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Adenine Nucleotides and Energy Charge during Fiber Elongation of two Cottons differing in their Lint Lengths

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Changes in adenylates (ATP, ADP and AMP) and energy charge have been investigated in elongating fibers of *Gossypium arboreum* L. cv. LD 133 (a short staple type) and *Gossypium hirsutum* L. cv. LH 372 (a long staple type) at different stages of cell growth. A high rate of dephosphorylation of ATP and low energy charge is observed in long staple cotton relative to the short staple when the growth rate of fibers is highest. This is discussed in the light of higher energy requirements and increased utilization of ATP by long fibers than their short counterparts. Pronounced oscillations along with significant differences in nucleotide ratios suggest rapid changes in energy metabolic sequences and different metabolic behaviour in the elongating fibers of two cotton types.

Key Words: Cell (fiber) elongation, Adenylate phosphates, Energy charge, Osmoregulation, Short staple cotton, Long staple cotton

Introduction

Botanically, cotton fiber is a hair or trichome: a unicelled extension from the ovular epidermis. It is a rather prodigious plant cell measuring 1000–3000 times longer than its diameter. The developing cotton fiber is an excellent research system for studying physiologic and biochemic changes accompanying cell elongation and/or maturation. Since the fiber originates and ends as a single cell, elongation can be studied free of any complications from cell division. The specific intent of the present study is to investigate the energetics of cell extension by measuring the pool sizes of adenine nucleotides (ATP, ADP and AMP) and energy charge in elongating fibers of two cottons, namely, *Gossypium arboreum* L. cv. LD 133 (a short staple type) and *Gossypium hirsutum* L. cv. LH 372 (a long staple type).

ATP, ADP and AMP have been identified in developing cotton fibers (Franz 1969 and Carpita & Delmer 1981) but systematic determinations at different fiber ages have not been carried out to correlate the changes in these components with the process of cell extension. ATP

plays a pivotal role in maintenance of cell integrity, regulation of cell turgor, phosphorylation of metabolic substrates, and various energy requiring biosyntheses. Obviously, the fiber metabolism must be fueled by an adequate energy supply to attain cell extension of considerable magnitude. We have assumed during the design of this study that energy constraints in the short staple cotton may be impeding its fiber growth as compared with the long staple cotton. Consistent with this possibility, we present data showing higher energy requirements and better energy transduction in elongating fibers of the long staple cotton relative to the short staple one.

Materials and Methods

Crop was raised according to the recommended practices for fertilizers, plant protection, weed control, and irrigations to optimize lint yield in the field. Individual flowers were tagged on the day of anthesis (designated as day 0) and bolls were harvested at 5, 10, 15, 20 and 25 days postanthesis (DPA). Fiber length was determined by the method of Gipson and Ray (1969) measuring from the ovule epidermis to the tips of fibers produced on the chalazal end of the ovules. Fiber length of ten randomly selected ovules per boll from five different bolls was measured and averaged.

Extraction of Nucleotides: Till 5 DPA, it was difficult to separate fibers from the seed. Hence the young ovules were used for analyses, while at 10 DPA and in subsequent periods, only fibers were used. Fresh ovule/fiber samples of known weight were ground in a glass homogenizer immediately after harvest in cold 7% (v/v) $HClO_4$. All steps of extraction were carried out at 0–4°C. The acid mixture was spun at 20,000 g for 20 minutes. The *p*H of the supernatant was adjusted to 6.7 ± 0.1 with 5M K_2CO_3. The neutralized extract was centrifuged as before and the supernatant used for assaying adenosine phosphates. Duplicate extracts were prepared in all cases and the values averaged. The assays were conducted spectrophotometrically at 340nm. Experiments were repeated three times.

Assay of Nucleotides

ATP: The assay mixture contained triethanolamine buffer, *p*H 7.6, 85 mM; $MgCl_2$, 5 mM; glucose, 5 mM; NADP, 0.1 mM; 10 μg glucose-6-phosphate dehydrogenase and 20 μg hexokinase. The sample volume was 1.0 ml in a total volume of 3.0 ml.

