designed as control (0 h), or kinetin (0 h) and then exposed to the light for 0.5, 1, 2, 4 or 6 h, respectively. Data points represent means ± SE of three replicates

The effect of inhibitors of Spd synthesis on chlorophyll accumulation

Increasing concentration of spermidine synthesis inhibitors MGBG and DCHA in the medium significantly reduced the level of chlorophyll in both water and kinetin-treated cotyledons (Table 1). In the cotyledons preincubated in the dark with 1 mM MGBG and then exposed to the light for 6 h the accumulation (synthesis) of chlorophyll was inhibited by about 49% in comparison with the control (Table 1). The kinetin-stimulated chlorophyll accumulation was inhibited by about 53% in the presence of 1 mM MGBG. MGBG concentrations higher than 1.0 mM were shown to be toxic and resulted in strong inhibition of the greening process. 10 mM DCHA inhibited the chlorophyll accumulation in the control and kinetin-treated cotyledons by about 45 and 25%, respectively (Table 1).

Discussion

This report deals with the effect of cytokinin on the greening process, in relation to endogenous free PA levels and their metabolism in cucumber cotyledons. Studying PAs metabolism in greening may help in evaluating the

possible application of these natural polycations to control kinetin-stimulated processes.

The cytokinin response was found to be accompanied by an increase in free Put content during the greening process (Fig. 1), which confirms the results of Suresh et al. (1978) and Walker et al. (1988). Similar results were also obtained by Legocka and Żarnowska (2000) who studied the effect of cytokinin on cucumber cotyledon enlargement. It is suggested that an increase in Put level occurs as a cellular response to acidification of the cytoplasm due to cation/anion imbalance and serves as a regulator of cellular pH during cytokinin-induced expansion of cotyledons (Altman and Levin 1993). The addition of KCl and $CaCl_2$ to the kinetin solution strongly depressed the accumulation of Put (Legocka and Żarnowska 2000). This implies a role for Put in cell homeostasis, by virtue of their polycationic nature.

The level of Spd, which increased at the beginning of the greening, was found to decrease at 6 h to its initial level (Fig. 1). The decrease of Spd content at 6 h in both control and cytokinin-treated cotyledons, in comparison with its level at 4 h, may be a result of its degradation or its binding to cellular structures. PAO activity increased at 4 h of light exposure (Fig. 2), in both control and kinetin-treated cotyledons, which may result in polyamine degradation. The second possibility also can take place, as it was shown in the earlier work, where higher levels of spermine bound to chromatin and a large increase in spermidine content bound to ribosomes were observed in etiolated cucumber cotyledons, after 24-h-incubation in the dark with the hormone (kinetin) in comparison to the water-control (Legocka and Żarnowska 2000).

The drop in Spd and Spm content might also be a result of their transport from the cytoplasm to cellular organelles, mainly chloroplasts. The data presently available allows us to suggest that polyamines may play a role in chloroplast development and function (Del Duca et al. 1994; Kotzabasis 1995; Del Duca et al. 2000; Dondini et al. 2003). Unfortunately, no information is available about the translocation of free polyamines; moreover, it has been demonstrated that oxidases take part in the transport of polyamines at the subcellular level (Martin-Tanguy 2001).

As it has been shown that inhibition of Put synthesis did not affect cytokinin promoted greening and enlargement of cucumber cotyledons (Walker et al. 1988; Legocka and Żarnowska 2000) the involvement of light and kinetin on Spd synthesis, mainly on one of the key enzymes which takes part in Spd and Spm synthesis—SAMDC, was studied.

The data presented in Fig. 3 is the first report that demonstrates the light and kinetin regulation of SAMDC gene expression. Both light and cytokinin significantly increased the level of SAMDC transcript at 30 min. Kotzabasis et al. (1999) suggested that intracellural level of polyamines is photoregulated. It was proposed two photoreceptor systems: a protochlorophyllide/blue-light photoreceptor regulating the polyamine decrease during chloroplast photodevelopment and red-light (PSII—reaction centre)/blue light photoreceptor for the control of the PA increase in green cell.

