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HORMONE-BINDING PLANT PROTEINS. ISOLATION, PHYSICOCHEMICAL PROPERTIES, AND RECEPTOR FUNCTION

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Published data on the isolation and physicochemical and functional properties of hormone-binding plant proteins are reviewed. These investigations are directed mainly at the isolation of pure proteins, the proof of their receptor activity, and an explanation of their role in hormonal regulation of plant metabolic processes.

Key words: auxins, cytokinins, ethylene, abscisic acid, gibberellins, phytohormone receptors, cotton.

Many previously discovered natural compounds act as hormones and regulate physiological processes in plants. These include brassins, dihydroconiferol, triacontanol, methyljasmonate, several phenolic compounds that enhance the effects of known phytohormones, and several inhibitors [1-4]. Despite the existence of a wide spectrum of compounds controlling many aspects of plant development, there are only five principal types of phytohormones: auxins, gibberellins, cytokinins, abscisic acid, and ethylene. They regulate certain physiological processes by interacting with specific targets.

Each type of phytohormone has a characteristic basic function [5]. Auxins stimulate cell replication and expansion; cytokinins, cell division; and gibberellins, stem growth. Abscisic acid suppresses growth and metabolic processes at various stages of ontogenesis. Ethylene regulates fruit ripening and leaf loss.

However, certain key aspects of hormonal signal transduction in the cell gene are not yet understood. This is due primarily to the varied spectrum of action of the hormones, which not only complement each other but also exhibit synergism. Furthermore, it is unknown if each phytohormone has a single or several mechanisms of action on various cellular levels. Specific proteins, phytohormone receptors, play a significant role in hormone signal transduction in membranes and genetic material of plant cells. These proteins are found in the corresponding target tissues and have highly specific sites for hormone binding. Biochemical reactions needed to effect a physiological response of a given hormone are initiated by the receptor as a result of the binding. The receptors facilitate the interaction of cells and hormones and are the principal, although not the only, factor determining the specificity of the reaction between the cell and a particular hormone [6].

Research on the mechanism of hormonal regulation in plants aims to reveal the nature of the hormone receptor, to define its location in the cell and the nature of its interaction with the hormone, and to study metabolic processes caused by the hormone—receptor interaction.

This area of research has recently developed extensively owing to the improvement of protein-chemistry methods. It must be noted that the isolation of highly purified phytohormone receptors is complicated because their content in plant tissue is exceedingly low. They are identified using isotopic tracers and biospecific chromatography.

The isolation and identification of specific receptors are also difficult because the proteins in cells that noncovalently bind phytohormones include receptors, protein-transporters, hormone-storage proteins, and enzymes that participate in the synthesis, modification, and destruction of phytohormones.

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AUXIN-BINDING PROTEINS

Numerous auxin-binding proteins (ABP) from membranes and water-soluble fractions have been studied. However, their functions, except for the auxin-transport one, have not been determined [7]. The molecular mechanism of action of auxin is treated in plant-cell biology as a problem involving the mechanism of signal recognition and processing in plant cells. Nevertheless, the molecular mechanism of the initial action of plant-growth substances is close to being determined thanks to the development of the receptor hypothesis and the use of techniques to identify receptors for plant-growth substances.

ABP have been observed in the cytosol and nuclei of plant cells, in the total and plasma membranes, and in the tonoplast and endoplasmatic reticulum. Various receptors may be functional in cells during various stages of ontogenesis or may be connected with molecular mechanisms fulfilling a variety of functions.

Auxin receptors in the membrane and in soluble or chromatin-bound proteins that regulate gene expression are studied to reveal the mechanism of action of auxin. Results from studies of the structure and function of ABP have been reviewed [7-10].

ABP from the membrane fraction of corn (*Zea mays*) coleoptiles were investigated thoroughly after high-affinity binding of auxin was first reported [11]. The isolation of highly purified ABP was reported [12]. The purification method was developed. Its function was studied. Antibodies were prepared. A polypeptide of mass 20 kDa (in SDS-PAAG) was isolated by chromatography over auxin—Sepharose and immuno-specific sorbents. Analysis of the binding activity of this protein with ^{3}H-IAA (indole-3-acetic acid) established the optimum binding at pH 5.5 and a binding constant of 5.7×10^{-8} M. The molecular weight of the ABP that was determined by gel filtration was 40-45 kDa [13]. Therefore, it is probably a dimer. Plasma membranes isolated from corn roots typically have a high level of auxin-binding activity compared with other plant sources. Other researchers have confirmed the ABP to be a polypeptide of mass 20 kDa by using various purification methods [14, 15]. Two oligonucleotides were synthesized based on the N-terminus amino-acid sequence of purified ABP [16]. A search of a c-DNA library found several subsequent clones, including some with the whole chain. Sequencing of the clones enabled the complete primary amino-acid sequence of ABP to be established. The protein consists of 163 amino-acid residues [16]:

Met-Ala-Pro-Asp-Leu-Ser-Glu-Leu-Ala-Ala-Ala-Ala-Pro-Ala-Arg-Gly-Ala-Tyr-Leu-Ala-Gly-Val-Gly-Val-Ala-Val-Leu-Leu-Ala-Ala-Ser-Phe-Leu-Pro-Val-Ala-Glu-Ser-Ser-Cys-Val-Arg-Asp-Asn-Ser-Leu-Val-Arg-Asp-Ile-Ser-Gln-Met-Pro-Gln-Ser-Ser-Tyr-Gly-Ile-Glu-Gly-Leu-Ser-His-Ile-Thr-Val-Ala-Gly-Ala-Leu-Asn-His-Gly-Met-Lys-Glu-Val-Glu-Val-Trp-Leu-Gln-Thr-Ile-Ser-Pro-Gly-Gln-Arg-Thr-Pro-Ile-His-Arg-His-Ser-Cys-Glu-Glu-Val-Phe-Thr-Val-Leu-Lys-Gly-Lys-Gly-Thr-Leu-Leu-Met-Gly-Ser-Ser-Leu-Lys-Tyr-Pro-Gly-Gln-Pro-Gln-Glu-Ile-Pro-Phe-Phe-Gln-Asn*-Thr-Thr-Phe-Ser-Ile-Pro-Val-Ser-Asp-Pro-His-Gln-Val-Trp-Asn-Ser-Asp-Glu-His-Glu-Asp-Leu-Gln-Val-Leu-Val-Ile-Ile-Ser-Arg-Pro-Pro-Ala-Lys-Ile-Pro-Leu-Tyr-Asp-Asp-Trp-Ser-Met-Pro-His-Thr-Ala-Ala-Val-Leu-Lys-Phe-Pro-Phe-Val-Trp-Asp-Glu-Asp-Cys-Phe-Glu-Ala-Ala-**Lys-Asp-Glu-Leu**.

Amino-acid analysis showed that ABP belongs to the family of proteins with the vacilin β-structure, contains a metal-binding cluster of amino acids bonded to the Trp residue in a side chain, and possesses properties of oxalate oxidases. Molecular modeling revealed that the Trp in the 44-position interacts with auxin. As a result, the receptor structure undergoes a conformational change and the hormone signal is transmitted in the cell gene [17].

