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Influence of ABA and Calcium on Dark Fixation of Carbon Dioxide in Cotton Fibres

By

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With 1 Figure

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Summary

BASRA A. S., DHILLON-GREWAL R., SARLACH R. S. & MALIK C. P. 1993. Influence of ABA and calcium on dark fixation of carbon dioxide in cotton fibres. – Phyton (Horn, Austria) 33 (1): 1–6, 1 figure. – English with German summary.

The influence of ABA and calcium on dark fixation of CO_2 by cotton fibres (*Gossypium hirsutum* L. cv. F 414) was studied at the stage of 15 days after anthesis, denoting the phase of primary wall development and elongation. Both ABA and calcium markedly inhibited the activity of PEP carboxylase and also the fixation of $H^{14}CO_3^-$ into organic acids. ABA inhibited carbon incorporation into amino acids as well but calcium was marginally promotory. Application of chlorpromazine, a calmodulin antagonist, did not reveal consistent effects. While it inhibited PEP caboxylase activity, the incorporation of label into organic acids and amino acids was slightly stimulated.

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Zusammenfassung

BASRA A. S., DHILLON-GREWAL R., SARLACH R. S. & MALIK C. P. 1993. Der Einfluß von ABA und Kalzium auf die Dunkelfixierung von Kohlendioxid in Baumwollfasern. – Phyton (Horn, Austria) 33 (1): 1–6, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

In Baumwollfasern (*Gossypium hirsutum* L. cv. F 414) wurde der Einfluß von ABA und Kalzium auf die Dunkelfixierung von CO_2 15 Tage nach der Blüte untersucht. Zu diesem Zeitpunkt erfolgt die Primärwandbildung und Zellstreckung. Sowohl ABA als auch Kalzium hemmte auffallend die Aktivität der PEP Carboxylase aber auch die Fixierung von $H^{14}CO_3^-$ in organischen Säuren. ABA hemmte auch den Kohlenstoffeinbau in Aminosäuren, wogegen Kalzium leicht fördernd wirkte. Der Zusatz von Chlorpromazin, ein Calmodulin Antagonist, ergab keine übereinstimmenden Ergebnisse. Während es die PEP Carboxylaseaktivität inhibierte, stimulierte es geringfügig den Einbau von markierter Substanz in organische Säuren und Aminosäuren.

1. Introduction

Cotton fibres are unicellular elongated cells which differentiate from the epidermal layer on the ovules (BASRA & MALIK 1984). The process of fibre growth involves both elongation and secondary wall deposition phases. Elongation of the primary cell wall occurs for a period of about three weeks after anthesis, followed by deposition of a thick cellulosic secondary wall (BASRA & MALIK 1984). An understanding of regulatory mechanisms of fibre elongation is both of inherent as well as applied scientific interest.

Elongating cotton fibres possess an active system for dark fixation of CO_2 catalyzed mainly by PEP carboxylase (DHINDSA & al. 1975, DHINDSA 1978, BASRA & MALIK 1983, NAYYAR & al. 1988, KAUR & al. 1990). Oxaloacetate once formed can either be reduced to malate or transaminated to aspartate. During the period of 5 to 15 DAA, the reduction of oxaloacetate to malate is the main route of the metabolism of fixed carbon which accumulates along with K^+ to achieve increased turgor for fibre extension (BASRA & MALIK 1983). Beside this, dark fixation of CO_2 is involved in linking other pathways of fibre metabolism (BASRA & MALIK 1985, NAYYAR & al. 1988).

ABA, which inhibits fibre growth, also inhibits the enzyme activities associated with dark metabolism of CO_2 (DHINDSA 1978). Conversely, cotton ovules cultured in the presence of fluridone, an inhibitor of ABA biosynthesis, responded with a marked increase in these enzyme activities (KAUR & al. 1990). Recently, the role of Ca^{2+} has been implicated in the action of ABA in other systems, such as stomatal closure and inhibition of respiration in protoplasts (DESILVA & al. 1985a, b; OWEN & al. 1987a, b; OWEN 1988; MANSFIELD & al. 1990). Recently, we obtained evidence for the involvement of calcium in regulating peroxidase and o-diphenol oxidase activities of cotton fibres and also studied its possible relationship to ABA (BASRA & al. 1992). As part of the continuing research programme, the

present study was conducted to determine whether calcium could also modulate the fibre capacity for dark fixation of CO_2 and be related to ABA action in some way.