ADP/AMP: The assay mixture contained triethanolamine buffer, *p*H 7.6, 16 μ mole; $MgCl_2$, 8 μ mole; KCl, 16μ mole; PEP, 2 μ mole; NADH, 20 n mole; lactate dehydrogenase, 15μg. The sample volume was 1.0 ml in a total volume of 3.0 ml. For ADP, 6 μg pyruvate kinase was added and for AMP, 6 μg myokinase was also added.

Calculations: The adenylate energy charge ratio (Atkinson 1968),

$$\frac{ATP + 0.5\ ADP}{ATP + ADP + AMP},$$

a measure of the energy-rich adenylates in a cell, was calculated from adenine nucleotides to assess the energy status of the fiber at a particular stage of development. In view of the control of key enzymes in metabolic sequences by nucleotide ratios (Atkinson 1977) some presumably relevant ones were also calculated.

Results

Growth Data: The cumulative increases in fiber length are shown in figure 1. In both, short and long-fibered cotton, there is little growth during the first 5 days, but thereafter growth rate increases. Fiber growth slows down after day 15 and a final length of about 1.7 and 2.5 cm is attained in the short and long-fibered cotton respectively. By day 15, 87 and 80% of elongation is completed in the short and long fibers respectively. By 25 DPA, fiber elongation is completed in both the cottons.

Adenine Nucleotides

In both the fibers, total adenylates increase significantly till 15 DPA and do not turnover appreciably thereafter (table 1). Total adenylates are marginally higher in the fibers of long staple cotton throughout the period of cell elongation. At day 10, 15 and 20, a relatively high proportion of adenosine phosphates is in the mono—and diphosphate form in the long staple cotton while the converse is true of the other cotton (table 1). ATP content in the long staple fibers fluctuates in the range of 272–400 n mole g^{-1} dry weight while in short staple from 349–422 n mole g^{-1} dry weight.

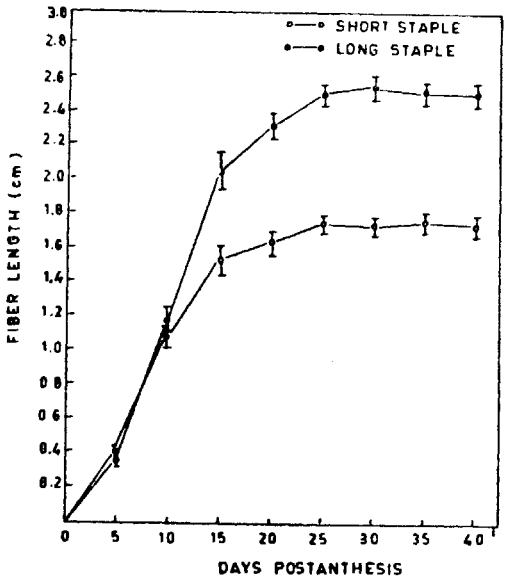

Figure 1 The cumulative increase in fiber length of two cottons at different fiber ages. ± represents standard error values of 50 replicates

(Energy Charge (EC)

Data in table 2 show that EC in the short staple fibers drops from 0.71 to 0.64 to 0.68 at 5, 10, 15 DPA and recovers to the initial value at 20 and 25 DPA. In the long staple cotton, EC values are relatively lower at all stages of fiber elongation. EC drops considerably from 5 DPA onwards and is found to be lowest at 15 and 20 DPA. It does not recover to the initial figure as observed in case of short staple cotton at later stages of fiber growth.

Adenylate Ratios

Large changes in ATP/ADP, ATP/AMP and ADP/AMP ratios are noted in both cottons at all fiber growth stages (table 2). ATP/ADP and ATP/AMP ratios are consistently greater in the short staple fibers during the entire period of fiber elongation except at 5 and 25 DPA where ATP/AMP and ATP/ADP ratio is greater in the long staple fibers respectively. On the other hand, opposite is true of ADP/AMP ratio except at 25 DPA.