Immunoblotting found that fresh tobacco (*Nicotiana*) leaves contain 0.7-1.2 μg/g of ABP. These quantities are comparable with those of ABP in corn roots and indicate that the ABP levels are similar in dicotyledonous and monocotyledonous plants. The ABP content in tobacco leaves increased 20 times upon expression of the recombinant gene and decreased to half of the initial value upon gene epxression [18]. However, ABP of mass 14 kDa was isolated from tobacco plasma membranes. This polypeptide may belong to a new group of ABP that is involved in auxin stimulation of a proton pump [19].

An ABP of mass 57 kDa exhibits high cross-reactivity with BSA antiserum. This was used to purify this protein in high yield. The IAA—ABP complex exhibited high affinity for H^+-ATP-ase, i.e., the ABP evidently has two binding sites [20].

A study of the dynamic interaction of recombinant tobacco ABP with monoclonal antibodies has shown that the monoclonal antibodies recognize the two hypothesized auxin-binding domains and the C-terminus sequence. The electrical response of tobacco protoplasts caused by auxin has been used as a functional test to show that the monoclonal antibodies are either auxin agonists or antagonists. These effects have confirmed that ABP are located in the plasma membrane and are involved in activating the auxin-dependent electrical response of tobacco protoplast that is activated, as proposed, by an ABP conformational change [21].

Soluble or chromatin-binding receptors are interesting owing to the hypothesis that auxin initially regulates gene activity. It is known that auxin in vivo can cause increased r-RNA synthesis and ribosome accumulation. The receptor associated with chromatin is apparently capable of entering the nucleus and increasing transcription in analogy with the receptor model for animal steroid hormones [6].

Two IAA-binding proteins were isolated from nuclei of the unripe endosperm of coconut (*Cocos nucifera*). A protein in the nucleoplasm was purified by affinity chromatography over carboxyl-2,4-poly(lysyl)-Sepharose. Its molecular weight determined by electrophoresis in SDS—PAAG was 94 kDa with a binding constant (K_d) for IAA of 7.5×10^{-6} M. The other IAA-binding protein (mass 70 kDa) was isolated from the fraction of chromatin nonhistone proteins and purified by affinity chromatography over the same sorbent. It exhibited high ($K_d = 5.8\times10^{-8}$ M) and low ($K_d = 8.2\times10^{-6}$ M) affinities for IAA. The quantity of IAA-binding sites for the purified ABP was 0.4 pM/μg and 5.1 pM/μg for the proteins with high and low affinity, respectively [22].

A suspension of tobacco cell culture yielded a soluble ABP of mass ~200 kDa. The optimal binding was observed at pH 7.5-7.8. The ABP concentration was very low, less than 0.2 pM/mg of protein. This hindered the study [23]. However, it has been noted that adding Mg^{2+} and ATP can increase the quantity of binding sites. The affinity of the receptor for IAA is evidently regulated by phosphorylation and dephosphorylation processes [24].

The isolated proteins with high IAA affinity ($K_d = 10^{-8}$-10^{-9} M) are oligomers of 3-4 subunits of mass ~50 kDa [25]. The locations of these ABP were not found. They may migrate between the nucleus and cytoplasm, accumulating in the cytoplasm upon depletion of auxins during the steady-state stage of culture growth. The proteins migrated from the cytoplasm into the nucleus upon addition of fresh auxins but not their inactive analogs to the medium. This activated in parallel nuclear synthesis of RNA. Auxin changed the transcription of specific types of m-RNA [26]. This effect could be reproduced in vitro upon adding to isolated nuclei preparations of partially purified ABP with auxins. Transcription was not stimulated in the absence of hormone or protein [25]. Such stimulation was not quantitative and stable.

Thus, the functional properties of soluble tobacco ABP are very similar to those of animal soluble hormone receptors, e.g., steroidal hormone receptors [6]. The similarity is highlighted by the fact that the auxin-binding activity of this protein is positively correlated with the extent of its phosphorylation [27].

The isolation of nucleoplasmatic and chromosomal fractions of IAA-binding proteins has been reported. The nucleoplasmatic fraction contains acidic proteins according to the amino-acid composition. The hormone—receptor complex affects the RNA-polymerizing activity of the in vitro system and the stimulating effect of the IAA—receptor complex compared with a control and IAA. It has been hypothesized that the chromosomal IAA-binding protein acts as an acceptor in the hormone—chromatin interaction process [28].

Research on the effect of IAA on transcription and translation leads to the conclusion that the effect of IAA on the synthesis of nucleic acids and protein is mediated by the formation of its complex with receptor proteins. The IAA can form both strong and labile complexes with the receptor. Some researchers view the formation of the IAA-receptor complex as a necessary condition for the manifestation of hormone regulatory activity [5, 8].

Hypotheses of the mechanism of auxin action at the cellular level propose the existence of several types of ABP. We investigated the location of ABP in fractions obtained from sequential centrifugation of homogenate from cotton (*Gossypium hirsutum* L.) sprouts. Preparations from hypocotyls are richest in IAA-binding proteins, which appear in the subcellular and soluble-protein fractions.

The specific binding of ^{3}H-IAA with the membrane preparation and the water-soluble proteins reached saturation within 15 min at 20°C. The optimum pH for binding with the membrane preparation was ~5.5; with the water-soluble proteins, ~7.5. The plots of specific binding of ^{3}H-IAA with membrane preparation show at least two types of binding sites with K_d values in the range 0.1-1.0 μM. The plot of ^{3}H-IAA binding with water-soluble proteins is consistent with a single type of binding site with $K_d = 6\times10^{-9}$ M and $B_{max} = 0.2\times10^{-12}$ M/mg of protein [29]. The effect of various compounds that exhibit auxin-like activity on ^{3}H-IAA binding was studied. The order of ^{3}H-IAA-binding inhibition by these compounds for the membrane-bound proteins was 1-naphthylacetic acid (1-NAA) > IAA > 2,3,5-triiodobenzoic acid (TIBA) > 2,4-dichlorophenoxyacetic acid (2,4-D) > indolyl-3-propionic acid (IPA); for water-soluble proteins, IAA > TIBA > 1-NAA > 2,4-D > IPA.

The order of inhibition of ^{3}H-IAA-binding with membrane preparations leads to the conclusion that membranes from cotton sprouts are rich in binding sites with properties similar to that called "site-1" that was described for membranes from corn, bean (*Faba*), and grass (Poales) coleoptiles. The water-soluble proteins from cotton sprouts are similar to those from corn,

beans, and tobacco cell culture [30]. The membrane fraction yielded a preparation of proteins that are solubilized in triton X-100 and retain a high affinity for ^{3}H-IAA binding. The order of inhibition of this binding with the aforementioned compounds is characteristic of the native membranes.

The ability of the water-soluble and solubilized-membrane protein fractions to stimulate RNA synthesis in vitro was studied. It has been found that only the preparation containing the soluble auxin-binding proteins significantly increases the incorporation of ^{3}H-UTP into RNA of chromatin together with IAA. This is consistent with ideas about the role of water-soluble ABP [31].

