Short Communication

Auxin-induced Changes in the Incorporation of \(^3\)H-Amino Acids into Soybean Ribosomal Proteins

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ABSTRACT

Auxin-induced activation of 80S ribosomes and polyribosome formation in mature soybean (Glycine max var. Hawkeye) hypocotyl (R. L. Travis, J. M. Anderson, and J. L. Key. 1973. Plant Physiol. 52: 608-612) in the presence of a mixture of radioactive amino acids correlates with an increased specific radioactivity of at least three ribosomal proteins; the labeling of one of these increased severalfold above the control level. Results of experiments with 5-fluorouracil and cycloheximide indicated that the proteins in question were synthesized in response to auxin and became associated with pre-existing ribosomes. Ribosomal dissociation experiments indicated that these proteins were associated with the 60S ribosome subunit.

We have reported that auxin enhances the rate of amino acid incorporation by ribosomes isolated from excised mature soybean hypocotyl (10). The enhanced level of protein synthesis is associated with an increase in the relative level of polyribosomes from about 30% up to about 65% of the total ribosome population. Evidence indicated that the increase in polyribosome level in response to auxin was preceded by, and dependent upon, the “activation” of 80S monoribosomes. Changes in the complement of ribosomal protein may be the mechanism by which ribosomes are activated by auxin. We report here studies on the differential accumulation of newly synthesized proteins by ribosomes from control and auxin-treated tissue.

MATERIALS AND METHODS

Plant Material. Soybean seeds (Glycine max var. Hawkeye) were germinated in darkness for 96 hr as previously described (1). All treatments consisted of duplicate 125-ml flasks containing 200 sections of basal hypocotyl (10 mm) excised at least 20 mm below the cotyledons.

Amino Acid Incorporation Studies. For \(^3\)H-amino acid incorporation, 1-cm hypocotyl segments (10 g) were incubated 5 hr at 30 C in 20 ml of medium containing 2% sucrose, 50 \(\mu\)g ml\(^{-1}\) chloramphenicol (to inhibit bacterial growth) and 0.1 mCi of each of the following amino acids: \(^3\)H-L-leucine (6 Ci/mmol\(^{-1}\)), \(^3\)H-L-lysine (60 Ci/mmol\(^{-1}\)), \(^3\)H-L-valine (7 Ci/mmol\(^{-1}\)), and \(^3\)H-L-tyrosine (7 Ci/mmol\(^{-1}\)). All experiments were repeated using \(^1\)C-amino acids with similar results. Auxin (2,4-D) and 5-fluorouracil were used at 7.5 \(\times\) \(10^{-5}\) M and 5 \(\times\) \(10^{-3}\) M, respectively.

Preparation of Ribosomes and Ribosomal Subunits. Free, or run-off, 80S monoribosomes were induced in hypocotyl sections by \(N_2\) gas dissociation of polyribosomes (6). Ribosomes were prepared as previously described (10), except that the discontinuous gradient step was omitted. Ribosomal subunits were prepared by KCl dissociation of monomeric ribosomes (11). Approximately 20 \(A_{260}\) units of monoribosomes were layered on 15 to 31% linear sucrose gradients (35 ml) containing 0.5 M KCl. Centrifugation was for 15 hr at 81,500g (Spinco SW 27 rotor). All steps were carried out at 0 to 4 C. The distribution of ribosomal subunits in the sucrose gradient was monitored with a continuous recording ISCO Model D density gradient fractionating system. Fractions containing 40S and 60S subunits were recovered and centrifuged at 221,000g for 4 hr (Spinco type 65 rotor) to pellet subunits.

Extraction and Polyacrylamide Gel Electrophoresis of Ribosomal Protein. Ribosomal protein was extracted from approximately 20 \(A_{260}\) units of resuspended ribosomes or ribosomal subunits (0.5 ml) as previously described (7). Polyacrylamide gel electrophoresis of ribosomal proteins was done essentially as described by Panyim and Chalkley (8) and according to Lin et al. (7).

Determination of Radioactivity Associated with Ribosomal Proteins. Gels were frozen on powdered dry ice, sliced into 1-mm sections and digested for 2 hr at 80 C in 0.2 ml of a 1:1 mixture of 0.1 N HCl and 30% H\(_2\)O\(_2\). Radioactivity was determined in a Packard liquid scintillation spectrometer.

RESULTS AND DISCUSSION

The apparent “activation” of 80S ribosomes and the conversion of pre-existing monoribosomes to polyribosomes following auxin treatment of mature soybean hypocotyl (10) were suggestive of possible hormone-induced changes in ribosome-associated proteins. To investigate this possibility, tissue slices were incubated in \(^3\)H-amino acids in the absence and presence of auxin followed by ribosome isolation and fractionation of ribosome-associated proteins by one-dimensional gel electrophoresis. About one-third of the 80 to 85 ribosomal proteins (2) were resolved in this study.