Discussion

The study shows dynamical energy status of elongating cotton fibers. Total adenylates increase with the fiber age in both the cottons, with the highest levels occurring at day 15. The peak content coincides with the period when most

Table 1 *Concentration of adenosine phosphates during fiber elongation of a short staple (SS) and a long staple (LS) cotton*

Adenylates (n mole g^{-1} dry weight ± S.E.)	Cotton	Fiber Age (DPA)				
		5	10	15	20	25
ATP	SS	353±14	349±20	392±25	423±15	413±19
	LS	362±17	320±16	272±29	330±14	401±16
ADP	SS	50±10	161±20	153±13	138±17	137±11
	LS	60±13	221±18	273±12	211±20	103±15
AMP	SS	128±16	152±18	151±19	120±12	127±20
	LS	136±14	160±15	201±18	113±15	217±21
ATP+ADP+AMP	SS	531	662	686	681	677
	LS	558	701	746	734	721

Total represents sum of means of three replications.

Table 2 *Adenylate ratios and energy charge during fiber elongation of a short staple (SS) and a long staple (LS) cotton*

Adenylate Ratio	Cotton	Fiber Age (DPA)				
		5	10	15	20	25
$\frac{ATP}{ADP}$	SS	7.0	2.1	2.5	3.0	3.0
	LS	6.0	1.4	.99	1.5	3.9
$\frac{ATP}{AMP}$	SS	1.8	2.3	2.6	3.5	3.2
	LS	2.6	2.0	1.6	3.0	1.8
$\frac{ADP}{AMP}$	SS	.39	1.0	1.0	1.1	1.0
	LS	.51	1.3	1.3	1.8	.49
$\frac{ATP + 0.5\ ADP}{ATP + ADP + AMP}$	SS	.71	.64	.68	.72	.71
	LS	.70	.61	.54	.59	.62

Adenylate contents used in ratio determinations represent mean of three replications

of the cell elongation is completed in both the fibers. It is noteworthy that during the same period, fibers of the long staple cotton contain a higher proportion of adenosine phosphates in the mono- and diphosphate form indicating a high rate of dephosphorylation of ATP i.e. there is an increased utilization of ATP. A similar situation does not exist for short counterparts.

We discuss the conclusion of increased utilization of ATP by long fibers in relation to their faster rate of growth. One of the most important cellular 'sinks' for ATP is attributable to the regulation of ionic fluxes (Hanson & Trewavas 1982). Potassium and malate act as important osmoregulatory solutes during turgor driven extension growth of the developing cotton fiber (Dhindsa et al. 1975, 1976 and Dhindsa 1978). A better provision of energy in the long fibers may be used to regulate internal ionic state of the fiber cell in terms of energization of K^+-sugar uptake and malate synthesis which, in turn, would impact upon a superior metabolic and turgor regulation of such fibers. Further-

more, other energy utilizing biosyntheses e.g. the synthesis of RNA, proteins, membrane lipids and nucleotide sugars potentially could also be rate limiting in fiber growth. It is suggestive therefore that rate of fiber elongation may be the result of ATP levels.

Energy charge values at all stages of fiber growth are lower in the long fibers than the short ones. One would expect that larger ATP utilization by long fibers may result into some drop of energy charge relative to the short fibers. Generally, growing and dividing cells maintain a high energy charge around 0.8, whereas senescing or dormant cells maintain an energy charge of less than 0.5 (Chapman et al., 1971). From our data, the critical energy charge threshold for fiber growth seems to be lower than that of bacterial cells.

Adenine nucleotides have a number of effects on respiration. The ratios of different adenylates is one way of knowing about cellular energy metabolism and its regulation (Atkinson 1977). More or less constant ratios indicate stable conditions in energy metabolism. In our study, pronounced oscillations in adenylate ratios during fiber elongation emphasize the point that energy charge can obscure large changes in individual adenylate ratios. Likewise, Lowry et al. (1971) have pointed out that for certain enzymes the ratios ATP/AMP and ATP/ADP may be the metabolically dominant factors rather than the energy charge per se. Therefore, the differences in adenylate ratios in the two cotton fibers during elongation could constitute a metabolic signal, which can modulate the activity of some key enzymes and thus, give rise to a different metabolic pattern in each case. In this context, a specific metabolic milieu of the two fibers may lead to differential fiber production in the respective cottons.

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