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A Comparative Study of the Effect of Abscisic Acid and cAMP on Protein Synthesis in Wheat Caryopses under Drought Conditions

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Abstract—The effect of exogenous abscisic acid and cAMP on synthesis of soluble proteins in wheat caryopses in drought has been studied. Both compounds affected the formation of the polypeptides whose synthesis was stimulated by dehydration: they increased the incorporation of the label into polypeptides of 13, 15, and 26 kD and decreased the incorporation of the label into polypeptides of 14, 64, and 77 kD. Abscisic acid and cAMP increased the level of the incorporation of [^{14}C]leucine into the low-molecular-weight polypeptides of 12, 17, and 19 kD whose synthesis was suppressed by drought. These data suggest that the cyclic adenylate signal system is probably involved in the effect of abscisic acid on protein synthesis in drought.

Key words: protein synthesis, drought, abscisic acid, cAMP, signal systems of plant cells

Under unfavorable conditions, particularly in drought, significant changes in the hormonal status of plants occur, the content of abscisic acid (ABA) increasing. In plant cells an intensive synthesis of ABA is observed due to induction of the expression of the gene for zeaxanthin oxidase, one of the key enzymes involved in the formation of this plant hormone from carotenoids [1, 2]. The increase in the level of ABA under stress conditions results in changes in genome expression, leading to changes in the composition of the proteins synthesized [3, 4]. It was shown that ABA affects the activity of the nuclear and cytosolic protein kinases [5] and causes phosphorylation of new proteins. Exogenous ABA was found to stimulate the protein kinase activity and protein phosphorylation in pea seedlings [6]. ABA increased the phosphorylation of a polypeptide of 9 kD in pea leaves at a low positive temperature [7].

There is some reason to consider that all these events are caused by triggering of signal systems in plant cells. In particular, the system of cyclic nucleotides functions effectively under stress conditions [8, 9]. A change in the level of cAMP results in changes in the cAMP-dependent phosphorylation of proteins involved in the control of genome expression. Key regulatory enzymes of different metabolic pathways as well as proteins regulating a number of extremely important intracellular processes are targets of cAMP-dependent phosphorylation catalyzed by protein kinases.

It should be noted that investigations of the molecular responses of plant cells to drought are usually performed on vegetative organs. However, plant seeds are also an interesting model for investigation of the mechanisms of drought resistance, since their germs are able to tolerate more than 80% decrease in water content while ripening [10, 11]. There is a viewpoint that the relative resistance of the translation and transcription of the "non-shock" mRNA to stress factors while ripening of seeds has a definite physiological sense, providing complete development of seeds and accumulation of reserve compounds that are necessary for successful germinating of the seeds and the formation of viable seedlings [12].

The goal of this study was to investigate the possible participation of the cyclic adenylate signal system in the mechanism of the effect of abscisic acid on protein synthesis in wheat caryopses under drought conditions.

MATERIALS AND METHODS

Caryopses of spring wheat *Triticum aestivum* L., variety Moskovskaya 35, were used to investigate the effect of drought on protein synthesis. The plants were grown in

Abbreviations: ABA) abscisic acid.

