Hormonal regulation of gene expression in barley aleurone layers

Induction and suppression of specific genes

Randall C. Nolan* and Tuan-Hua David Ho**

Department of Biology and Plant Biology Program, Division of Biology and Biomedical Sciences, Washington University, St. Louis, MO 63130, USA

Abstract. As part of our investigation of the mode of action of plant hormones in barley (Hordeum vulgare L.) aleurone layers, we have studied the expression of five identified and three unidentified mRNA species in the presence of exogenous gibberellic acid (GA3) and abscisic acid. Three of the mRNAs are GA3-inducible, three are suppressed by GA3, and two are constitutive. The extent of the GA3 effect differs considerably for both inducible and suppressible mRNAs. For example, a tenfold higher concentration of GA3 (10^{-8} M) is required for full induction of the high-pI group α-amylase mRNA than is required for the low-pI α-amylase mRNA (10^{-9} M). Temporal regulation of mRNA abundance also varies between the two α-amylase isoenzyme groups. The three GA3-suppressible mRNA species studied, alcohol dehydrogenase (ADH1), a probable amylase and protease inhibitor, and an unidentified barley mRNA species also varied in response to GA3. The ADH1 mRNA decreased drastically within 8 h of GA3 treatment, whereas the other two began to decrease in abundance only after 12–16 h of GA3 treatment. Abscisic-acid treatment counteracted the GA3 effects for both the inducible and suppressible mRNA species. Comparison of α-amylase-mRNA levels and α-amylase-synthesis rates showed a strong correlation between the two parameters, the only exception being a lack of α-amylase synthesis in the presence of α-amylase mRNA at low GA3 concentrations. Therefore, the expression of α-amylase seems to be regulated primarily by its mRNA levels.

* Present address: Department of Biology, Indiana University, Bloomington, IN 47401, USA

** To whom correspondence should be addressed

Key words: Abscisic acid and gene expression – Aleurone – α-Amylase – Gene expression (barley aleurone) – Gibberellin and gene expression – Hordeum (gene expression).

Introduction

Barley aleurone layers have provided a model system for the study of the regulation of gene expression by plant hormones. Gibberellic acid (GA3), synthesized in the germinating barley embryo, diffuses to the aleurone layer cells, where it triggers the synthesis and secretion of several hydrolytic enzymes. These hydrolases then break down storage materials in the endosperm into smaller components, which are used by the seedling to maintain growth until photosynthesis commences. In isolated aleurone layers, GA3 has been shown to induce synthesis of α-amylase isoenzymes (EC 3.2.1.1), proteases, nuclease (EC 3.1.30.2), β-1,3; 1,4-glucanases (EC 3.2.1.73), and other hydrolases (Jacobsen and Higgins 1982; Callis and Ho 1983; Hammerton and Ho 1986; Brown and Ho 1986; Stuart et al. 1986). The mRNA for α-amylase, β-1,3; 1,4-glucanase, and a putative thiol protease, aleurain, have also been shown to be induced by GA3, (Rogers 1985; Muthukrishnan et al. 1983; Chandler et al. 1984; Stuart et al. 1986; Rogers et al. 1985). Jacobsen and Beach (1985) have demonstrated a tenfold stimulation of transcription of α-amylase genes by run-on transcription studies using nuclei from GA3-responsive aleurone protoplasts. Abscisic acid (ABA) blocks this stimulation of α-amylase transcription. In fact, ABA has been found to antagonize GA3 action in all of the events investigated thus far in barley aleurone layers (Ho 1983).
A major goal of our research is to determine the patterns of control that GA₃ and ABA exert on gene expression in barley aleurone layers. To this end, the effects of these hormones on the levels of eight different mRNAs have been determined. Northern blot experiments were carried out in which RNA samples were subjected to electrophoresis and then hybridized with specific cDNAs (copy DNAs) for the different mRNA species (Maniatis et al. 1982; Church and Gilbert 1984). Three of these mRNA species, encoding alcohol dehydrogenase (ADH₁, EC1.1.1.1), a probable α-amylase/protease inhibitor (PAPI) and an unidentified mRNA have been found to be GA₃-suppressible, whereas three mRNAs (high-pI α-amylase, low-pI α-amylase, and aleurain) are GA₃-inducible, as has previously been reported (Rogers 1985; Rogers et al. 1985). Two other transcripts were found to be essentially constitutive in expression. A complex pattern of regulation is evident for both the suppressible and inducible mRNAs, as shown by time course, GA₃ dosage, and GA₃ versus ABA experiments. Comparison of α-amylase-isoenzyme synthesis and the mRNA levels for the two groups of isoenzymes shows, with one possible exception, a strong correlation between the two parameters. This is further indication that α-amylase expression is regulated primarily at the mRNA level.

