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STRUCTURAL–FUNCTIONAL CHARACTERISTICS OF SOME PROTEIN-PEPTIDE REGULATORS OF THE TRANSMISSIONS OF A HORMONAL SIGNAL IN THE COTTON PLANT

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Water-soluble cytokinin- and ethylene-binding proteins have been isolated from cotton plant shoots, and some of their physicochemical characteristics have been studied. It has been shown that, by a number of criteria, the proteins isolated must be assigned to the phytohormone receptors.

It is known that a large role in the transmission of a hormonal signal in the plant cell at the membrane and genetic levels is played by specific protein receptors of phytohormones [1]. However, the nature of the majority of receptor proteins and the mechanism of the interaction of the hormone-receptor complexes with chromatin have still not been studied in full measure. Isolation of the "true" receptor proteins will provide the possibility of studying not only the mechanism of the formation of the hormone-receptor complexes but also their cell, tissue, species, and interspecies specificities and, further, their participation in the cascade of biochemical reactions and interactions determining the transmission of a hormonal signal into the cell genome and the physiological response to it.

To solve this problem it is necessary to investigate the totality of hormone-receptor systems of a concrete plant material taking into account their close correlative link due to the polyfunctionality of phytohormones.

We have investigated the receptor systems of the cotton plant for three types of phytohormones — auxin, cytokinin, and ethylene —, since it is basically just these which control leaf shedding, not to speak of their influence on the growth and development of the plants. In the present paper we give results on the isolation and identification of cytokinin- and ethylene-binding proteins (CBP and EBP, respectively) of the cotton plant.

Three-day shoots of a *Gossypium hirsutum* cotton plant were investigated. To isolate the CBPs, the total water-soluble proteins were chromatographed on a sorbent with an immobilized cytokinin in an NaCl gradient. Each fraction was tested for its degree of binding with a labeled cytokin, ^{3}H-BAP. The greatest affinity was possessed by a fraction eluted with 1 M NaCl. HPLC analysis showed that this fraction was not homogeneous, and we therefore separated it on TSK gel HW-65 Ph.

On electrophoretic analysis, the fractions eluted by 70% ethanol migrated in the form of a single band with *M* 43 kDa. The protein present in this fraction exhibited a high affinity for ^{3}H-BAP with K_d = 4.3 nM and, in a complex with the cytokinin, activated RNA polymerase by 40% in comparison with a control, i.e., it possessed pronounced receptor properties [2, 3].

Electric focusing (Fig. 1, *a*) showed that the protein under investigation was an acidic protein (*pI* 3.6) and also confirmed its individuality.

According to the results of amino acid analysis, the protein contained 16 amino acids, among which Lys, Leu, Gly, and Asx predominated (Table 1). Calculation based on the amino acid analysis gave an integral value that was a multiple of the molecular mass determined electrophoretically, which may be yet another confirmation of the homogeneity of the protein under investigation.

To isolate the ethylene-binding protein we made use of the property of ethylene for forming complexes with the receptor proteins that are stable for a long time. The total water-soluble proteins of the cotton plant were first separated

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TABLE 1. Amino Acid Composition of the Water-soluble Cytokinin-binding Protein from Cotton Plant Shoots

Amino acid *	Amount of residues, nmole	Amino acid *	Amount of residues, nmole
Asx	48.80	Arg	19.34
Glx	50.90	Tyr	15.10
Ser	1.00	Val	20.62
Gly	20.00	Met	0.32
Thr	1.34	Ile	11.72
Ala	33.10	Leu	35.34
Pro	16.56	Phe	15.72
His	3.10	Lys	39.90

*Trp not determined.

TABLE 2. Amino Acid Composition of the Water-Soluble Ethylene-Binding Protein from Cotton Plant Shoots

Amino acid	Amount of residues, nmole	Amino acid	Amount of residues, nmole
Asx	15.2	Cys	6.2
Glx	14.0	Tyr	15.9
Ser	2.6	Val	7.4
Gly	9.5	Met	7.9
Thr	5.7	Ile	22.9
Ala	7.9	Leu	15.5
Pro	14.2	Phe	18.2
His	1.2	Lys	14.6

*Trp was not determined.

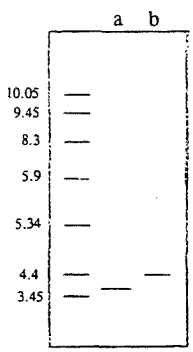

Fig. 1. Electric focusing of the cytokin-binding protein (*a*) and the ethylene-binding protein (*b*).

according to hydrophobicity, and the fractions obtained were analyzed with respect to their affinity for ^{3}H-ethylene. The greatest binding activity was shown by a fraction eluted by 70% ethanol. This fraction was saturated with labeled ethylene and, after elimination of the unbound radioactivity, it was chromatographed on TSK gel HW-50 F. Isotopic analysis of the fractions obtained permitted the identification of a protein with *M* 24 kDa possessing a high affinity for ethylene (K_d = 1.2 nM), and activating the synthesis of RNA in the nuclear chromatin of the cotton plant [4].

The absence of additional bands on isoelectric focusing confirmed the homogeneity of this protein (Fig. 1., *b*). From the value of the isoelectric point (*pI* 3.9) it, like the CBP, can be assigned to the acid proteins. In this protein we detected (without taking Trp into account) 16 amino acid residues, among which Ile and Phe predominated (Table 2). As in the case of the CBP, the homogeneity of the protein was shown by the fact that the calculated value of the mass of the amino acid residues was a multiple of the mass determined electrophoretically.

Thus, we have isolated from a cotton plant for the first time ethylene- and cytokinin-binding proteins, have studied some of their physicochemical characteristics, and have obtained a number of indices of their receptor nature.

EXPERIMENTAL

The HPLC analysis of the amino acid composition of the proteins was conducted on an Ultrasphere C_{18} column, 0.46 × 25 mm, with, as eluent, 14 mM NaOAc in acetonitrile—water (1:1).

The CBP and EBP were isolated by methods described previously [3, 4]. Isoelectric focusing was performed on standard PAAG plates with a pH gradient from 6.5 to 9.5 (LKB, Sweden) by a modified procedure [5].

The amino acid analysis of the CBP and EBP was conducted as in [6].

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