The data from Fig. 3 shows positive correlation between the *SAMDC* transcript level (30 min), the enzymatic activity (2 h) and spermidine biosynthesis (4 h) in the control, whereas in kinetin-treated cotyledons higher levels of *SAMDC* mRNA showed no correlation to enzymatic activity which was lower (2 h). Lack of correlation between high messenger RNA level and low enzyme activity appears difficult to explain other than through some form of post-transcriptional and/or translational regulation (Malberg and Cellins 1994; Xiong et al. 1997; Franceschetti et al.2001; Zosi et al. 2003). The same phenomenon was observed under dehydration and high salinity stress. The increase in the expression of the main genes involved in polyamine biosynthesis during stress treatments did not correlate with accumulation of all polyamines (Alcázar et al. 2006). The abundance of transcript even when enzyme activities are relatively low suggests that mRNAs may be stored (Sari-Gorla 1992).

Light quality has also previously been shown to influence the activity of polyamine biosynthetic enzymes, with red light promoting the activity of arginine decarboxylase (ADC) in pea buds (Dai and Galston 1981), and SAMDC in *Pharbitis nil* seedlings (Hirasawa and Shimada 1994). In both instances, the increase in enzyme activity was reversed with the application of far-red light, implicating phytochrome in the regulation of PA biosynthetic enzymes (Dai and Galston 1981; Yoshida et al.2002). Additionally, blue light also promoted SAMDC activity in *Pharbitis nil*, although this was not reversible with the application of far-red light, indicating the involvement of a separate blue-light receptor (Yoshida et al. 2002).

It should be pointed out that expression of SAMDC may also be affected by internal circadian rhythm. In detailed experiments, Dresselhaus et al. (1996) observed that the transcript level of barley *SAMDC* gene changed with circadian rhythm.

Inhibitors of Spd biosynthesis, MGBG and DCHA have been used to investigate the physiological functions of PAs by altering their endogenous content. The antileukaemic agent MGBG is a competitive inhibitor of SAMDC (Slocum and Flores 1991). Cyclohexylamine (CHA) is a competitive and reversible inhibitor of Spd/Spm synthase (Batchelor et al. 1986). Experiments with MGBG and DCHA showed that both inhibitors depressed the chlorophyll accumulation in the greening cucumber cotyledons. It is necessary to underline that the effect of depression of chlorophyll accumulation in the presence of inhibitors of

Spd biosynthesis is a result of general reduction of the level of Spd that is involved in cell organization (membranes, DNA, RNA, ribosomes, proteins) (Slocum and Flores 1991). These results additionally, but indirectly, confirm that PAs are important factors in greening and can play some role in kinetin action.

Our results indicate that changes in the level of free PAs and their metabolism during cytokinin-stimulated greening are not essential for plant hormone action. It leads to the conclusion, also suggested by Bouchereau et al. (1999) and Martin-Tanguy (2001), that bound forms, of PAs, appear important for plant developmental processes. A possible role of bound polyamines during the greening is discussed, and it needs further biochemical studies, which are in progress, to solve the overall function of these forms of PAs in chloroplasts throughout the process of cytokinin-stimulated greening.

Acknowledgments This work was partly supported by a Research Project of the Dean of the Faculty of Biology, Adam Mickiewicz University, Poznań—PBWB-413/2002. We acknowledge Heidi Nicholl of City University, London for the linguistic correction of the manuscript.

References

Alcázar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P, Tiburcio AF, Altabella T (2006) Involvement of polyamines in plant response to abiotic stress. Biotechnol Lett 28:1867–1876

Altman A (1989) Polyamines and plant hormones. In: Bachrach U, Heimer YM (eds) The physiology of polyamines. CRC, Boca Raton, pp 122–145

Altman A, Levin N (1993) Interaction of polyamines and nitrogen nutrition in plants. Physiol Plant 89:653–658

Bagni N, Barbieri P, Torrigiani P (1983) Polyamine titer and biosynthetic enzymes during tuber formation of *Helianthus tuberosus*. J Plant Growth Regul 2:177–184

Batchelor K, Smith RA, Watson NS (1986) Dicyclohexyl-amine is an inhibitor of spermidine synthase. Biochem J 233:307–308