Affinity chromatography was used to purify total ABP. A study of the structures of compounds with auxin-like activity showed that the auxin analogs should have a free carboxylic acid for binding with the auxin receptor. Therefore, an affinity sorbent with IAA-acetamidohexylsepharose in which the IAA was bonded through the imino group to the spacer of AH-sepharose 4B was synthesized to purify the ABP. The desalted extract of water-soluble proteins was passed over the affinity column. Bound proteins were eluted by 1 M NaCl from the affinity column as a single peak and exhibited specific binding to ^{3}H-IAA.

HPLC over a Glass Pacc TSK HW-3000 (8×300 mm) analytical column (LKB) was used to purify further the proteins and estimate their molecular weights. Five main peaks from 200 to 8 kDa were observed. Each of these was studied for ^{3}H-IAA binding and ability to stimulate ^{3}H-UTP incorporation into chromatin RNA. Only the protein of apparent molecular weight 200 kDa was capable of stimulating RNA-polymerase activity. It bound specifically ^{3}H-IAA at a concentration of 30×10^{-9} M with a density of binding sites of at least 150×10^{-12} M/mg of protein.

Electrophoresis in SDS—PAAG has shown that the protein consists of four subunits of 43 kDa. Determination of the N-terminus amino acid in the ABP of mass 200 kDa identified Pro as the 1-dimethylaminonaphthalene-5-sulfonyl derivative. This is one criterion of its homogeneity [32]. Later, an ABP of similar molecular weight of 44 kDa was isolated from pea (*Pisum sativum*) using the photoaffinity label 5-azido-[7-^{3}H]-IAA [33].

ABP from cotton sprouts had two principal receptor properties. It bound specifically and with high affinity a mediator molecule (IAA) and activated a response in the presence of a mediator. A protein of approximately the same molecular weight and capability to activate IAA-dependent RNA synthesis was isolated from tobacco cell culture [27]. Apparently, ABP-1 of mass 190 kDa that consists of subunits of mass 48 kDa from water-soluble proteins of mung bean (*Phaseolus aureus*) sprouts [34] is also analogous to this protein although it was not investigated for ability to activate RNA synthesis.

ABP from cotton sprouts were used in primary screening of new plant-growth regulators. Thus, the animal thyroid hormone thyroxine competed directly with auxin for ABP and was used to develop the growth stimulator T4. It inhibited competitively ^{3}H-IAA binding to soluble ABP with $K_{inh} = 3\times10^{-9}$ M. Like IAA, thyroxine in the presence of soluble ABP activated RNA synthesis in vitro in isolated cotton-sprout chromatin. The auxin-like activity explains the stimulation by thyroxine of cotton-seed germination [35].

CYTOKININ-BINDING PROTEINS

It has been proposed that the physiological response to the action of cytokinins occurs through their interaction with certain proteins [36]. A mediator protein was observed in pea-chromatin preparations. It stimulated RNA synthesis in vitro in a system containing *E. coli* polymerase and homologous pea DNA.

Differential centrifugation was used to characterize the binding of BAP (6-benzylaminopurine) with fractions from tobacco callus. It has been found that fractions precipitated at 80,000 g have two binding sites [39]. One of these has low affinity for cytokinins (K_d 7.7×10^{-6} M) and was not specific for biologically active analogs. For the second high-affinity site (K_d 1.4×10^{-7} M), the competition of cytokinin analogs with BAP for binding sites correlated with their biological activity. The subcellular location of these sites was not determined.

A new affinity probe of zeatin riboside ([9R]Z) conjugated to a carrier protein was developed to isolate membrane-bound cytokinin-binding protein (CBP) from *Arabidopsis thaliana*. This probe was used to detect CBP by IFA. Its effectiveness was compared with that of model proteins exhibiting cytokinin activity such as monoclonal anti-[9R]Z-antibody, which bound cytokinin conjugate. Proteins incapable of binding cytokinin (e.g., BSA) were inactive. The affinity probe has shown that CBP are present in the membrane fraction of *A. thaliana* cells cultivated in vitro [40].

Free biologically active cytokinins or monoclonal anti-[9R]Z-antibodies inhibit binding, which indicates that the

binding is highly specific. CBP are partially solubilized if membranes are treated with KCl. This indicates that they are located in peripheral membranes. Two different CBP were obtained by separating solubilized microsomal proteins using anion-exchange chromatography [41]. Anion-exchange chromatography over DEAE-cellulose and affinity chromatography over BAP-bound sepharaose-4B gave CBP-1 and CBP-2 from the soluble fraction of tobacco callus proteins [42]. Their purification factors are 270- and 600-fold; masses, 34 and 26 kDa; and BAP binding dissociation constants (K_d), 8.9×10^{-6} M and 1.1×10^{-6} M, respectively. Zeatin and kinetin but not adenine, ATP, or IAA inhibited BAP binding to CBP-2. The optimum pH for BAP binding to CBP-1 and CBP-2 was approximately 6.5 and 7.5. The primary structure of CBP-1 is significantly homologous (90%) to endochitinase; that of CBP-2, to osmotin-like protein. Therefore, CBP-2 was proposed to be a stress protein.

CBP of mass 17 kDa was isolated from etiolated mung bean (*P. aureus*) sprouts. The protein was specifically bound to phenylurea derivatives, which typically have high cytokinin activity, K_d 4×10^{-10} M [43].

Cytokinin receptors are most probably soluble proteins observed in vegetative organs of grasses [44]. It has been found that leaf cells contain CBP outside of chromatin [45]. These are cytoplasmic cytokinin receptors that transfer the hormonal signal to chromatin [46].

Affinity chromatography with immobilized BAP as a ligand was used to isolate a protein fraction capable of activating transcription in vitro [47]. However, CBP from barley (*Hordeum*) leaves could not be characterized by this method because of a large amount of proteins bound nonspecifically to the BAP-sepharose [48].

A highly purified CBP fraction was obtained from barley leaves by combining differential precipitation by ammonium sulfate, gel chromatography over Sephadex G-150, and affinity chromatography over epoxysepharose with immobilized BAP [49]. The protein in this fraction had mass 42±0.5 kDa, bound to ^{3}H-trans-zeatin with K_d 10^{-7}-10^{-8} M, and did not interact with other classes of phytohormones. Zeatin binding to the protein was reversible. The affinity of several cytokinin derivatives correlated well with their physiological activity. CBP isolated from vegetative organs of wheat and barley had common properties: acidic proteins, easily extracted from cells, not glycoproteins, optimum hormone binding at pH 7-8. The zeatin binding strength depended on ionic strength and medium properties whereas the ligand binding specificity changed insignificantly and was the same for proteins from various grass species. Two CBP of masses 220 and 27 kDa that have high affinity for ^{3}H-BAP were isolated by affinity chromatography from cotton ovaries [50].