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pots under optimal ground moistening (total moisture capacity, 70%). On the stage of earring, drought was created by cessation of watering to reach 30% total moisture capacity. The intensity of protein synthesis in caryopses was determined using 2-[^{14}C]D,L-leucine (concentration 2 mM, specific activity 1.2-1.4 MBq/g). The caryopses removed from wheat ears were placed into a solution of [^{14}C]leucine. In the case of the experimental samples, the solution contained cAMP (0.1 mM) or ABA (0.05 mM). The concentrations used were taken from the literature data or chosen in preliminary experiments. Time of incubation was 1 h. After the incubation in the presence of the label, the caryopses were washed free from the radionuclide with 1 mM unlabeled leucine. The samples were fixed in liquid nitrogen, dried by lyophilization, and ground. Before isolation of proteins, the plant material was treated with ethyl ether to remove lipids and pigments. Proteins were extracted with phosphate buffer containing 1 M NaCl, pH 7.5, at 4°C [13-15]. The content of soluble proteins was determined by the Lowry method [16]. One-dimensional electrophoresis of proteins in 12% polyacrylamide gel in the presence of 2% SDS was performed in vertical gel plates as described by Laemmli [17]. The following proteins (Serva, Germany) were used as the markers: bovine serum albumin (67 kD), ovalbumin (45 kD), chymotrypsinogen (25 kD), myoglobin (17.8 kD), cytochrome *c* (12.3 kD). Equal amounts of proteins were applied on the starting position of the gel. After fixation, the gels were stained with 0.1% solution of Coomassie Brilliant Blue R-250 (Serva). Molecular weights of polypeptides were determined as described by Weber and Osborn [18]. Quantitative assay of protein bands was performed by scanning of the gels on an ISCO densitometer (USA) at 580 nm. The boundary of the protein bands were marked on the densitogram. The content of the polypeptides was determined considering that the area of the peak on the densitogram is proportional to the content of the corresponding protein. Knowing the amount of the protein applied to the lane, the content of the separate polypeptides was determined from the calculated areas of the peaks [19]. Radioactivity of samples was measured in dioxane-based liquid scintillator (ZhS-8) using a Delta-300 counter (Tracor Analytic, USA). The counting efficiency determined using ^{14}C-standards (Amersham, England) was 80%. Quenching of samples was removed using two-channel counting as described by Bush [20]. To study the character of distribution of the radioactive polypeptides in the gel, the lane was cut into fragments of length equal to the bases of the corresponding peaks on the densitogram. The fragments were dried, and then each of them was incubated in 1 ml of a mixture containing 30% H_2O_2 and 1% NH_4OH for 4-6 h at 65°C. After the gels were completely dissolved, the solutions were counted in dioxane scintillator. All the experiments were done three times, each single sample containing 15 plants. Statistical analysis was performed using the Statgraf program. Graphs were plotted using the Microsoft Graph program.

RESULTS AND DISCUSSION

The effect of each unfavorable factor of the environment results in different structural and functional changes in plant cells that are mostly determined by the ratio of synthesis and decomposition of different compounds including proteins. The specific radioactivity of polypeptides newly formed in the presence of labeled amino acids is a measure of the intensity of their synthesis and decomposition. We have observed previously that the intensity of protein synthesis in the fractions of different solubility of wheat caryopses decreases in drought [21]. The role of soluble proteins is of special importance in realization of the cell respond to stress factors, since the qualitative composition of these proteins is often affected by these factors. In our investigations, soil drought did not affect the qualitative composition of the polypeptides synthesized from [^{14}C]leucine, but it affected the distribution of the radioactivity among these polypeptides. Drought decreased the specific radioactivity of a significant number of polypeptides, mainly those of 38, 40, and 67 kD (4-7-fold), and increased the specific radioactivity of the polypeptides of 13, 14, 15, 26, 64, 70, and 77 kD (Fig. 1).

Determination of the level of the endogenous cAMP in wheat caryopses under conditions of different moistening showed that deficiency in moisture results in an increase in the content of cAMP [22]. The same effect was observed at the reduced temperature [23, 24], on separating of the tissue from the whole plant [25], or on removing of the cell wall [26].

Investigation of the effect of exogenous cAMP on synthesis of some polypeptides demonstrated that cAMP affected differently the intensity of the formation of the polypeptides whose synthesis was stimulated by drought (13, 14, 15, 26, 64, 70, and 77 kD) (Fig. 2). The incorpo-

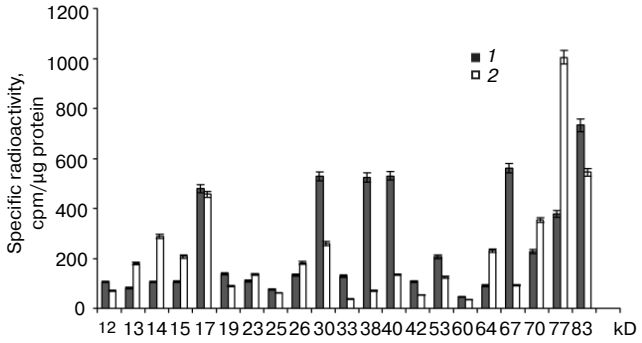

Fig. 1. Effect of drought on the incorporation of [^{14}C]leucine into polypeptides: *1*) control; *2*) drought.