Bouchereau A, Aziz A, Larther M, Martin-Tanguy J (1999) Polyamines and environmental challenges: recent development. Plant Sci 140:103–125

Bradford MM (1976) A rapid sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein dye binding. Anal Biochem 72:248–254

Brault M, Maldiney R (1999) Mechanisms of cytokinin action. Plant Physiol Biochem 37(5):403–412

Cho SC (1983) Effect of cytokinin and several inorganic cations on the polyamine content of lettuce cotyledons. Plant Cell Physiol 24:27–32

Dai YR, Galston AW (1981) Simultaneous phytochrome controlled promotion and inhibition of arginine decarboxylase activity in buds and epicotyls of etiolated peas. Plant Physiol 67:266–269

Del Duca S, Tidu V, Bassi R, Esposito C, Serafini-Fracassini D (1994) Identification of chlorophyll-a/b proteins as substrates of transglutaminase activity in isolated chloroplasts of *Helianthus tuberosus* L. Planta 193:283–289

Del Duca S, Dondini L, Della Mea M, Muňoz de Rueda P, Serafini-Fracassini D (2000) Factors affecting transglutaminase activity catalysing polyamine conjugation to endogenous substrates in the entire chloroplast. Plant Physiol Biochem 38:429–339

Dondini L, Del Duca S, Dall'Agata L, Bassi R, Gastaldelli M, Della Mea M, Di Sandro A, Claparos I, Serafini-Fracassini D (2003) Suborganellar localisation and effect of light on *Helianthus tuberosus* chloroplast transglutaminases and their substrates. Planta 217:84–95

Dresselhaus T, Barcelo P, Hagel C, Lörz H, Humbeck K (1996) Isolation and characterization of a Tritordeum cDNA encoding *S*-adenosylmethionine decarboxylase that is circadian-clock-regulated. Plant Mol Biol 30:1021–1033

Franceschetti M, Hanfrey C, Scaramagli S, Torrigiani P, Bagni N, Burtin D, Michael AI (2001) Characterization of monocot and dicot plant *S*-adenosyl-L-methionine decarboxylase gene family including identification in the mRNA of a highly conserve pair of upstream over-lapping open reading frame. Biochem J 353:402–409

Franceschetti M, Fornale S, Tassoni A, Zuccherelli K, Mayer MJ, Bagni N (2004) Effects of spermidine synthase overexpression on polyamine biosynthetic pathway in tobacco plants. J Plant Physiol 161:989–1001

Galston AW (1983) Polyamines as modulators of plant development. BiosScience 33:382–383

Galston AW, Kaur-Sawhney R (1995) Polyamines as endogenous growth regulators. In: Davies PJ (ed) Plant hormones: physiology, biochemistry and molecular biology. Kluwer, Dordrecht, pp 158–178

Hirasawa E, Shimada A (1994) The photoresponse of *S*-adenosylmethionine decarboxylase activity in leaves of *Pharbitis nil*. Plant Cell Physiol 35(3):505–508

Hiscox JD, Israelstam GF (1979) A method for extraction of chlorophyll from leaf tissue without maceration. Can J Bot 57:332–1334

Kakkar RK, Sawhney VK (2002) Polyamine research in plant—a changing perspective. Physiol Plant 116:281–291

Kotzabasis K (1995) A role for chloroplast-associated polyamines? Botanica Acta 109:5–7

Kotzabasis K, Fotinou C, Roubelakis-Angelakis KA, Ghanotakis D (1993a) Polyamines in the photosynthetic apparatus. Photosystem II highly resolved subcomplexes are enriched in spermine. Photosynth Res 38:83–88

Kotzabasis K, Christakis-Hampsas MD, Roubelakis-Angelakis KA (1993b) A narrow-bore HPLC method for the identification and quantitation of free conjugated and bound polyamines. Anal Biochem 214:484–489

Kotzabasis K, Navakoudis E, Tsolakis G, Senger H, Dörnemann D (1999) Characterization of the photoreceptor(s) responsible for the regulation of the intracellular polyamine level and the putative participation of heterotrimeric G-proteins in the signal transduction chain. J Photochem Photobiol B 50:38–44