CBP in tobacco callus cells was successfully determined using fluorescent anticytokinins [51]. Three proteins were identified. Two were soluble. One was ribosomal. One of the soluble proteins had high affinity (K_d 2×10^{-8} M for zeatin) and showed selectivity for a series of compounds according to their biological activity, like the ribosomal protein (K_d 7.8×10^{-8} M for zeatin). The second soluble protein had relatively low affinity for zeatin (K_d 1.2×10^{-6} M). The method using fluorescent probes is promising for investigating receptor proteins owing to its high sensitivity. However, it was unfortunately not further developed.

CBP found in embryonic wheat (*Triticum*) and other grasses are characterized in most detail. Antiserum to CBP has shown that protein is localized in tissues of protein bodies surrounding the embryonic shaft and cross-reacts with proteins of other plants [52, 53]. Antiserum formed precipitates with translation products in vitro such as CBP (monomer with mass 54 kDa in SDS—PAAG) and proteins of higher molecular weight. However, highly purified antibodies interacted only with the protein of mass 54 kDa. Therefore it was assumed that global post-translation changes did not occur. The protein accumulated in 2-4-week embryos and made up 9% of soluble protein. Properties of CBP have also been discussed [54]; however, not as a receptor but as a protein that sequesters cytokinins and regulates their concentration. The similarity with bean proteins that store zeatin was also noted.

A peptide that was assumed to be a part of CBP was identified using a photoactive cytokinin [55]. It has been found that the photoactive tracer bound to a single histidine residue. Protein-chemistry methods and tritium-labeled photoactive cytokinins with specific affinities are valuable for studying other CBP. Production of antibodies to the peptide containing the binding site is also promising. They should be capable of recognizing other CBP. Little information is available on the distribution of specific CBP in plants. Research on CBP from barley leaves and wheat embryos should be mentioned. It was observed that CBP isolated from barley leaves was highly specific for pea proteins but had no strict age or species specificity and could in a cytokinin complex increase the matrix activity of chromatin and the RNA-polymerase activity [56]. CBP from wheat embryos, CBP-1, was also studied systematically [52, 53]. These proteins can bind isopentyladenine and synthetic cytokinin, BAP. However, a number of their properties differ from those described [57] for zeatin-binding proteins (ZBP) from barley leaves. These include molecular weight and ligand-binding specificity. CBP-1, in contrast with ZBP, has low affinity for natural cytokinin and zeatin. According to immunocytological analysis, it is located in wheat embryos in external tissues

such as scutellum, coleoptile, and coleorhisa and inside cells in protein bodies that are known to contain reserve seed proteins. CBP-1 disappeared after seed germination and was not detected in vegetative plant organs even by immunological methods. Only at the start of flowering could it again be identified in gynoecium tissues.

Therefore, the physiological and cytological properties of the studied CBP are more typical of a receptor function than those of CBP-1.

Later CBP of mass 67 kDa was isolated from barley leaves by IFA using competition of cytokinin with anti-idiotypic antibodies against zeatin antibodies [58]. Monoclonal antibodies to CBP with mass 70 kDa from corn sprouts exhibited cross-reactivity with CBP (67 kDa) and inhibited activation of RNA-polymerase-1 from barley chromatin that was inhibited by CBP and *trans*-zeatin.

Determination of the cytokinin-binding activity of plant proteins [57] was used to detect CBP in various grass organs and to establish quantitative trends of their localization. It can be stated confidently that CBP occur in leaves, roots, and coleoptiles of grasses and in leaves during ontogenesis. The ontogenesis stage or plant parts where CBP would be absent have not yet been observed. This is consistent with the significance of CBP and is correlated with the sensitivity to cytokinins of various plant tissues. CBP are also present in cells of the stomatal apparatus, the operation of which is modulated by cytokinins [59]. They are detected reliably on the subcellular level only in cytosol. This is consistent with the predominant cytoplasmic location of endogenous cytokinins in higher plants [60]. The optimum hormone binding pH of 7.5 is consistent with the cytoplasmic location of these proteins [61, 62]. Based on these data, the presence of mobile CBP in any cellular structures (organelles) is possible. Purified CBP were first isolated from water-soluble protein extracts of 5-day corn roots and purified approximately 500 times by anion-exchange chromatography, gel filtration, HPLC, and hydrophobic chromatography. The molecular weight of the polypeptide was determined as about 46 kDa by electrophoresis [63].

The biochemical properties of CBP that have been found to date are consistent with their possible receptor function. The cytoplasmic location of CBP indicates that they may affect other cellular organelles. Calmodulin, which is located mainly in cytoplasm [64] but affects various aspects of cell metabolism [65], provides an example of such regulatory proteins. One of the possible explanations of the multifunctional action of cytoplasmic CBP primarily on protein biosynthesis is the suggestion that they are involved in cytokinin-dependent regulation of the activity of certain t-RNA [66]. However, the combined structural and functional features of CBP must be explained to understand why CBP are present in cells. This, in turn, can reveal the molecular bases of action of cytokinins in plants.

A study of the specific binding of ^{3}H-BAP with crude membranes from cotton sprouts has shown that two binding sites with K_d in the range 8.5×10^{-6} and 6×10^{-9} M are present [67]. The water-soluble proteins also exhibit high affinity with K_d 4.3×10^{-9} M. Then, water-soluble CBP whose location in the cytosol suggests that it affects the cell genetic apparatus was studied [68]. Several physicochemical parameters were determined for this protein [69]. According to electrophoresis, the molecular weight under denaturing conditions is 43 kDa; the apparent molecular weight determined by HPLC, 170 kDa, i.e., electrophoresis in SDS leads to the conclusion that CBP has a subunit structure and consists of four subunits. The isolated protein is an acidic protein with an isoelectric point of 3.6. Amino-acid analysis by HPLC has shown that it contains 16 amino acids with Lys, Leu, Gly, and Aspx dominating. Use of dansyl has shown that the terminal amino acid of the CBP is valine. Amino-acid analysis of the water-soluble CBP from cotton sprouts by HPLC over a C_{18} Ultrasphere column gave the following quantitative composition (in nmole): Asx 48.80(49), Arg 19.34(19), Glx 50.90(51), Tyr 15.10(15), Ser 1.00(1), Val 20.62(21), Gly 20.00(20), Met+, Thr 1.34(1), Ile 11.72(12), Ala 33.10(33), Leu 35.34(35), Pro 16.56(17), Phe 15.72(16), His 3.10(3), Lys 39.90(40), not counting Trp [70].

The physicochemical properties confirm that the isolated CBP is homogeneous [70].

CBP of grass cultures have been most extensively studied at the Institute of Plant Physiology of the Russian Academy of Sciences by O. N. Kulaeva. Thus, collaboration with M. Venis (Great Britain) resulted [71] in 500-fold purification of CBP from corn sprouts by ion-exchange, gel-filtration, and hydrophobic chromatography. The molecular weight of the isolated protein was 46 kDa with $K_d = 11.5\times10^{-9}$ M, i.e., parameters close to those for CBP from cotton sprouts. Unfortunately, other properties could not be compared because an amount of protein sufficient to determine the amino-acid composition could not be isolated and the N-terminal amino acid is blocked.