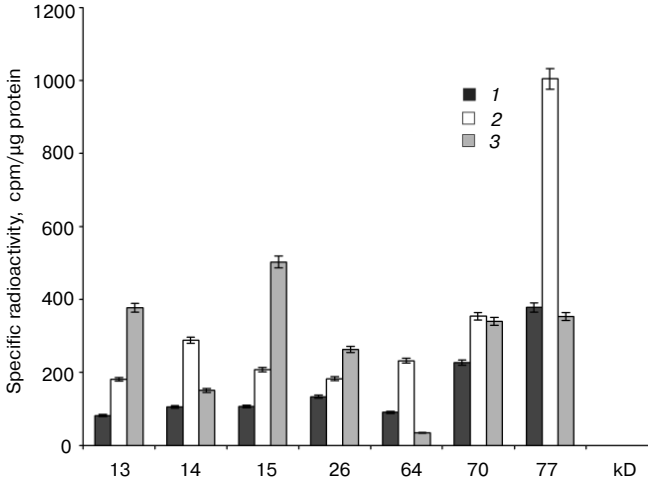

Fig. 2. Effect of cAMP on the radioactivity of the drought-stimulated polypeptides: *1*) control; *2*) drought; *3*) drought + cAMP (0.1 mM).

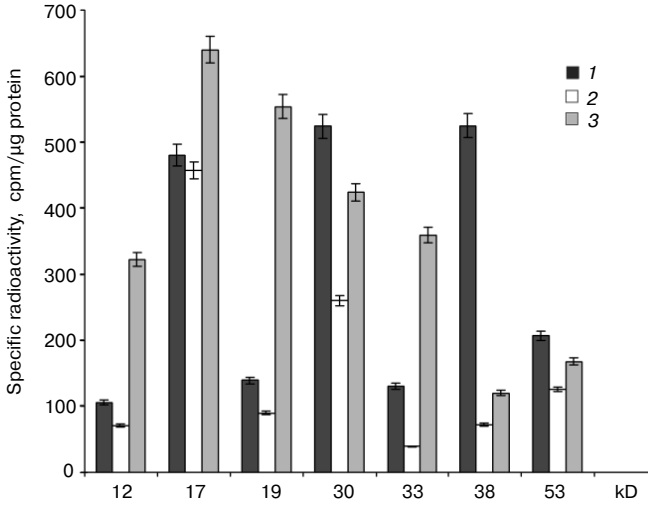

Fig. 3. Effect of cAMP on the incorporation of [^{14}C]leucine into the drought-suppressed polypeptides: *1*) control; *2*) drought; *3*) drought + cAMP (0.1 mM).

ration of [^{14}C]leucine in the low-molecular-weight polypeptides of 13, 15, and 26 kD increased in the presence of cAMP, while the intensity of the formation of the polypeptides of 14, 64, and 77 kD decreased. The level of the synthesis of the polypeptide of 70 kD remained virtually constant, corresponding to that in drought. The specific radioactivity of some polypeptides whose synthesis was suppressed by drought increased 2-3-fold in the presence of cAMP (Fig. 3). Exogenous cAMP affected the formation of both high- and low-molecular-weight polypeptides, but mostly the low-molecular-weight ones (12-33 kD) were affected.

Abscisic acid is considered by some researchers as an internal signal arising in cells in response to dehydration and inducing a cascade of stress-defensive reactions. Exogenous ABA can trigger this program in the absence

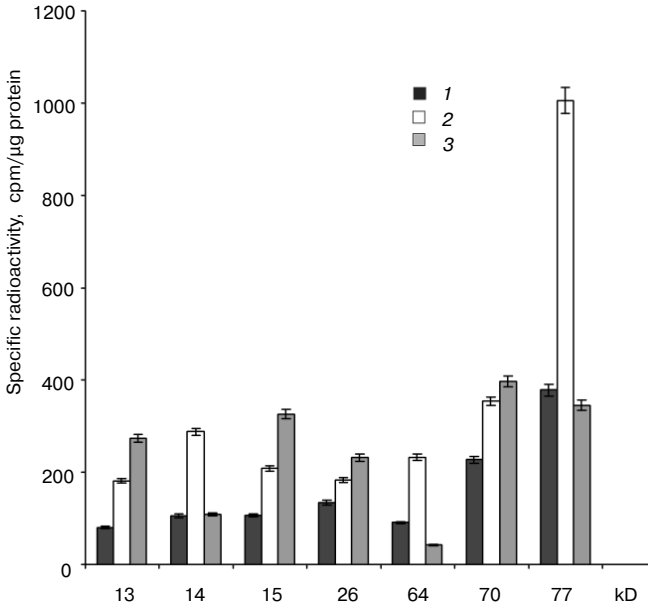

Fig. 4. Effect of ABA on the radioactivity of the drought-stimulated polypeptides: *1*) control; *2*) drought; *3*) drought + ABA (0.05 mM).

