Water Stress and Protein Synthesis

I. DIFFERENTIAL INHIBITION OF PROTEIN SYNTHESIS

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ABSTRACT

Water stress causes both a qualitative change in the types of proteins produced by Avena coleoptile cells as demonstrated by a double-labeling ratio technique, and a quantitative reduction in the rate of incorporation of leucine into proteins. The osmotica mannitol and Carbowax-4000 cause similar changes in the pattern of protein synthesis showing that these effects are due to water stress rather than to a particular osmoticum.

Plants undergo frequent periods when the availability of water is limited. Under conditions of water stress, growth of plants is inhibited (2, 6), and a variety of morphological and biochemical modifications are produced (8). For example, water stress is reported to inhibit the incorporation of amino acids into proteins (1, 14) and to cause a decrease in the protein content of the tissues (15, 16). There has been little indication as to whether water stress causes a general decrease in the rate of synthesis of all proteins, or whether it has a specific effect on the synthesis of only certain proteins. The decrease in polysome levels noted by Hsiao (7) in water-stressed tissues could be interpreted either way.

The following experiments were carried out in order to distinguish qualitative from purely quantitative effects of water stress on protein synthesis. The use of a double labeling ratio technique allowed us to detect small changes in the pattern of protein synthesis (4, 11, 13). It will be shown that water stress caused by either mannitol or Carbowax-4000 causes a major alteration in the pattern of protein synthesis in Avena coleoptile.

MATERIALS AND METHODS

The plant material consisted of 1-cm sections cut from 2.5- to 3.2-cm defoliated coleoptile of Avena sativa, cv. Victory. Seedlings were grown, and sections were prepared by methods already described (3). Sections were preincubated for 1.5 hr before the start of any treatment. All solutions contained 2.5 mM potassium maleate buffer, pH 4.7, with addition of osmoticum of 0.3 m mannitol (Difco) or 21% (w/w) CBW (Mann) when stress was desired.

For measurement of the quantitative effects of water stress on protein synthesis, groups of 10 sections were incubated for 3 hr in buffer + osmoticum and with leucine-4,5-3H (5 or 29.8 Ci/m mole). The sections were then washed briefly in buffer containing 0.2 mg/ml leucine and killed by boiling for 5 min in 10 ml of 80% ethanol + 0.2 mg/ml leucine. This ethanol extract was saved and used to compare soluble radioactivity. The sections were then treated for 5 min with 10 ml of each of the following: boiling 80% ethanol + 0.2 mg/ml leucine, hot 5% trichloroacetic acid, 90% ethanol, 100% ethanol, 100% ethanol + anhydrous ether (3:1), and anhydrous ether. After drying, the sections were extracted overnight in 2 ml of 1 N NaOH at 37 C. Aliquots of the extracts were used to determine the protein content by the method of Lowry et al. (12) and to count radioactivity by liquid scintillation using Permafluor (Packard Instrument Co.) as scintillator.

The double labeling ratio technique coupled with gel electrophoresis (4, 11, 13) was used to study the quantitative effects of water stress on protein synthesis patterns. Groups of 50 sections were incubated for 3 hr in 3 ml of buffer ± osmoticum and with 180 µCi leucine-4,5-3H (53 Ci/m mole) or 150 µCi leucine-UL-14C (180 mCi/mmole). The sections were then washed and homogenized with a ground glass homogenizer in 1 ml of 0.1 M tris buffer, pH 8, containing 0.5 M sucrose, 5 mM cysteine and dithiothreitol, and 6 mM ascorbic acid. The homogenate was centrifuged at 40,000g for 1 hr and the supernatant was used as the source of soluble proteins.

Equal volumes of 14C- and H-labeled proteins were mixed, and 75-µl aliquots were placed on acrylamide gels consisting of a 7.5% separation gel (7.5 cm) and a 2.4% spacer gel (2 cm). The separation gel buffer was 0.37 M tris, pH 8.3, and the spacer gel buffer was 0.18 M tris, pH 6.9. Ammonium persulfate and N,N,N',N'-tetramethylethlenediamine were added as catalyst and initiator, respectively. The electrophoresis was run at 0 to 4 C, using 5 mM tris-glycine, pH 8.3, as the electrode buffer. The current was 2 mamp/gel for the first 20 min and 3.5 mamp/gel for the next 150 min.