The functional characteristics of CBP isolated from cotton, barley, and corn sprouts were also very similar [66].

We obtained much experimental evidence for the receptor nature of water-soluble CBP from cotton sprouts [72, 73]. Thus, it has been determined that CBP is bound highly specifically and tightly to the synthetic cytokinin ^{3}H-BAP with K_d = 4.3 nM. The number of binding sites B_{max} = 5.8 pM/mg protein. Other phytohormones do not compete with ^{3}H-BAP.

Compounds with cytokinin activity competitively replaced ^{3}H-BAP from CBP binding sites in the following order: BAP > dropp > IPA > serotonin > kinetin > adenine.

The hormone—receptor interaction was studied using a complex of fixed structure with optimized energy parameters. Molecular models of complexes produced by computer modeling using the PCM-MMX program show that the studied molecules of kinetin and serotonin interact with a histidine residue through a water molecule to form one H-bond whereas BAP and isopentyladenine form two and cytodef forms three [74, 75].

The functional activity of CBP was studied in a model system with isolated chromatin. This system is widely used to reproduce in vitro the initial stage of molecular action of a phytohormone [76]. The BAP—CBP complex activates transcription in the model system whereas addition of other phytohormones to CBP does not produce such stimulation. This confirms that the recognition by CBP of its own class of phytohormones is highly specific. The interaction of the hormone—receptor complex with the nuclear genome can be viewed as one of the stages of hormonal regulation of gene expression. However, the plant cell contains mitochondrial and chloroplast genetic apparatuses also. It has been noted previously [77, 78] that phytohormones regulate their activities also. However, it is unclear whether chloroplasts are activated independently by cytokinins or through a certain regulatory factor formed in the nucleus. Addition of BAP, CBP, and their complex to a system with isolated chloroplasts substantially stimulated RNA and protein synthesis. The hormone—receptor complex induced the synthesis of five new polypeptides of molecular weight from 18 to 60 kDa, presumably proteins of the Rubisco complex [79]. Our results and those published in the literature [8] lead to the conclusion that a cytoplasmic mediator (receptor) coordinates the response to the hormone of the nuclear and chloroplast genomes. In fact, it is known that cytokinins activate the synthesis of RBPC (ribulosobiphosphatecarboxylase), one of the subunits of which is coded in the nucleus and is synthesized in the cytoplasm, another of which is coded and synthesized in chloroplasts [80, 81].

Thus, the functional activity of water-soluble CBP has been studied in sufficient detail. However, the combined structural and functional features of CBP must be determined in order to reveal the actual presence of CBP in cells. This improves the understanding of the molecular bases of action of cytokinins in plants.

ETHYLENE-BINDING PROTEINS

Attempts are being made to explain the binding mechanism of ethylene and its synthetic analogs with receptors by analogy with cytokinins and other phytohormones. It is assumed that the ethylene concentration in the intercellular space of plant tissues reflects the concentration of this plant regulator inside the cell and that these concentrations correlate with the ethylene distribution coefficient between air and the cell contents. About 98% of radioactive ethylene accumulated by tissues is slowly released into air (1-10% per hour). However, heating to 45-50°C caused rapid release of ethylene. It has been found that cotyledons have a high affinity for this gas and that ethylene analogs, e.g., propylene and vinyl chloride, competitively inhibit its binding in proportion to their physiological effects in developing plant systems. An attempt was made to isolate ethylene-binding protein (EBP) by differential centrifugation [82, 83]. It has been found that the radioactivity is concentrated in the supernatant after incubation of plant-tissue homogenate with ^{14}C-ethylene followed by centrifugation at 1000 g and is completely precipitated at 61,000 g. Analogous results were obtained from a study of tobacco and mung bean [84]. Incubation of EBP from *P. vulgaris* with increasing amounts of free ethylene in the presence of a constant radioactivity concentration showed that the binding is reversible and saturated. Scatchardt analysis of these and other data showed the presence of only one binding site with K_d in the range 6×10^{-9}-1×10^{-10} M. Then it has been determined that the concentration of binding sites is at least ten times less than the actual dissociation constant obtained from the Scatchardt curves [85] and is not a linear function of the receptor concentration.

The binding constant is very high compared with that for other types of phytohormones and is close to those for animal hormones [86, 87]. These data explained previous results [82, 83] indicating that ethylene bound in vivo remains attached to the binding sites even after prolonged purification.

These factors also explain why continuous purging of the binding sites with nitrogen increased the binding because endogenous ethylene bound to EBP was removed before their stabilization.

It has been also shown [88] that the ethylene-binding system is thermally stable and sensitive, at least partially, to proteolytic enzymes. The system has a wide optimum-pH range between 7.5 and 9.5. A study of the effect of ethylene structural analogs on the binding has been found that alkanes are ineffective. On the other hand, a wide range of alkenes and alkynes

inhibit binding. In all instances the inhibition was competitive and reversible. This is consistent with the presence of EBP that apparently involve receptors. Judging from band centrifugation, EBP are localized in the endoplasmic reticulum (ER). The fact that the EBP-site band is located in the medium with density 1.175 g/cm^3 and decreases upon treatment with EDTA, which dissociates ribosomes and coarse ER, confirms this. Furthermore, the two basic marker enzymes ER-NADH and NADPH-cytochrome C reductase are also detected in these bands. Results from differential and band centrifugation were confirmed by autoradiography [89]. Developing cotyledons were treated with ^{14}C-ethylene. Tissue slices were fixed by OsO_4, which reacts with C=C double bonds, to immobilize radioactive ethylene in the tissue after nonbound ethylene was removed. Investigation of the slices by light- and electron-microscopic autoradiography unambiguously has confirmed that EBP are located in ER and protein globules of the membranes.

Solubilization was used for further isolation and purification of EBP located in the membrane [90, 91]. Solubilized preparations with the ability to bind ethylene were produced in experiments with triton X-100, triton X-114, and octyl glucoside. The properties of the solubilized EBP were similar to those of membrane-bound proteins except that the sensitivity to a pH change was decreased and the dissociation and association rates decreased. The affinity and specificity for ethylene and its analogs did not change.

A determination of the Stokes radius and the sedimentation coefficient of the complex of the binding sites with detergent indicates that the molecule is highly asymmetric with a molecular weight of 50 kDa although the molecular weight of the protein itself may be significantly less if associated detergent molecules are taken into account. Highly purified EBP are available at present. The isolation involves a combination of size-exclusion chromatography over sephacryl, fractionation in the presence of detergents, pH precipitation, and HPLC [92].

Efforts to isolate and characterize EBP continued. For EBP in particular, it has been found that several compounds compete with ^{14}C-ethylene for the binding site without inducing a physiological response, e.g., 1,3-butadiene. However, furan acted as an ethylene analog by both its competition with ^{14}C-ethylene and its physiological response [93, 94].

