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Water Stress and Protein Synthesis

II. INTERACTION BETWEEN WATER STRESS, HYDROSTATIC PRESSURE, AND ABSCISIC ACID ON THE PATTERN OF PROTEIN SYNTHESIS IN *AVENA* COLEOPTILES[1]

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ABSTRACT

Water stress causes a reduction in hydrostatic pressure and can cause an increase in abscisic acid in plant tissues. To assess the possible role of abscisic acid and hydrostatic pressure in water stress effects, we have compared the effects of water stress, abscisic acid, and an imposed hydrostatic pressure on the rate and pattern of protein synthesis in *Avena* coleoptiles. Water stress reduces the rate and changes the pattern of protein synthesis as judged by a double labeling ratio technique. Abscisic acid reduces the rate but does not alter the pattern of protein synthesis. Gibberellic acid reverses the abscisic acid-induced but not the stress-induced inhibition of protein synthesis. The effect of hydrostatic pressure depends on the gas used. With a 19 : 1 N_2-air mixture, the rate of protein synthesis is increased in stressed but not in turgid tissues. An imposed hydrostatic pressure alters the pattern of synthesis in stressed tissues, but does not restore the pattern to that found in turgid tissues. Because of the differences in response, we conclude that water stress does not affect protein synthesis via abscisic acid or reduced hydrostatic pressure.

Protein synthesis is one of the biochemical processes that are affected by water stress in plants (12, 19). Tissues that have been subjected to water stress generally show a reduction in protein synthesis as measured by amino acid incorporation (3, 13, 18). We have recently shown that a steady state water stress causes a qualitative change in the pattern of protein synthesis as monitored by double labeling ratio technique coupled with disc electrophoresis (7). Little is known as to how water stress exerts its effects. Water stress might operate directly or indirectly. It has been suggested (12) that it may act directly by mechanisms involving a reduction in chemical potential of water, or through a reduction in hydrostatic pressure in the cells, or through an increase in the concentration of cell solutes. Alternatively, water stress could act indirectly, its effects being mediated by some chemical compound(s) that becomes increasingly available during water stress and that then brings about inhibition in protein synthesis. One possibility is abscisic acid, which accumulates rapidly in plant tissues subjected to water stress (16, 20, 22) and which inhibits protein synthesis.

In order to assess the possible role of ABA and reduced hydrostatic pressure in the stress-induced changes in protein synthesis, we have compared the effects of water stress, ABA, and hydrostatic pressure on the rate and pattern of protein synthesis in *Avena* coleoptiles. We will show here that each agent exerts its own distinct effects on protein synthesis.

MATERIALS AND METHODS

The plant material consisted of 1-cm sections cut from 2.5- to 3.2-cm defoliated coleoptiles of *Avena sativa*, cv. Victory. Seedlings were grown and sections were prepared by methods already described (6). 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 as osmoticum of 0.3 M mannitol (Difco) or 21% (w/w) Carbowax-4000 (Mann) when stress was desired.

The incorporation of ^{3}H-leucine into total proteins was used to measure the quantitative effects of water stress on protein synthesis. The double labeling ratio technique coupled with gel electrophoresis was used to study the qualitative effects of water stress on protein synthesis patterns. Details of both of these techniques are presented in an accompanying paper (7). It should be remembered that in the double labeling ratio technique, two types of protein mixtures are used in each experiment. The first (control mix) consisted of proteins labeled with ^{14}C- and ^{3}H-leucine under identical conditions (either no osmoticum or osmoticum in both). The second mixture (treat mix) consisted of proteins labeled with ^{14}C-leucine under control conditions (*i.e.* no osmoticum) and proteins labeled with ^{3}H-leucine under different conditions (*e.g.* + osmoticum). When the ^{14}C/^{3}H 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 will be obtained for the treat mix gels if the treatment has had no qualitative effect on the pattern of protein synthesis; if a change in the pattern of protein synthesis has occurred, considerable variation in the ratios will be encountered and the resulting curve will have peaks and valleys instead of being level.

For labeling of proteins under hydrostatic pressures, the incubation container was placed in a Scholander pressure bomb (portable model, PMS Instrument Co., Corvallis, Ore.) and a hydrostatic pressure of 118 p.s.i. (8.2 atm) was imposed using N_2, air or a 19:1 mixture of N_2-air. After the 1.5-hr preincubation, the pressure was released, the radioactive leucine was added to the solution and the pressure was quickly reimposed. At the end of the incubation period the pressure was again released, and the sections were quickly washed, killed, and treated as described previously (7).

