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ABA-BINDING PROTEIN OF THE COTTON PLANT *Gossypium hirsutum* AND THE SPECIFICITY OF ITS BINDING WITH ABA

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It has been established by electrophoresis in PAAG that in all the organs and tissues of the cotton plant Gossypium hirsutum *L. ^{3}H-ABA binds with a protein having MM 19—20 kDa. Scatchardt analysis has shown that the binding is specific and receptor-like, with $K_d = 5 \times 10^{-10}$ M.*

The growth inhibitor abscisic acid (ABA) takes part in practically all important physiological processes of plants. It has been shown that the synthesis of ABA increases under water and salt stresses [1] and also during the ageing of various plant organs [2]. ABA participates in the processes of inducing and maintaining the period of dormancy of the seeds and buds [3] and the synthesis of reserve proteins in seeds [4], and it influences the synthesis of DNA and RNA and also the transport of certain ions through the plasmalemma, so counteracting the effects of other phytohormones [5].

In spite of the large number of reports devoted to the action of ABA on plants and their individual organs, the question of the molecular mechanisms of the action of ABA remains obscure. Contradictory suggestions have been put forward on the nature and location in the cell of the specific targets mediating all the responses to the action of the hormone [6—8].

In view of this, the task of the present investigation included the isolation of proteins specifically binding ABA in the cotton plant in a receptor-like manner.

The binding of ^{3}H-ABA with the proteins of various organs and tissues of the cotton plant was studied in vitro. In an electrophoretic study of an extract of proteins from various organs and tissues of the cotton plant that had been incubated with ^{3}H-ABA, it was found that in all cases the ABA bound with only a single protein having MM 19—20 kDa (Fig. 1).

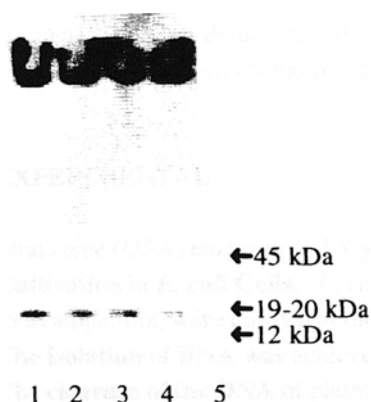

Fig. 1. Electrophoregram of an extract of the proteins of the cotton plant *G. hirsutum* L. that had been incubated with ^{3}H-ABA for 45 min—1 h: 1) seeds; 2) leaf blades; 3) seedlings; 4) separate layer of cuttings; 5) markers.

To reveal the specific nature of the interaction of ABA with its protein we made an analysis of binding constants. A Scatchard graph showed the presence of two types of binding sections: with a low capacity and high specificity ($K_d = 5 \times 10^{-10}$

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M), and with a high capacity and low specificity ($K_d = 3 \times 10^{-5}$ M) (Fig. 2). The difference in the binding constants is obviously due to the presence in cells of a nonspecific acceptor for ABA, since the work was performed with a total protein fraction.

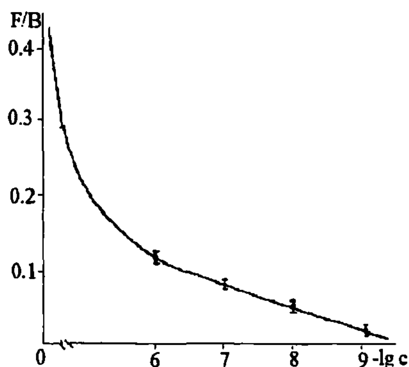

Fig. 2. Scatchard graph for the ABA-binding protein (incubation with ^{3}H-ABA for 45 min—1 h): *F*- free hormone; *B* — bound hormone.

Consequently, the protein found binds ABA with a fairly high affinity, which shows the receptor-like nature of this binding.

Thus, in all the organs and tissues of the cotton plant *G. hirsutum* we have found a protein with MM 19—20 kDa specifically binding ABA in receptor-like manner with $K_d = 5 \times 10^{-10}$ M. However, the absence of information on the specifically biological properties of this protein do not permit its definitive characterization as an ABA receptor.

EXPERIMENTAL

The following reagents were used: ^{3}H-ABA (Amersham, England), ZhS-107 standard toluene scintillator, and other "Reakhim" reagents with the grades kh.ch ["chemically pure"] and ch.d.a. ["pure for analysis"], and also a Gamma-1 scintillation counter (LKB, Sweden).

Hybrids of the cotton plant *Gossypium hirsutum* L. were provided by the Institute of Plant Genetics and Experimental Biology, Academy of Sciences of the Republic of Uzbekistan.

Seeds were treated with concentrated sulfuric acid to eliminate the hairy coating, the testa was removed, and defatting was carried out by Folch's method. Chlorophyll was extracted from the leaves with isopropyl alcohol three times. Seedlings were obtained by germinating seeds on 0.7% agar with the addition of mineral salts in Petri dishes at 37°C.

The proteins were isolated by the procedure of Markova et al. [9]. Aliquots of the protein extracts were incubated with ^{3}H-ABA having a specific activity of 21 Ci/mmole at room temperature for 1 h. The proteins were separated by Laemmli electrophoresis in 10% PAAG at 80 V, 2.5 mA, for 9 h. After electrophoresis the gel was cut into 0.5-cm sections, which were dissolved in a mixture of H_2O_2 and 10% NH_3 (99:1). In the first 24 h of the experiment, the pieces of gel were dried at 37°C, after which 0.5 ml of the mixture of H_2O_2 and 10% NH_3 was added to each test-tube which was then kept at 45°C overnight. On the following day the temperature was raised to 60°C for 2—3 h. After the dissolution of the gel the mixture was transferred into scintillation bottles each containing 3 ml of the standard toluene scintillator ZhS-107, and the radioactive label was determined on a Gamma-1 counter.

Results for plotting a Scatchard graph were obtained by paper chromatography in the three-component system of solvents butan-1-ol—glacial acetic acid—water (50:25:25). The tracks were cut into 0.5-cm pieces and the radioactive label was counted. In this system ABA bound to protein remained at the start, and the R_f value of the free hormone was 0.98. All the experiments were carried out in triplicate.

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