At the termination of electrophoresis, gels were soaked in 12.5% trichloroacetic acid for 1 hr and then stained overnight in a solution of 0.125% Coomassie brilliant blue and 0.25% Analine blue black in 7.5% acetic acid. The excess stain was removed by washing the gels in 7.5% acetic acid-25% methanol for 30 min and destained electrophoretically for 20 min. The gels were scanned for OD at 560 nm and were then cut into 1-mm slices. Each slice was incubated in a scintillation vial with 0.5 ml of NCS (Amersham/Searle) for 2 hr at 45 to 48 C. 10 ml Permafluor were then added, and 14C and 3H were determined for each vial. The ratio of cpm 14C to 3H was calculated for each slice and was plotted against slice number starting from the top of the gel.

Two types of protein mixtures were used in each experiment. The first (control mix) consisted of proteins labeled with 14C-
and H-leucine under identical conditions (either no osmoticum or osmoticum in both). The second mixture (stress mix) consisted of proteins labeled with 14C-leucine under control conditions (i.e. no osmoticum) and proteins labeled with 3H-leucine under water stress conditions (i.e. + osmoticum). When the 14C/3H ratio for the gels was calculated, the ratio was expected to be nearly identical for all slices of the control mix gels. Similar ratios throughout the gels were obtained for the stress mix gels if the treatment had no qualitative effect on the pattern of protein synthesis; if a change in the pattern of protein synthesis occurred, considerable variation in the ratios were encountered, and the resulting curve had peaks and valleys instead of being level.

RESULTS

Quantitative Effect of Water Stress on Protein Synthesis. The quantitative effect of water stress on incorporation of leucine into proteins is shown in Figure 1. Mannitol concentrations in excess of 0.05 M cause an inhibition in the rate of leucine incorporation with a maximum inhibition (about 80%) occurring at 0.3 M. The rate of incorporation was nearly constant with time, with or without mannitol (Fig. 2). The differences in incorporation were not due to differences in rate of uptake of the label (5). These data agree with those obtained earlier for coleoptiles with proline as the amino acid (2). Water stress clearly causes a significant reduction in the over-all rate of protein synthesis in this tissue.

Qualitative Effect of Water Stress on Protein Synthesis. Qualitative effect of water stress on leucine incorporation into proteins is shown by the results of the double labeling ratio experiments. When the proteins from control sections (incubated without mannitol) were separated on polyacrylamide gels, about 40 bands could be detected which varied in labeling and intensity of staining (Fig. 3). But when the proteins from control mixture were separated, and the 14C/3H ratio was determined for each gel slice, nearly identical ratios were obtained (Fig. 4). This is expected, since the proteins in the presence of either of the labels were being made under identical conditions, i.e. without mannitol in both cases. When the proteins of the stress mixture are separated, a similar curve with nearly constant ratios will be obtained if water stress causes an equal inhibition of the synthesis of all proteins, but variation in 14C/3H ratio will result if the effect of water stress on the rate of protein synthesis varies with the proteins. The experimental curve (Fig. 4) shows considerable variation in the 14C/3H ratio along the whole length of the gel. The average ratio was higher in the experimental curve, because stress inhibits the incorporation of 3H-leucine and thereby increases the ratio. Three separate gels on which the aliquots from the same protein mixture were placed, gave identical double label ratios. The experiments were repeated twice, and characteristic results were reproduced. It should, however, be pointed out that these ratios do not necessarily pertain to single protein species but to groups of proteins.

Mannitol and Carbowax-4000 Cause Similar Changes in Pattern of Protein Synthesis. We have considered the possibility that the effects on protein synthesis noted when mannitol was used as the osmoticum might be a specific response to the osmoticum rather than to the resulting water stress. To check this, the experiments were repeated using CBW as the osmoticum. Water stress produced by CBW inhibits protein synthesis as effectively as water stress produced by mannitol (data not

Fig. 1. Inhibition of leucine incorporation by water stress induced by mannitol. Groups of 10 sections were pretreated for 1.5 hr with 0 to 0.5 M mannitol in 2 ml of medium, then 3 μCi of 3H-leucine added. After 3 hr, the sections were harvested as described in text, and incorporation of leucine into proteins was determined.