Carbon dioxide also inhibits ethylene activity in vivo but does not inhibit ^{14}C-ethylene binding in vitro. Research on ethylene binding with bean-runner tissues detected three binding proteins that differ in dissociation rates [95]. The protein with the shortest dissociation half-life (2-15 min) disappeared before homogenization; that with a half-life of 1 h, after homogenization. The protein with the longest dissociation half-life (50 h) was present in seeds and was similar in many ways to the well characterized membrane-bound protein from *P. vulgaris* [96]. The long half-life facilitated purification and enabled radiolabeled ethylene to be used to determine EBP in vivo. Despite the hydrophobicity of the protein, researchers [97] achieved a 162-fold purification of EBP by anion-exchange chromatography (DEAE-sepharose and FPLC-mono Q) of proteins solubilized by triton. Pure protein was obtained after chromatography over an immuno-selective column to remove traces of phytohemagglutinin. According to the literature, this is a copper-containing glycoprotein. It has been found previously [98] that ethylene is bound highly specifically to protein receptor sites by a transition metal, presumably copper, located at the active center.

Antibodies to this protein were used to study the ethylene-binding sites of pea [99]. However, highly purified material could not be obtained without affinity chromatography. Ethylene-sensitive tissues containing EBP were also found in apple (*Malus pumila*) and bindweed (*Convolvulus*). However, the sensitivity to ethylene and the degree of binding to it were not correlated [100]. Ethylene-sensitive and -insensitive mutants of *A. thaliana* [101] are apparently promising as effective tools for studying ethylene receptors. Five receptors were determined in the ethylene-sensitive mutants. These are two-component signal proteins [102]. The hydrophobic N-terminus of the receptor site binds etheylene whereas a mutation in this domain prevents ethylene binding and makes the plant insensitive to it. The C-terminus of the receptor site is similar to bacterial histidinekinases. However, differences are noted. The site of phosphorylation includes His^{353} at the receptor whereas histidinekinase involves histidinic acid. A model was proposed based on genetic data wherein ethylene binding inhibits signal transduction. However, the exact function of these receptors is unclear. They participate in detecting the hormonal signal by regulating cell responses to ethylene. The receptors may ensure that the ethylene is detected accurately. Each of the proteins has a different binding affinity for ethylene and activity level, e.g., when acting as kinases or phosphatases during ethylene binding [103]. Certain EBP and ethylene can interact with other molecules.

The main problem after isolation of receptor proteins is to elucidate the mechanism of their action at the subcellular or biochemical level. In contrast with animal hormones and their receptors, the relationship of the observed physical parameters of EBP in plants and the properties of the functional receptor must be found. With respect to affinity and specificity, binding proteins in *P. vulgaris* and *P. aureus* can be viewed as likely candidates for functional receptors for which an ethylene binding

constant of 6.4×10^{-10} M in the aqueous phase corresponds to an ethylene concentration of 0.14 μL/L in the gas phase.

Ethylene concentrations in the gas phase that usually give the threshold, half-maximum, and saturated response in the systems are about 0.01, 0.1, and 1.0 μL/L, respectively. Thus, a concentration of 0.14 μL/L, which corresponds to the half-saturated binding sites, agrees well with the expected 0.1 μL/L.

Analogously, the inhibition constant for structural analogs is close to the concentrations required to give the half-maximum effect in physiological tests in pea [98] and other plants [104]. Furthermore, alkanes, which do not influence development, are equally ineffective at inhibiting ethylene binding to the corresponding EBP sites.

The association and dissociation rate constants do not agree with those for ethylene receptors in certain test systems such as root growth, where the influence of ethylene is apparent soon after applying the growth regulator and ceases when the regulator is removed [105]. In other test systems the influence of ethylene develops during the same time interval but not as quickly.

The subcellular location of binding sites is also consistent with many known effects of ethylene on the biochemical level where the secretion or synthesis of proteins determines, e.g., leaf wilting [106]. Moreover, it can be assumed that ethylene-binding activity in *P. vulgaris* is associated with the initiation of a specific biochemical response. The binding sites can then be viewed as functional receptors.

The situation is complicated by the fact that the function of ethylene in the system described above is unknown. Two test systems were investigated. These were the zones of falling and ripening fruits, in which certain primary effects initiated by ethylene are reliably known [107]. The concentration of binding sites in these tissues was very low, which prevented using them as model systems. A significant affinity between proteins from the falling and fruit tissue zones was found only by greatly increasing the detection limit for binding sites, e.g., by using antibodies to EBP from *P. vulgaris*.

Water-soluble EBP until now have not been investigated. It has been noted previously that EBP are located mainly in cytoplasmic membranes and are not transported into the nucleus. Water-soluble EBP that transfer freely into cytosol should exist along with membrane-bound ones. We simultaneously with other researchers [108, 109] have found that such proteins exist. However, proteins located in the cytosol fraction of pea sprouts were rapidly dissociating receptors (lifetime ~5 min). Water-soluble proteins of the cytosol fraction from cotton sprouts were precipitated by adding ammonium sulfate up to 70% of saturation and were isolated on hydrophobic sorbent Polykhrom-1 using a stepped ethanol gradient. The resulting fractions were analyzed for affinity for ^{3}H-ethylene. The fraction eluted by 70% ethanol had the greatest affinity. EBP isolated fron cotton sprouts formed complexes that were stable over time with labeled ethylene. This property was used to isolate EBP using ^{3}H-ethylene as a radioactive tracer.

The fraction eluted by 70% ethanol was saturated with ^{3}H-ethylene, incubated for 12 h, purged with inert gas to remove nonbound ethylene, and isolated on a TSK HW-50F column. The protein was determined spectrophotometrically and radiometrically. The radioactivity level showed that the fourth peak had affinity for ethylene.

Fraction 4 migrated upon electrophoresis in the presence of SDS as a single band of molecular weight about 24 kDa. The EBP binding constant with ^{3}H-ethylene determined in Scatchardt coordinates was 1.2×10^{-9} M. The numbrer of binding sites was 12.4×10^{-12} M/mg of protein. The isoelectric point of the isolated protein was 3.9. Amino-acid content of the water-soluble EBP from cotton sprouts was analyzed using HPLC over a C_{18} Ultrasphere column. The quantity of amino-acid residues (nmole) was: Asx 15.2(15), Tyr 15.9(16), Glx 14.0(14), Val 7.4(8), Ser 3.0(3), Met 7.9(8), Gly 9.5, Ile 22.9(23), Thr 5.7(6), Leu 15.4(14), Ala 7.9(8), Phe 18.2(18), Pro 14.2(14), Lys 14.6(15), His 1.2(1), Cys 6.2(6), without counting Trp [70].

The isolated protein can be considered a probable candidate for the ethylene receptor owing to its high affinity for it. The occurrence of the EBP in the membrane and the cytoplasm of plant cells suggests the existence of independent mechanisms of action of ethylene in the membrane and in the cell genetic apparatus [73].

Thus, the incorporation of these levels into the response of the cell to the hormone is characteristic of ethylene, like for other phytohormones. A study of the interaction of EBP with membranes labeled with fluorescent probes has shown that the protein, although exhibiting a bias toward the membrane, in contrast with CBP, is not a membrane receptor [110].