Kumar A, Taylor M, Mad Arif SA, Davies AW (1996) Potato plants expressing antisense and sense *S*-adenosylmethionine decarboxylase (*SAMDC*) transgens show altered levels of polyamines and ethylene antisense plants display abnormal phenotypes. Plant J 9:147–158

Kumar A, Altabella T, Taylor MA, Tiburcio AF (1997) Recent advances in plant polyamine research. Trends Plant Sci 2:124–130

Legocka J, Żarnowska A (1999) Role of polyamines in the cytokinin-dependent physiological processes. I. Effect of benzyladenine on polyamine during chloroplast differentiation in the tissue culture of *Dianthus caryophyllus*. Acta Physiol Plant 21:349–354

Legocka J, Żarnowska A (2000) Role of polyamines in the cytokinin dependent physiological processes. II Modulation of polyamine levels during cytokinin-stimulated expansion of cucumber cotyledons. Acta Physiol Plant 22:395–401

Legocka J, Żarnowska A (2002) Role of polyamines in the cytokinin-dependent physiological processes. III Changes in polyamine levels during cytokinin-induced formation of gametophores buds in *Ceratodon Purpureus*. Acta Physiol Plant 24:303–309

Malberg RL, Cellins ML (1994) Arginine decarboxylase of oats is activated by enzymatic cleanage into two polypeptides. J Biol Chem 269:2703–2706

Martin-Tanguy J (2001) Metabolism and function of polyamines in plants: recent development (new approaches). Plant Growth Regul 34:135–148

Masgran C, Altabella T, Fascas R, Flores D, Thompson AJ, Besford RT, Tiburcio AF (1997) Inducible overexpression of oat arginine decarboxylase in transgenic tobacco plants. Plant J 11:463–473

Rorat T, Grygorowicz WJ, Berbezy P, Irzykowski W (1998) Isolation and expression of cold specific genes in potato *(Solanum sogarandinum*). Plant Sci 133:57–67

Roy M, Gosh B (1996) Polyamines, both common and uncommon, under heat stress in rice (*Oryza sativa*) callus. Physiol Plant 98:196–200

Sari-Gorla M (1992) Gene expression during the male gametophytic phase. Giorn Bot Ital 126:99–109

Sergiev LG, Alexieva VS, Karanov EN (1995) Cytokinin and anticytokinin effect on growth and free polyamines content in etiolated and green radish cotyledons. J Plant Physiol 145:266–270

Slocum RD, Flores HE (1991) Biochemistry and physiology of polyamines in plants. CRC, Boca Raton

Smith TA, Best GR (1977) Polyamines in barley seedlings. Phytochem 16:841–843

Suresh MR, Adiga PR (1977) Putrescine-sensitive (artifactual) and insensitive (biosynthetic) *S*-adenosyl-L-methionine decarboxylase activities of *Lathyrus sativus* seedling. Eur J Biochem 31:511–518

Suresh MR, Ramakrishna S, Adiga PR (1978) Regulation of arginine decarboxylase and putrescine levels in *Cucumis sativus* cotyledons. Phytochemistry 17:57–63

Walden R, Cordeiso A, Tiburcio A (1997) Polyamines: small molecules triggering pathways in plant growth and development. Plant Physiol 113:1009–1013

Walker MA, Roberts DR, Dumbroff EB (1988) Effect of cytokinin and light on polyamines during the greening response of cucumber cotyledons. Plant Cell Physiol 29:201–295

Xiong H, Stanley BA, Tekwani BL, Pegg AE (1997) Processing of mammalian and plant *S*-adenosylmethionine decarboxylase proenzymes. J Biol Chem 272:342–348

Yoshida I, Yamagata H, Hirasawa E (2002) Signal transduction controlling the blue- and red-light mediated gene expression of *S*-adenosylmethionine decarboxylase in *Pharbitis nil*. J Exp Bot 53(373):1525–1529

Zosi V, Scaramagli S, Bregoli AM, Biondi S, Torrigiani P (2003) Peach (*Prunus persica* L.) fruit growth and ripening: transcript levels and activity of polyamine biosynthetic enzymes in the mesocarp. J Plant Physiol 160:1109–1115