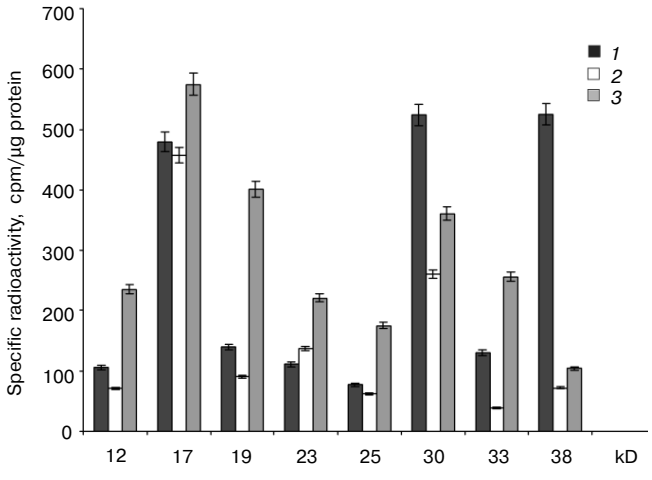

Fig. 5. Effect of ABA on the incorporation of [^{14}C]leucine into the drought-suppressed polypeptides: *1*) control; *2*) drought; *3*) drought + ABA (0.05 mM).

of a stress factor as well [27, 28]. It was demonstrated that exogenous ABA results in the expression of the defensive genes and, as a consequence, accumulation of a great number of the stress metabolites: inhibitors of proteinases [29], phytoalexins [30], free proline [31], and others.

Our previous experiments demonstrated that the dehydration of the plants resulting in the loss of 14% of water increased the level of ABA in the caryopses by 11% on the stage of earring and by 72% at the milky stage [32].

In our experiments, exogenous ABA did not result in changes in qualitative composition of the soluble proteins in wheat caryopses. Abscisic acid decreased the incorporation of [^{14}C]leucine into the stress polypeptides of 14, 64, and 77 kD and stimulated synthesis of the polypeptides of 13, 15, 26, and 70 kD (Fig. 4). Abscisic acid stimulated formation of those polypeptides whose synthesis was inhibited by drought (especially the low-molecular-weight polypeptides of 12, 17, 19, 30, and 33 kD) (Fig. 5).

The effect of ABA on plants is mediated through the different cell signal systems, providing the effective transmission and amplification of the external signals [33]. Abscisic acid activates the lipoxygenase [34], MAP-kinase [35, 36], superoxide synthase [37], calcium [38-41], and phosphatidic [42, 43] signal systems.

It is of special importance to reveal the involvement of the cAMP messenger system in the realization of the effects of ABA, since the adenylate cyclase signal system play an important role in the response reactions of plants to stress factors. The results of some works suggest the possibility of the involvement of the cAMP system in the mechanism of action of ABA under stress conditions (low temperature) [6, 7]. The data on negative regulation by cAMP-dependent protein kinase of the synthesis of the hydrophilic polypeptide homologous to the Lea-proteins of higher plants that is induced by both osmotic stress and ABA indicated the involvement of the cAMP system in the mechanism of action of ABA [44]. Dehydrin isolated from tomato plants subjected to salt stress was accumulated in the presence of ABA and can be phosphorylated by cAMP-dependent protein kinase *in vitro* [45]. A study of the promoter regions of the *rab*-genes encoding the proteins of 15.5-16.8 kD in rice plants under conditions of osmotic stress revealed the presence in these genes a conservative sequence that is homologous to the cAMP-recognizing site. Fragments of this sequence were also revealed in cotton plants in the promoter regions of the genes controlled by ABA [46]. The fact that the formation of similar polypeptides of the soluble fraction are stimulated (12, 13, 15, 17, 19, and 26 kD) or inhibited (14, 64, and 77 kD) by exogenous ABA and cAMP suggests that the cAMP-messenger system can be involved in the mechanism of action of abscisic acid in drought.

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