In fact, the highest level of RNA synthesis in a model system with isolated chromatin is attained upon adding the hormone—receptor complex to the incubation medium. The activation of these processes is less evident upon adding ethylene or EBP and is caused by formation of hormone—receptor complexes using endogenous EBP or ethylene and depends on their concentrations.

The results for the functional activity of water-soluble cotton EBP that indicate a high affinity for ethylene and activation of transcription processes in a model in vitro system confirm that the isolated protein acts as a receptor [111].

GIBBERELLIN-BINDING PROTEINS

More than 70 gibberellins (GBA) are known [112]. Two factors are responsible for the existence of so many forms and their different activity levels. In different plants, GBA metabolism can occur differently and can determine the species-specific GBA. Gibberellins regulate plant ontogenesis stages such a growth stimulation, flowering, parthenocarpy induction, sex expression, etc. Their effect on formation of cotton fiber is especially interesting. The following gibberellins were isolated from cotton: GBA_1, GBA_3, GBA_4, GBA_7, GBA_9, and GBA_{13} [113].

IAA, GBA_3, and kinetin simulate the incorporation of ^{14}C-glucose into cellulose of the primary (7 days after flowering) and the secondary (22 days after flowering) cell wall of cotton fiber [114]. A study of the effect of auxins and gibberellins on fiber formation in cotton has shown that these phytohormones affect the formation and growth of fiber independently of each other [115, 116]. A series of studies on the role of GBA in the regulation of cotton-fiber formation demonstrated the importance of gibberellin. It has been found that GBA_3 can stimulate significantly fiber formation [117-119]. The significance of GBA was studied in most detail [115, 120]. It has been found that GBA_3 stimulates the synthesis of cotton-fiber cellulose and blocks inhibition by kinetin and abscisic acid (ABA). IAA under these same conditions stimulates to a lesser extent cellulose synthesis and blocks inhibition by only ABA. The dominant role of GBA in the regulation of cotton-fiber cellulose synthesis has been determined [121].

The mechanism of action of GBA, like other phytohormones, is apparently similar and analogous to the action of steroidal hormones in animals [6]. Gibberellins, like other phytohormones, affect the genetic apparatus of plant cells [122-124].

The relationship of GBA_3 physiological activity to DNA synthesis from lettuce hypocotyls was first described by Ginzburg and Kende [125]. Apparently GBA_3 acts through interaction with specific gibberellin-binding proteins (GBP). A ^3H-GBA_1-GBP complex was isolated using ^3H-GBA_1 of specific activity 50 Ci/mmol as a marker. This complex is stable for a long time. The dissociation half-life is 38.5 h at 0°C and 9 h at 18°C. Treatment with DNA-ase, RNA-ase, and phospholipase did not decrease the binding level. However, treatment with protease and heating destroyed the complex. Also, competitive release of ^3H-GBA_1 from the complex was observed with addition of a 100-fold excess of labeled GBA_1 and GBA_5 whereas biologically inactive GBA_8 did not have this capability [126].

Water-soluble GBP from hypocotyls of cucumber (*Cucumis sativus*) were studied in more detail. Proteins from supernatant obtained after centrifugation of homogenate at 100,000 g were precipitated by 60% ammonium sulfate and chromatographed over Sephadex G-50 [127]. The binding activity of these proteins with ^3H-GBA_4 was investigated. A correlation of the competitive activity of several giberellins with their physiological activity was observed. It is interesting that IAA at a concentration of 6×10^{-8} M inhibited by 40% ^3H-GBA_4 binding to GBP. Analysis of the Scatchardt curves revealed the presence of saturated ($K_d = 10^{-7}$ M) and unsaturated components at the GBP site. The concentration of binding sites was 0.37×10^{-12} M/mg of soluble proteins or 0.78×10^{-12} M/g of fresh tissue. The dissociation half-life was much shorter than for GBP from pea, about 10 min at 0°C. The specific activity was maximal at pH 7.5.

The concentration of GBA-binding sites was 1/1000 of the concentration of animal steroidal hormone receptors. Studies of GBP must differentiate receptor proteins from proteins involved in phytohormone metabolism. Thus, GBP from corn can catalyze conversion of GBA_1 into GBA_8.

The assumption that gibberellins can act as quasisteroidal hormones led to a search for GBP in membranes.

The action of phytohormones including GBA on plant cells is considered to involve specific receptors in plasmic membranes whereas hormone binding changes the structure of certain membrane sites [128, 129]. It was hypothesized [130] that the GBA_3 binding site contains membrane lipids and that the membrane receptors of phytohormones are proteins. Other researchers suggest that gibberellins, auxins, and cytokinins influence the functional activity of chromatin and activate differently the gene through the receptor [131, 132]. The receptor molecule is apparently a protein that, on one hand, recognizes (binds) photohormones and, on the other, acts as chromatin acceptor where the activated genes are located [133].

Reviews about the isolation and function of GBP were published in the 1980s [15, 134, 135]. Structural features of soluble cytoplasmic GBP were first described in 1985. However, little progress has been made in purifying and characterizing GBP [136]. Binding of GBA to soluble protein from corn leaves has also been reported. However, it is irreversible and exhibits a low selectivity for active and inactive GBA [137].

Attempts to identify GBP by photoaffinity labeling using azido-GBA_4 were also unsuccessful. Only 15% of the incorporated radioactivity was found on labeled GBA_4. The hormone—receptor complex also could not be identified by electrophoresis and autoradiography [138].

Antiserum agglutinated upon immunization by monoclonal antibodies to GBA derivatives and during GBP screening in these monoclones with aleurones of oat protoplasts. It also inhibited GBA induced synthesis of α-amylase [139].

The research results lead to the conclusion that GBP are located on the external side of plasma membranes. GBA stimulates the appearance of protein that binds to the DNA genome in rice [140].

GBP from cotton ovaries were isolated by affinity chromatography. GBA_3 was selected as the ligand. Its function is most studied in plants, including cotton [141]. The primary criterion of GBP biological activity is activation of RNA synthesis in the presence of GBA.

An alternative method for isolating GBP is based on the affinity of the hormone—receptor complex for DNA. The total acidic soluble proteins were separated by affinity chromatography over two columns with DNA—cellulose. DNA-binding proteins (DBP) with an affinity for immobilized DNA regardless of the presence of gibberellins in the fraction were sorbed on the first DNA—cellulose column. Proteins not bound to sorbent in the first column were placed on the second column after preliminary addition of gibberellin to a concentration of 10^{-5} M. The DNA—cellulose in the first column bound practically all DBP. Therefore, the GBP fraction was sorbed in the second column.

Electrophoresis in SDS—PAAG of proteins from the GBA_3—DBP fraction has shown that they consist of one protein with mass 160 kDa. Under reducing conditions (with β-mercaptoethanol), this decomposes into two polypeptide fragments of mass 71 and 78 kDa. The isoelectroc point of the isolated protein is 4.6.