2. Material and Methods

The cotton crop (*Gossypium hirsutum* L. cv. F 414) was raised in the field following recommended agricultural practices. The flowers were tagged on the day of anthesis in the morning and the bolls were harvested at 15 days after anthesis (DAA). At this stage, the fibres are invested with a primary cell wall and exhibit dark CO_2 fixation capacity (Basra & Malik 1983). Seed clusters with intact cotton fibres were isolated and incubated *in vitro* for 3 hr at 28° C in Hepes buffer (20 mM, pH 7.5). After incubation, the fibres were detached from the seeds and extracted for PEP carboxylase activity as described earlier (Basra & Malik 1983). The enzyme activity was assayed by the modified method of Downton & Slatyer (1971), coupled with malate dehydrogenase. Total assay volume of 3 ml had: 100 µmole Tris-HCl buffer, pH 8; 50 µmole $NaHCO_3$; 30 µmole $MgCl_2$; 0.5 µmole dithiothreitol; 5 µmole PEP; 0.5 µmole NADH; 4.2 U malic dehydrogenase and 0.2 ml enzyme extract. The decrease in absorbance was measured at 340 nm. For $H^{14}CO_3^-$ fixation studies, seed clusters with adhering fibres were similarly used containing 5 µCi $NaH^{14}CO_3^-$ (specific activity 45 mCi/ml) in the incubation medium. Fixation into organic acid and amino acid fractions was followed as per Dickson (1979). Various chemicals, viz. ABA (10 µM), $CaCl_2$ (5 mM), EGTA (2 mM) and chlorpromazine (10 µg/ml) were incorporated in the medium to observe their effects on PEP carboxylase activity and HCO_3^- fixation into organic acids and amino acids. The results presented are mean values from duplicate determinations. Enzyme activity was expressed as µmoles/min/fibres of one seed and incorporation of radioactivity as cpm/fibres of one seed.

Abbreviations: ABA – abscisic acid; DAA – days after anthesis; PEPC – phosphoenolpyruvate carboxylase; CP – chlorpromazine; EGTA – ethylene glycol-bis-(β amino ethyl ether) N, N′-tetraacetic acid.

3. Results and Discussion

As compared with the control, both ABA and calcium treatment reduced PEP carboxylase activity (Fig. 1, Table 1). The activity was further reduced when ABA and $CaCl_2$ were applied together. Dhindsa (1978) has reported inhibition of PEPC activity by ABA but this is the first observation showing inhibition by calcium as well. Pertinently, Gavalas & Manetas (1980) have shown that 1 mol m^{-3} calcium is able to inhibit the *in vitro* activity of PEPC in stomatal guard cells.

Calcium can exert its effect either *per se* or through its binding with calcium-binding proteins particularly calmodulin (Allan & Trewavas 1987). In the present study, application of chlorpromazine, a calmodulin antagonist, did not relieve the inhibition of PEPC activity implying that the gross effects of high exogenous Ca^{++} probably do not involve calmodulin (Fig. 1). Incubation in the presence of EGTA which chelates wall-bound calcium did not cause an appreciable change in enzyme activity, but ABA-mediated inhibition was slightly relieved by simultaneous application of

Table 1

PEP carboxylase activity measurements of cotton fibres at 15 DAA as influenced by ABA, calcium, EGTA and chlorpromazine

Treatment	PEP carboxylase activity (μmoles/min/fibres of one seed)
Control	8.04
ABA (10 μM)	2.41
$CaCl_2$ (5 mM)	1.93
EGTA (2 mM)	7.43
Chlorpromazine (10 μg/ml)	4.82
ABA (10 μM) + $CaCl_2$ (5 mM)	1.11
ABA (10 μM) + EGTA (2 mM)	4.02
ABA (10 μM) + Chlorpromazine (10 μg/ml)	2.41

EGTA (Fig. 1). ABA + chlorpromazine treatment was, however, not effective.