Material and methods

Tissue preparation. Aleurone layers were prepared from barley (Hordeum vulgare L. cv. Himalaya) caryopses as described by Nolan et al. (1987). Seeds were from the 1981 harvest at Washington State University, Pullman, USA. Ten aleurone layers were incubated in 2 ml of buffer (20 mM Na-succinate pH 5.0, 20 mM CaCl₂, 10 μg/ml chloramphenicol) for protein-synthesis studies and 75 aleurone layers were incubated in 15 ml of buffer for RNA extraction.

Protein analysis. For analysis of protein synthesis, aleurone layers were labelled with 9.4 μCi/ml of a mixture of [³⁵S]methionine and [³⁵S]cysteine (Trans 35S-label from ICN, Irvine, Cal., USA) for the last hour of incubation. Prior to labelling they were rinsed twice with incubation buffer. The layers were then ground with acid-washed sea sand and 100 μl of 10 μM leupeptin in a chilled mortar. Non-denaturing gel sample buffer (30% glycerol, 10 mM 2-amino-2-(hydroxy-methyl)-1,3-propanediol (Tris)-HCl, pH 6.8, 0.01% Bromophenol blue) was added to the extracts and trichloroacetic-acid (TCA)-precipitable counts were determined as described by Mans and Novelli (1960). Synthesis of α-amylase isoenzymes was analyzed by non-denaturing polyacrylamide gel electrophoresis, followed by fluorography, as described in Nolan et al. (1987). Aliquots containing equal TCA-precipitable counts were added to each gel lane. Intensity of α-amylase bands on fluorograms therefore indicates their percentage of total protein-synthesis activity (differences between lanes do not necessarily reflect differences in the absolute rates of α-amylase synthesis).

Analysis of RNA. Total RNA was isolated as described by Belanger et al. (1986). Northern analysis was performed as described in Nolan et al. (1987). Briefly, 10 μg of total RNA was subjected to electrophoresis in formaldehyde-agarose gels as described in Maniatis et al. (1982) and blotted onto “Gene Screen” membranes (New England Biolabs, Boston, Mass., USA), using 10 × SSC (3 M NaCl, 0.3 M Na-citrate). The membranes were baked at 80°C for 2 h and hybridized to nick-translated cDNAs as described by Church and Gilbert (1984). Relative levels of specific mRNAs were then shown by autoradiography of the filter hybridizations as described by Nolan et al. (1987). Barley cDNAs for high-pI α-amylase (pM/C), low-pI α-amylase (clone E), a putative thiol protease termed aleurain (G71), and a probable amylase/protease inhibitor (A1F) were obtained from Dr. J. Rogers, Washington University Medical School (Rogers 1985; Rogers and Millman 1984; Rogers et al. 1985; Munsey and Rogers 1986). Adh-1 cDNA from maize (pZmL793) was obtained from Dr. M. Sachs, Department of Biology, Washington University (Dennis et al. 1984). A ribosomal-RNA cDNA probe from soybean (pGmr-1) was obtained from Dr. L. Zimmer, Department of Biochemistry, Louisiana State University, Baton Rouge, USA. Three other cDNAs were obtained from a pUC 18cDNA library from 4-h ABA-treated barley aleurone layers and had previously been found to be unaffected by ABA treatment (B. Hong and S. Ukanes, Washington University, personal communication). They have been termed pHUV-1, pHUV-2, and pHUV-3.

Results

Time course of GA₃-mediated gene expression. Gibberellic acid has varying effects on the expression of different genes in barley aleurone layers as shown in Fig. 1. Barley α-amylase consists of two groups of isoenzymes, with almost identical molecular weight (44 kilodaltons, kDa) but different isoelectric points (Jacobsen and Higgins 1982; Callis and Ho 1983). Messenger RNA for a high-pI α-amylase group (pM/C probe) was virtually undetectable in the absence of GA₃, became detectable by 4 h of GA₃ treatment, and reached a maximum level by 12–16 h of GA₃ treatment. As shown previously (Nolan et al. 1987), high-pI α-amylase mRNA started to decline after around 24 h of GA₃ treatment and was greatly reduced by 40 h of GA₃ treatment (Fig. 1). Low-pI α-amylase has been shown to be constitutively expressed at a low level (Rogers 1985). We found that low-pI α-amylase mRNA (clone E probe) was evident in the 1-h time-point samples both in the presence and absence of GA₃ (Fig. 1). Levels of low-pI α-amylase mRNA decreased with time in the absence of added GA₃, but increased greatly in abundance by 8 h of GA₃ treatment. After 24 h of GA₃ treatment there was no appreciable decline in the levels of low-pI α-amylase transcripts with time as there was for high-pI α-amylase mRNA. High-pI and low-pI α-amylase mRNA therefore differed both in degree of induction by GA₃ and in their expression over