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INTERACTION OF ABSCISIC-ACID-BINDING COTTON (*Gossypium hirsutum*) PROTEIN AND PHYTOHORMONES

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The interaction of representatives of three classes of phytohormones, abscisic acid (ABA), the synthetic auxin 1-NAA, and the synthetic cytokinin 6-BAP, with ABA-binding cotton protein was studied. Binding of ABA with its protein was shown to be specific and receptor-like. Competitive protein binding showed that 1-NAA (15 ± 3%), 6-BAP (82 ± 3%), and ABA (95 ± 3%) replace ^{3}H-ABA from the protein complex. The possibility of reacting ABA-binding protein with various classes of intracellular phytohormones is discussed.

Key words: ABA, specific binding, cotton, competitive replacement.

The plant-growth inhibitor abscisic acid (ABA) is an antagonist of cytokinins and other growth stimulators in the regulation of many physiological processes. Thus, it inhibits the growth and greening of the cotyledon of pumpkin [1] and yellow lupine [2] and the synthesis of protein and m-RNA [3]. According to the contemporary conceptualization of hormonal regulation of seed germination, ABA is viewed as one of the "down-regulating" factors and cytokinins, "up-regulating." [4].

The physiological action of phytohormones is determined by their ratio [1]. Despite the large volume of literature on the action of ABA and other phytohormones on plants in general and their individual organs and on biochemical processes, the molecular mechanisms of their action and interaction still remain unclear. For example, it was established that nucleic and cytosolic proteinkinases (PK) are activated in the presence of cytokinins, ABA, and auxins [6]. Whereas ABA stimulated PK activity, BAP completely suppressed stimulation by ABA. We previously found using PAAG electrophoresis that ^{3}H-ABA in all organs and tissues of cotton is bound to a single protein of mol. wt. 19-20 kDa [7].

In the present article results are presented from a study of the interaction of ABA-binding cotton protein with representatives of three classes of phytohormones: ABA, synthetic auxin 1-naphthylacetic acid (1-NAA), and synthetic cytokinin 6-benzylaminopurine (6-BAP).

The binding of ABA to its protein in cotton is specific and receptor-like. This was demonstrated by competitive replacement by "cold" hormone incubated with ^{3}H-ABA-protein. Experiments showed that unlabeled ABA replaces labeled hormone by 95 ± 3%. Skatchardt analysis revealed a specific binding site with $K_d = 5\times10^{-10}$ M, which we have already reported [7].

Competitive protein binding of ^{3}H-ABA and the 1-NAA analog of indolylacetic acid showed that 1-NAA replaces ABA by only 15 ± 3%. This again confirms the specific nature of ABA binding to its protein. Results of the competitive interaction of ABA-binding cotton protein with phytohormones are presented below:

Phytohormone	Radioactivity, counts/min per 1 mg protein	Replacement, %
^{3}H-ABA	41187±1640	100
^{3}H-ABA + ABA	2059±62	95
^{3}H-ABA + BAP	7413±223	82
^{3}H-ABA + NAA	35008±1050	15

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Interesting results were obtained from a study of the competitive interaction of ^{3}H-ABA and 6-BAP. It was shown that 6-BAP replaces labeled hormone from the protein complex by 82 ± 3% (see above). This suggests that the protein has a high affinity for both ABA and 6-BAP.

Data for the antagonistic effects of these hormones upon interaction with the same receptors have been reported. Thus, a common receptor protein for ABA and cytokinins that is localized in the cytoplasm of pumpkin has been found [8]. It was observed that ABA stimulates proteinkinase from the cytosol of barley leaves whereas BAP has no effect on proteinkinase activity over a wide concentration range (10^{-8}-10^{-4} M) but completely inhibits the stimulatory activity of ABA [9].

Apparently the plant receptor proteins that respond to various phytohormones can be polyfunctional, like the phytohormones themselves. Such polyfunctional receptor proteins are known. For example, they include the thyroid hormone receptor in animals and man or the Ca-binding protein calmodulin, which has been observed in all currently known cells of plants and animals [10]. These proteins have several active centers for binding their ligands. For example, calmodulin has four centers. Depending on how many active centers a ligand occupies at a given moment, the receptor adopts the corresponding conformations, as a result of which various cell-target responses occur. The blood-serum protein prealbumin, which binds thyroxine, has binding sites both for thyroxin and triiodothyronin [11, 12] and for retinol-binding protein [13].

It is highly probable that such processes occur also in plant cells where ABA and cytokinins exert their effect by binding to one protein or another. The nature of the physiological response of a plant in this instance is determined by the stage of ontogenesis because the phytohormone balance in tissues during plant ontogenesis is rhythmic in nature [1].

Thus, the protein discovered by us binds ABA with rather high affinity, suggesting that the binding is receptor-like. It is very similar to the synthetic cytokinin 6-BAP. The physiological function of this protein in the plant probably occurs by interaction with ABA and cytokinins. The nature of the cell-target response is determined by both the phytohormone balance at various stages of ontogenesis and the actual phytohormone that is bound by the protein at the moment.

EXPERIMENTAL

The following instruments and reagents were used: ^{3}H-ABA with specific activity 7×10^{-4} mCi/mmol (INP, AS Rep. Uz.), 1-NAA and 6-BAP (Serva, Germany), standard toluene scintillant ZhS-107 (Reakhim), nitrocellulose filters "Synpor" (Chemapol, Czech Rep.) of 20 mm diameter with pore size 0.23 μ, other reagents of chemically pure and analytically pure grades from Reakhim, and a Beta-1 scintillation counter (LKB, Sweden).

The starting material for the experiments was hydrid cotton *Gossypium hirsutum* L. that was supplied from the genetic collection of the Institute of Genetics and Experimental Biology of Plants, Academy of Sciences of the Republic of Uzbekistan.

Seeds were treated with conc. H_2SO_4 to remove fibers and coating and were defatted by the Folch method [14]. Then seeds were germinated for 5-6 days in daylight at 37° C in Petri dishes on 0.7% agar with added Mg and Na salts. Chlorophyll from the leaves was extracted three times with isopropanol.

Proteins were isolated by the literature method [15] by adding PMSF (1M, 200 μl) and extraction buffer (100 ml). Aliquots of the protein extracts were incubated in a solution of ^{3}H-ABA at room temperature for 1 h and centrifuged with a suspension of activated carbon at 10,000 rpm for 15 min to adsorb unbound hormone.

The radioactivity of the resulting material was determined by liquid scintillation counting. Aliquots of the supernatant were first filtered under vacuum through Synpor nitrocellulose filters and washed three times with CCl_3CO_2H (10%).

The radioactivity was counted in the Beta-1 counter.

The degree of specific ABA binding to its protein was determined using competitive replacement of ^{3}H-ABA by an excess of "cold" hormone. Aliquots of protein that had been incubated with ^{3}H-ABA were treated with a 1000-fold excess of unlabeled ABA solution (0.1 M), incubated for 1 h at room temperature, and centrifuged with activated carbon at 10,000 rpm. The radioactivity of the resulting material was determined by liquid scintillation counting, first placing aliquots of the solution on Synpor filters, as described above.

Competitive protein interaction of ^{3}H-ABA, 6-BAP, and 1-NAA was performed as described above for ABA.

All experiments were repeated three times.

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