Dark fixation of carbon dioxide by PEPC channelizes carbon mainly into organic acids or amino acids (BASRA & MALIK 1985) and especially the former in case of elongating cotton fibres (BASRA & MALIK 1983). This is evident from increased $H^{14}CO_3^-$ incorporation into organic acids compared with amino acids in the controls (Table 2). It is worth noting that ABA and particularly calcium drastically reduced incorporation into the organic acid fraction. Interestingly, EGTA treatment markedly stimulated the incorporation proving the inhibitory role of calcium in carbon fixation. Furthermore, chlorpromazine application also led to a slight enhancement

Table 2

Influence of ABA and calcium on $H^{14}CO_3^-$ incorporation into organic acids and amino acids during incubation *in vitro* of intact cotton fibres at 15 DAA

Treatment	$H^{14}CO_3^-$ incorporation (cpm/fibres of one seed)	
	Organic acids	Amino acids
Control	8126 (100)	490 (100)
ABA (10 μM)	3111 (38.3)	444 (90.6)
$CaCl_2$ (5 mM)	159 (1.9)	534 (109)
EGTA (2 mM)	12553 (154.5)	443 (90.4)
Chlorpromazine (10 μg/ml)	9330 (114.8)	606 (123.7)

Figures in parentheses are relative percentages.

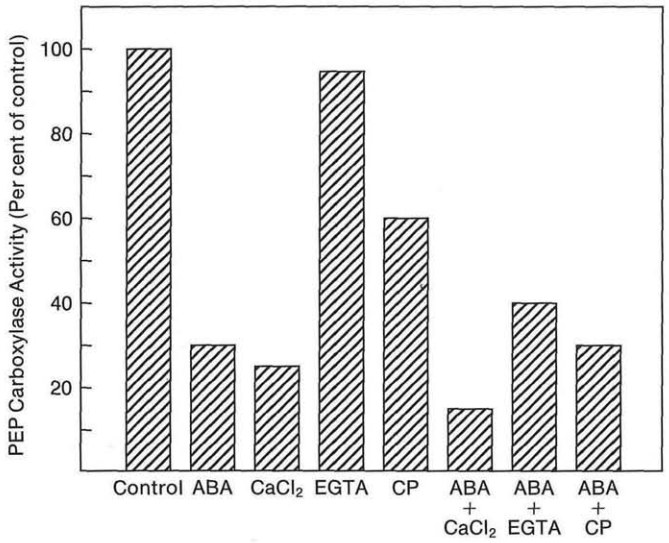

Fig. 1. Relative percentages of PEP carboxylase activity of cotton fibres at 15 DAA, as influenced by ABA, calcium, EGTA and chlorpromazine.

of incorporation into organic acids (Table 2). However, these effects of EGTA and chlorpromazine were not reflected in PEPC activities (Fig. 1).

As regards $H^{14}CO_3^-$ incorporation into amino acids, ABA reduced incorporation in this fraction as well but calcium tended to be slightly promotory (Table 2). Conversely, EGTA treatment caused diminished incorporation. However, application of chlorpromazine also promoted incorporation into amino acids which is unexpected if the calcium effect is mediated through calmodulin. The observed response may be due to a direct effect of calcium or its binding with an alternative receptor. Probably, the calcium-mediated increased incorporation into amino acids might occur at the expense of organic acid incorporation sparing carbon skeletons for amino acid synthesis.

Apparently, ABA as well as calcium could modulate the fibre capacity for dark fixation of CO_2 and thereby the rate of fibre elongation. The regulatory aspects need further investigation in this interesting biological system.

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