RESULTS

Comparison of ABA and Water Stress-induced Inhibitions of Protein Synthesis. Abscisic acid inhibits the incorporation of

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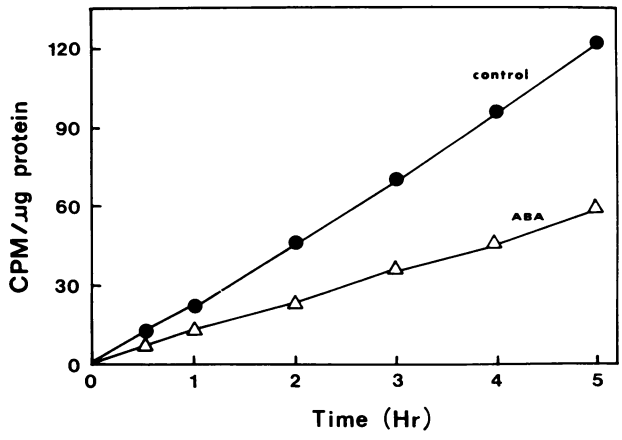

FIG. 1. Linearity of incorporation of ^{3}H-leucine in control and 100 µg/ml ABA-treated sections. Incorporation was started after a preincubation of 1.5 hr. In each case 5 ml of medium contained 60 sections and 20 µCi of ^{3}H-leucine. Each point is an average of two replicates.

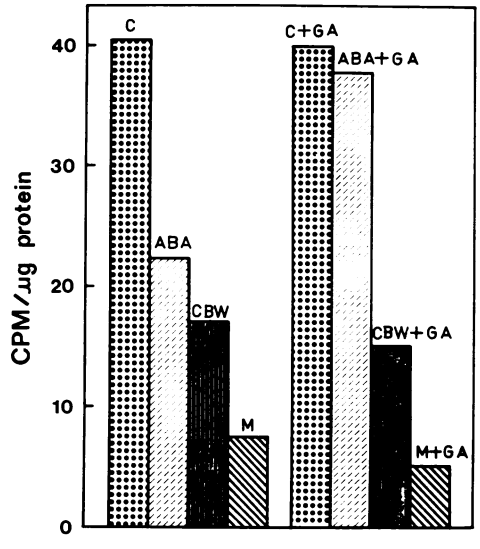

FIG. 2. Difference between ABA and water-stress inhibitions of protein synthesis in reversibility by GA. Groups of 10 sections incubated 1.5 hr. with 100 µg/ml ABA, 21% CBW, 0.3 M mannitol (M) or in control (C) alone, ± 20 µg/ml GA, then 1 µCi of ^{14}C-leucine was added and incubation was continued 3 hr. Incorporation of leucine into proteins was then determined.

leucine into *Avena* coleoptile proteins with a 50% inhibition occurring at about 100 µg/ml (data not shown). This is a steady state inhibition of protein synthesis (Fig. 1). The ABA-induced inhibition is distinct from that produced by water stress in two important ways. First, 20 µg/ml GA almost completely reverses the inhibitory effect of 100 µg/ml ABA on protein synthesis, but has no effect on the inhibitions induced by either CBW[3] or mannitol (Fig. 2). Second, ABA has, at best, only a slight effect on the pattern of protein synthesis as judged by the double labeling ratio technique (Fig. 3), in contrast to the major changes in pattern induced by water stress. These differences indicate that ABA and water stress must act independently in inhibiting protein synthesis and make it unlikely that the effects of water stress are due to any stress-induced ABA.

[3] Abbreviation: CBW: Carbowax-4000.

Effect of Hydrostatic Pressure on Rate and Pattern of Protein Synthesis in Water-stressed Tissues. Addition of 0.3 M mannitol or 21% CBW to *Avena* coleoptiles causes a reduction in their hydrostatic pressure of about 7.3 atm. The possibility that this reduction in hydrostatic pressure is the actual cause of the change in pattern of protein synthesis observed in water-stressed tissues has been checked by subjecting turgid and water-stressed tissues to an imposed hydrostatic pressure of 8.2 atm. This imposed pressure should restore both the rate and pattern of protein synthesis if the reduced pressure is actually the inhibiting agent.