Results of \(^3\)H-amino acid incorporation experiments indicate that the association with 80S ribosomes of at least three newly synthesized ribosomal proteins was increased in auxin-treated hypocotyl segments relative to the control tissue (Fig. 1). The bands representing these proteins have been designated P\(_1\), P\(_2\), and P\(_10\). The most significant increase in amino acid incorporation occurred in P\(_1\), where the specific radioactivity, reflecting only a change in radioactivity and not actual amount of the protein, from auxin-treated tissue was nearly 3-fold greater than...
Fig. 1. Polyacrylamide gel electrophoresis of ribosomal proteins from control (upper) and auxin-treated basal hypocotyl. Incubation of tissue, preparation of ribosomes, and extraction of ribosomal protein are described under “Materials and Methods.” Gels were electrophoresed at 1 mamp per tube for 4.75 hr, stained in 7% acetic acid containing 0.1% amido black, destained in 7% acetic acid, and scanned at 600 nm.

that of control tissue. While incorporation into P₁ and P₁₀ was greater in the auxin-treated tissue relative to control tissue, the results were less striking than those obtained with P₁. Although the most significant changes occurred in protein bands P₁, P₁₀, and P₁₀₀, all proteins were labeled in both auxin-treated and control tissue during the incubation period.

The above results indicate that the population of ribosomal proteins was altered during incubation in auxin. This may be a reflection of proteins associated with newly synthesized ribosomes and/or an alteration of the protein complement of pre-existing ribosomes. The ³H-amino acid incorporation experiment was repeated in the presence of 5-fluorouracil in order to determine whether incorporation represented protein synthesis associated with new ribosome synthesis or turnover synthesis of protein associated with pre-existing ribosomes. Fluorouracil selectively inhibits the synthesis of ribosomal RNA (4), and thus new ribosome formation. The pattern of radioactive amino acid incorporation was not significantly affected by 5-fluorouracil (Fig. 2) although under these conditions the effect of auxin on P₁ was magnified relative to other protein bands. Thus, auxin apparently influenced the protein complement of pre-existing ribosomes. In the presence of cycloheximide, incorporation of labeled amino acids into ribosomal protein in both control and auxin-treated tissue was prevented (Fig. 2), indicating that ³H-amino acid incorporation represented actual protein synthesis.

Ribosomal proteins have been studied extensively in bacterial and mammalian systems. Each system has been characterized relative to the total number of proteins associated with each ribosomal subunit (3, 9). In addition, numerous reports exist which identify several bacterial proteins relative to their function in regulating ribosome activity. Much less is known about plant ribosomal proteins. Jones et al. (2) have identified 80 to 85 individual 80S cytoplasmic ribosomal proteins in wheat. No attempt was made to fractionate the ribosomes into subunits to facilitate further classification of individual proteins. A previous report from this laboratory noted that free or run-off 80S ribosomes could be dissociated into biologically active subunits by treatment with KCl in the presence of Mg²⁺ (6). It seemed of interest to further identify the specific proteins affected by auxin relative to their location within the ribosome structure. Monoribosomes were dissociated into subunits on sucrose gradients containing 0.5 M KCl as previously described (6). Gradient fractions representing 40S and 60S subunits were pooled and ribosomal proteins were extracted and resolved by one-dimensional gel electrophoresis (Fig. 3). The results indicate that the protein bands which showed enhanced ³H-amino acid labeling during incubation in auxin were associated with the 60S subunit.

The results presented in this communication suggest that the control of ribosome activity through changes in specific ribosomal proteins may be a mechanism by which protein synthesis is regulated during auxin-induced growth transitions. The mechanism by which the proteins identified in this report might regulate subunit activity is not yet understood. It is impossible to conclude from the current evidence that these proteins do in fact regulate subunit activity. Rather, a correlation is demonstrated between the auxin-induced change in the level of at least one and possibly three ribosomal proteins and enhanced ribosome activity. A very rapid effect of androgens on the initiation of protein synthesis in rat prostate has been noted (5). These and other similar observations are suggestive of the involvement of hormones in the activation of ribosome activity.

Fig. 2. Effect of 5-fluorouracil (FU) and cycloheximide (CHI) on auxin-enhanced incorporation of ³H-amino acids into ribosomal protein.

Fig. 3. Polyacrylamide gel electrophoresis of ribosomal subunit protein. Subunit preparation is described under “Materials and Methods.”