Fig. 2. Linearity of leucine incorporation with time in stressed and turgid coleoptiles. Sections pretreated 1.5 hr in medium ± 0.3 M mannitol, then 50 μCi 3H-leucine added. Groups of 10 sections removed after 0.5 to 8 hr and leucine incorporation into proteins determined.

Fig. 3. Separation of soluble proteins on polyacrylamide gels and distribution of radioactivity among them. Fifty coleoptile sections were incubated for 3 hr in 3 ml of medium containing 75 μCi of (UL-14C)-leucine (180 mCi/mmol). Sections were then washed and homogenized in 1 ml of 0.1 M tris buffer, pH 8, containing 0.5 M sucrose, 5 mM dithiothreitol, 5 mM cysteine, and 6 mM ascorbic acid. The 40,000g supernatant of this homogenate was used as a source of soluble proteins. A 75-μl aliquot was subjected to electrophoresis on 7.5% polyacrylamide gel. The gel was stained with a mixture of Coomassie brilliant blue and Amido black, scanned at 560 nm and cut into 1 mm slices. Each slice was solubilized and its radioactivity determined.
coleoptile proteins. Inhibitions of amino acid incorporation have been noted before in water-stressed material (1, 10, 14), but as these experiments were not conducted under conditions of steady state water stress, it was not certain that the inhibitions were actually due to the water stress. In the study reported by Ben-Zioni et al. (1), the amino acid incorporation was studied in leaf discs sampled 2 hr after the plants had been removed from conditions of water stress and the leaves had regained turgidity. Furthermore, the incorporation was carried out under nonstress conditions. It seems reasonable to suspect that the stress-induced effects might have been modified during the 2 hr before the leaf discs were sampled as well as during the incorporation.

The double labeling ratio technique has been used here to assess the qualitative effect of water stress on protein synthesis. The technique was used because of its sensitivity, but its limitations need to be mentioned. When 14C control/3H-treated mixtures are used, peaks in the 14C/3H ratio curve indicate that the synthesis of one or more of the proteins in that slice is inhibited in treated tissues. A particular slice may contain many proteins, and there is no way to determine by this technique whether the synthesis of all proteins in the slice is equally affected by water stress. The synthesis of a minor protein might be stimulated by water stress, but this could be masked by an inhibition of synthesis of a major protein. A second problem is that one cannot determine whether the inhibition in the synthesis of any particular protein is complete or only partial. In order to answer such questions, the identity of individual proteins will have to be determined, the proteins will have to be purified, and the incorporation of amino acids into the specific proteins will have to be determined.

Despite these limitations, the present results clearly indicate that water stress causes a differential inhibition of the synthesis of Avena coleoptile proteins, with the synthesis of some proteins being affected to a greater extent than the synthesis of others. The fact that water stress has a differential effect on enzyme levels has been demonstrated for several tissues (9, 17–19). Hufnaker et al. (9) showed that water stress caused a greater decrease in the level of nitrate reductase than of ribulose-1,5-diphosphate carboxylase in barley leaves. But in these cases it could not be determined whether the changes in enzyme levels were due to differences in synthesis or degradation of the enzymes. The changes reported did not occur under steady state condition of water stress. In the present experiments the differences are clearly due to a differential effect on synthesis.

When plant cells exist under water stress, it must be recognized that their biochemical activity will be different from that of turgid cells. For example, the biochemical capacity of protoplasts will be different from the cells from which the protoplasts were derived, since protoplasts must be maintained under conditions of water stress to prevent osmotic rupture. At this point it would be interesting to know the identity of those enzymes whose activities are most severely inhibited by water stress. These tests will be made to identify some of the enzymes present under the most prominent peaks in the double labeling ratio curves (e.g. slice 8 in Fig. 4).

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LITERATURE CITED