The response to an imposed hydrostatic pressure depends on the type of gas used in the pressure chamber (Table I). With a 19:1 N_2-air mixture the imposed pressure stimulates protein synthesis in water-stressed but not turgid tissues. The rate of protein synthesis is not restored by the pressure to that of

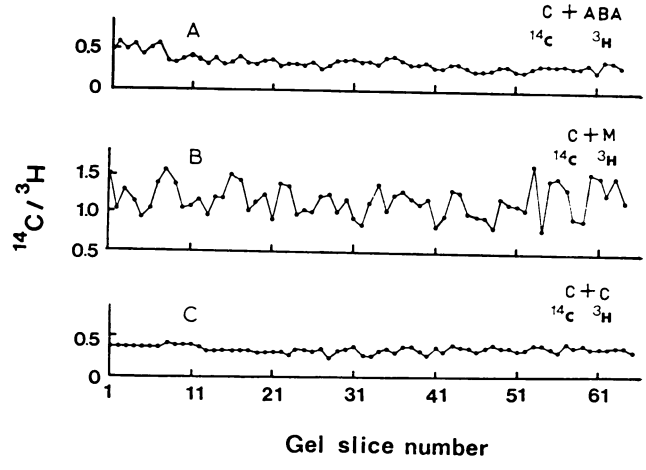

FIG. 3. Demonstration that ABA does not significantly alter the pattern of protein synthesis in *Avena* coleoptiles. Groups of 50 sections were preincubated for 1.5 hr. in 3 ml of medium with or without 100 µg/ml ABA or 0.3 mannitol. Then 150 µCi of ^{14}C-leucine or 180 µCi of ^{3}H-leucine added, sections incubated 3 hr and soluble proteins isolated. Aliquots of soluble proteins of ^{14}C-labeled control + ^{3}H-labeled ABA-treated tissues were mixed (upper curve, A), or ^{14}C-labeled control + ^{3}H-labeled mannitol-treated tissues were mixed (middle, B), or ^{14}C- and ^{3}H-treated control tissues were mixed (bottom, C) and proteins were separated by gel electrophoresis. The gels were cut into 65 slices numbered from the origin, ^{14}C and ^{3}H content of each was determined, and ^{14}C/^{3}H ratios were calculated.

Table I. *Effect of Imposed Hydrostatic Pressure on Rate of Leucine Incorporation into Avena Coleoptile Proteins*

Sections were pretreated 1.5 hr in medium with or without 0.3 M mannitol or 21% CBW. Pressure (118 p.s.i.) was applied to one group, no pressure to second. Leucine (7.5 µCi ^{3}H in A, 3 µCi ^{3}H in B, 1 µCi ^{14}C in C) was added, and incubation continued for 3 hr. Incorporation into proteins was then determined. 100% control values, in cpm/µg protein: 480 in A, 34 in B, 116 in C.

Pressure	Incorporation Rate		
	Control	+ Mannitol	+ CBW
	% control		
A. None	100	25	46
118 p.s.i. N_2-air (19:1)	103	39	85
B. None	100	20	37
118 p.s.i. N_2	122	25	61
C. None	100	33	53
118 p.s.i. air	86	27	41

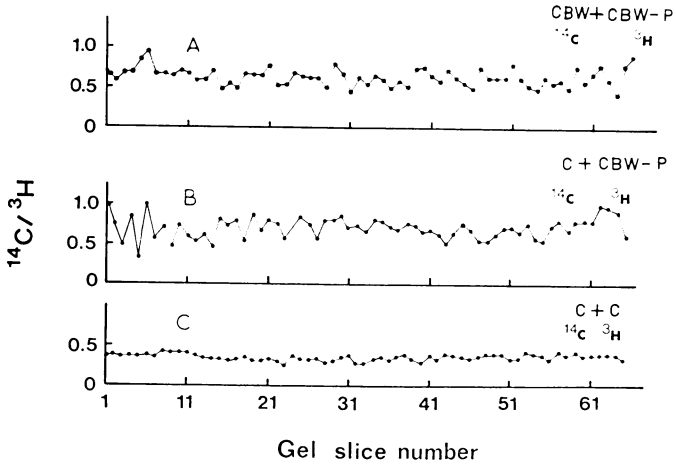

FIG. 4. Effect of imposed hydrostatic pressure on the pattern of protein synthesis. Conditions same as in Fig. 3 except that the upper curve (A) is a mixture of proteins labeled with ^{14}C-leucine in the presence of 21% CBW + 118 p.s.i. of 19:1 N_2-air, middle curve (B) is a mixture of proteins labeled with ^{14}C under control conditions or ^{3}H in the presence of 21% CBW + imposed pressure, and lower curve (C) is a mixture of proteins labeled with ^{14}C or ^{3}H under control conditions. If the applied pressure restored the pattern of protein synthesis to that of turgid tissues, curves B and C should be similar in linearity.

turgid cells, especially in the presence of mannitol. When N_2 was used as the gas, a slight stimulation of protein synthesis was obtained in both stressed and turgid cells, whereas with air as the gas, protein synthesis was inhibited in all cases.

