Effect of abscisic and gibberellic acids on malate synthase transcripts in germinating castor bean seeds

D. Rodriguez, J. Dommes and D.H. Northcote*

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW, UK (*author for correspondence); Present address: Laboratoire de Physiologie, Département de Botanique, -B22, Université de Liège, Sart Tilman, B-4000 Liège, Belgium

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Abstract

Several clones complementary to malate synthase mRNA have been identified in a complementary-DNA library to mRNA from castor bean endosperm. One of these clones has been used as a probe to measure levels of transcripts during seed germination and the effects of gibberellic acid and abscisic acid on these levels have been examined.

Malate synthase transcripts increased during germination and GA$_3$ advanced their appearance in the endosperm. Exogenously applied ABA inhibited the accumulation of transcripts over a time course of germination but the addition of GA$_3$ counteracted its inhibitory effects. The data confirmed previous reports which indicated that the action of both growth regulators was on transcript accumulation and that there is a coordinated induction of the enzymes involved in the lipid metabolism in oil seeds.

Abbreviations: ABA - abscisic acid; GA$_3$ - gibberellic acid; poly(A$^+$)RNA - polyadenylated RNA; SDS-PAGE - sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

Introduction

The mobilization of lipids during germination of fat-storing seeds such as castor bean, requires a specific set of enzymes which are generally not active at other stages of plant development. These are the enzymes of the glyoxylate cycle, located in the glyoxysome. Among them, two enzymes are unique to this process: isocitrate lyase (ICL) and malate synthase (MS) [1]. Both enzymes have been used as markers of germination [21, 25, 8, 9] and as indicators of glyoxysome formation in castor bean and related species [4, 5, 19].

Gibberellic acid, when applied exogenously to germinating castor bean seeds, advanced germination and increased the rate of lipid mobilization [21]. This stimulation corresponded to an increase in activities and amounts of some of the enzymes involved in the glyoxylate cycle, especially isocitrate lyase [21, 22]. This increase was associated with an acceleration in the appearance of isocitrate lyase transcripts [23]. Both the level of isocitrate lyase transcripts and its activity declined at a later stage in germination and this decline was advanced and accelerated when seeds were treated with gibberellic acid.

On the contrary, abscisic acid is a well-known inhibitor of germination [26] but its mode of action...
is not completely understood. It has been suggested that it acts at the level of translation [27] or transcription [30] or both [35, 7, 11].

Exogenous abscisic acid inhibited the appearance of germination-specific enzymes [15, 16]. In castor bean seeds it had an inhibitory effect on isocitrate lyase and malate synthase activities and Dommes and Northcote [8] have reported that the action of abscisic acid on isocitrate lyase synthesis is either to inhibit the transcription of mRNA or to increase the turnover of transcripts.

In order to investigate the action of the growth factors on gene regulation during germination it is necessary to compare their actions on the enzymes which are specifically induced during germination. We have already examined in some detail the action of the growth factors on isocitrate lyase and prepared the appropriate cDNA probes for this enzyme. The present study extends the work to malate synthase and opens up the possibility to investigate the genomic clones for the two enzymes so that a comparison of the regulation sequences is possible. The cDNA for malate synthase which we have now obtained will allow the sequence of the enzyme to be directly compared with that of isocitrate lyase.

cDNA probe

Poly(A +)RNA isolated from endosperms of 4–5-day-old seeds was used to prepare cDNA probes labelled with 32P. The single-stranded cDNA was synthesized from 6 μg of poly(A +)RNA in a reaction mix containing 100 μg ml⁻¹ oligo-dT₁₂₋₁₈, 50 mM Tris-HCl pH 8.3, 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM dNTP, 140 mM KCl, 4 mM Na₂pyrophosphate, reverse transcriptase 2.5 units ml⁻¹ and 0.74 MBq [α-32P]dATP (111 TBq mmol⁻¹). The mix was incubated at 43 °C for 30 min and EDTA was added to a final concentration of 20 mM to stop the reaction. The sample was purified by phenol-extraction and ethanol-precipitation.

Materials and methods

Germination of seeds

Castor bean (Ricinus communis L.) seeds were surface sterilized and germinated as described by Martin and Northcote [24]. Seeds were imbibed and grown in different media which were 1) H₂O (used as control), 2) 30 μM gibberellic acid (GA₃), 3) 30 μM abscisic acid (ABA) and 4) 30 μM GA₃ + 30 μM ABA.

All the media were autoclaved or sterilized by filtering through a Minisart NML filter, pore size 0.2 μm (Sartorius-Instrument Ltd., Surrey, UK). Glassware was baked at 180 °C for 12 h.

RNA extraction

Total RNA was extracted from castor bean endosperms germinated from 0 to 7 days as described by Martin and Northcote [24]. Its concentration was calculated by measuring the absorbance at 260 nm.

Polyaolenylated RNA (poly(A +)RNA was isolated from the total RNA by affinity chromatography on poly(U)-Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden), [6].