The imposition of hydrostatic pressure to water-stressed tissues causes a change in the pattern of protein synthesis as judged by the double labeling ratio technique (Fig. 4, upper curve). The pattern of protein synthesis is not restored to that found in unstressed tissues (Fig. 4, middle curve). It was not possible with any conditions tested to date to convert the pattern of protein synthesis in stressed tissues back to that of the control tissues.

DISCUSSION

The mechanism by which water stress causes qualitative changes in protein synthesis has not yet been established. One possibility is that the effects are indirect, mediated by stress-induced ABA. It is known that ABA inhibits protein synthesis (1, 5) and that plants can accumulate large amounts of ABA when subjected to water stress (16, 20, 22). For example, the ABA content of wheat leaves increases more than 7-fold after only 4 hr of mild water stress (21). It is not known whether water stress induces an accumulation of ABA in *Avena* coleoptiles, but if this occurs, exogenous ABA should have the same effects on protein synthesis as does water stress. We have shown that the two agents exert different effects. First, in contrast to the effect of water stress, ABA alters only the rate of protein synthesis without changing the pattern of synthesis. It is of interest to note that in other tissues ABA appears to have a more specific effect on the pattern of protein synthesis. For example, in barley aleurone cells ABA appears to inhibit primarily the secreted proteins such as α-amylase (5), while having little or no effect on intracellular enzymes such as nitrate reductase (8). The reason for this difference is not known. Secondly, GA reverses the ABA-induced inhibition of protein synthesis in *Avena* coleoptiles, but it has no effect on the stress-induced effect. We conclude that water stress and ABA exert their effects on protein synthesis independently.

Hsiao (12) has listed several ways in which water stress might exert a direct effect on biochemical processes. Certain of these possibilities, such as a decreased chemical potential of H_2O or a decreased shell of hydration around macromolecules, seem unlikely because of the small changes in H_2O concentration. Another possibility is via the reduction in hydrostatic pressure: the pressure in *Avena* coleoptiles stressed with 0.3 M mannitol is only about 3.5 atm, as compared with 10.8 atm in unstressed cells. Changes in hydrostatic pressure have been shown in a few cases for plants to cause an alteration in biochemical and physiological processes. For example, the uptake of salt into *Valonia* cells is highly sensitive to the turgor pressure (10). The *in vitro* activity of a cold-sensitive potato ATPase is increased 3-fold by a 5-atm increase in hydrostatic pressure (15).

We have attempted to assess the role of hydrostatic pressure by restoring the original hydrostatic pressure with gas under pressure. It must be recognized that such a procedure cannot reproduce the conditions that exist in the turgid tissue. The increased pressure alters the partial pressures of dissolved gases, with the result that the stressed tissues subjected to an imposed pressure will certainly have a different O_2 level than the turgid cells. The present results, however, suggest that in water-stressed *Avena* coleoptiles, the imposition of hydrostatic pressure can increase the rate of protein synthesis and can alter the pattern of protein synthesis. But the imposed hydrostatic pressure does not appear to restore the pattern of protein synthesis to that found in unstressed tissues. Thus, it seems unlikely that the reduced hydrostatic pressure can be the cause of the stress-induced changes in protein synthesis.

Finally, the possibility should be considered that water stress inhibits the synthesis of only those proteins produced on membrane-bound polysomes. In animals different classes of proteins appear to be produced on free and membrane-bound polysomes (9). In barley aleurone cells, where water stress has no effect on leucine incorporation into total proteins but does inhibit the incorporation into specific proteins (4, 14), there is a decrease in the number of membrane-bound polysomes, in response to water stress (2). A decrease in total polysomes in response to water stress occurs in corn leaves (11) and roots (17). The reason for the decrease in polysomes is not clear. Lack of messenger RNA (11), destruction of ribosomes (2), inactivity of ribosomes (2), and lack of membrane synthesis (2) have all been ruled out. We suggest that the decrease in membrane-bound polysomes may be due to the reduction in surface area of cellular membrane that occurs during water stress. In *Avena* coleoptiles, turgid cells transferred to 0.3 M mannitol lose over 10% of their volume; the area of cellular membrane might be expected to undergo a similar reduction. Even this modest shrinkage of the membranes may render them incapable of acting as attachment sites for polysomes.

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