A study of the effect of the resulting fraction on RNA synthesis by isolated nuclei has shown that its complex with gibberellin stimulates RNA synthesis by isolated cotton cell nuclei. In protoplasts, it induces a group of unidentified proteins and stimulates cellulose synthesis by the cell membrane [142].

The N-terminus amino acid residues for the light and heavy chains are Asp and Glu, respectively. The amino-acid composition of GBP determined by HPLC of amino-acid TFA-derivatives (nmole) is as follows: Asx 130.3, Met 10.2, Thr 49.7, Ile 50.8, Ser 51.2, Leu 69.0, Glx 118.3, Tyr 23.1, Pro 38.0, Phe 136.0, Gly 61.1, His 26.6, Ala 67.2, Lys 70.1, $^{1/2}$Cys* 17.1, Arg 41.2, Val 41.4, Trp 14.1. Trp was determined by hydrolysis with toluenesulfonic acid; Cys*, by extrapolation to zero time (after hydrolysis in 5.6 N HCl for 12, 18, and 24 h).

The quantitative properties of GBP affinity for GBA_3 were found by determining the hormone—receptor dissociation constants of the complex by affinity chromatography. According to frontal analysis, K_d of the complex is 1.1×10^{-4} M; K_d of free GBA_3, 0.85×10^{-7} M.

The resulting data [143] indicate that GBP from cotton seeds is a glycoprotein that contains D-mannose and 2-amino-2-deoxy-D-galactosamine in approximately equal amounts. The overall sugar content is 27%.

At present definite proof that GBP are receptors and not proteins involved in metabolism and gibberellin biosynthesis has not been obtained. This question can be resolved by using labeled GBA of high specific activity. The performance of these studies should consider that many tissues rapidly metabolize ^{3}H-GBA_1. Also, the difference between physiological GBA concentrations that stimulate growth and the affinity of the hormone for the receptor should be explained.

ABSCISIN-BINDING PROTEINS

Abscisic acid is a phytohormone that acts as an inhibitor and is present in practically all plant tissues. It is an antagonist of growth-stimulating hormones. ABA is distinctly polyfunctional. It suppresses growth, induces aging processes, participates in the regulation of seed and bud death, and also is involved in adaptation of plants to stress. Studies of the molecular mechanisms of action of ABA can provide valuable information about the genetics, selection, and biotechnology of plants. These data can be used to produce new varieties with economically valuable features such as early leaf loss, increased salt resistance, resistance to pathogens, etc. [143].

The regulation of aging and loss of leaves and other organs is of special interest for agriculture. It has been proposed that ABA is a compound that triggers these processes. The literature contains limited data suggesting the existence of specific binding proteins for this acid that are receptors in various tissues and organs. Thus, proteins binding ABA were observed in the plasmalemma of stomatal cells of *Vicia faba* [144]. These proteins were later characterized as ABA receptors. The amount of ABA bound to them increased not only upon water stress but also under the influence of proline [145].

ABA-binding proteins (ABP) with K_d for ABA of $3\text{-}4\times10^{-9}$ M were observed in protective cells of *V. faba* epidermis [146]. Electrophoresis in SDS—PAAG revealed three types of binding proteins: A (mass 20.2 kDa), B (mass 19.3 kDa), and

C (mass 24.3 kDa). ABA in alkaline medium was bound primarily to protein A. Most ABA at acidic pH interacted with proteins B and C. This result agrees well with the fact that ABA induces stomata closure at both alkaline and acidic pH values.

A single receptor protein common to ABA and 6-BAP that is localized in the cytoplasm of pumpkin cotyledons has been found [147, 148].

Two types of ABA receptors for rapid and slow plant responses to hormones have been proposed [149]. The rapid responses (<5 min) are induced only by 1-ABA. The site of action is the plasmalemma, i.e., cell membranes. The slow responses (>30 min) include RNA and protein synthesis. For this, S- and B-ABA are equally effective. Therefore, it was proposed that receptors for these types should differ and a rapid response to ABA should not always be preceded by a slow one.

Affinity probes for detecting and purifying ABP were prepared by combining ABA with carrier proteins (ovalbumin or BSA) through the C_1 or C_4' carboxylic groups. IFA found that these ABA—protein conjugates bind to fractions of solubilized microsomal proteins of *A. thaliana* but do not interact with soluble proteins. Heating or proteolysis inhibits conjugate binding. This indicates that the binding site is a protein. It has been observed after purifying the microsomes that the binding sites are located mainly in the plasmic membranes. Conjugate binding is independent of the carrier protein and method of ABA—protein binding but anti-ABA monoclonal antibodies compete and inhibit it. Furthermore, competitive inhibition to conjugate binding by a phytohormone, inactive ABA methyl ester, has been shown that the binding is specific and saturated. Conjugate binding was clearly correlated with the ABA/carrier-protein mole ratio. This confirmed that the affinity of the conjugates for ABP increases upon increasing the amount of phytohormone [150].

ABP were isolated using several photoaffinity agents based on anthracenone. These compounds exhibited hormonal activity similar to ABA. However, they inhibited binding at concentrations 3-4 times less than ABA [151].

A protein fraction with affinity for ^{3}H-ABA was isolated from water-soluble proteins of 3-day cotton sprouts by gel filtration over Sephadex G-50 and affinity chromatography over an ABA-agarose A-6 conjugate. Further separation of this fraction by hydrophobic chromatography over Polykhrom-1 using an isopropanol gradient produced three proteins, of which Γ_3 had the greatest affinity for ^{3}H-ABA. According to electrophoresis, Γ_3 gives a single band for a protein of mass 48 kDa. The protein in the hormone complex did not activate RNA-polymerase, i.e., was not a true ABA receptor. However, the membrane fraction yielded a protein of mass 70 kDa with high affinity for ^{3}H-ABA ($K_d = 5\times10^{-9}$ M) [152, 153].

Affinity chromatography over biospecific ABA-sepharose 4B also produced ABP from cotton sprouts [154]. Electrophoresis in PAAG has been shown that this fraction contains ABP of mass 19-20 kDa. Scatchardt analysis revealed two ABA binding sites, one with low capacity and high specificity ($K_d = 5\times10^{-10}$ M) for ABA binding; the other, high capacity and low specificity ($K_d = 3\times10^{-5}$ M).

The interaction of cotton ABP with three classes of phytohormones was studied [155]. It has been found that ABP bind to 1-NAA nonspecifically; to 6-BAP, specifically. A correlation between the content of ABP and the periods of leaf loss for varieties and lines of cotton sprouts with different periods of leaf loss was observed. Varieties and lines of cotton sprouts with early leaf loss contain on average 1.5-2 times more ABP than those with medium leaf loss and up to 40 times more than those with late leaf loss [156, 157].

Thus, methods for studying the structure and function of hormone-binding plant proteins are analyzed based on literature data. The use of phytohormones labeled with radioactivity made it possible to identify specific hormone-binding proteins in plant tissues. It has been found that higher plants contain two classes of receptor proteins, membrane-bound and cytosolic. Their identification revealed certain molecular aspects of phytohormone uptake and signal transduction. Further success in this area depends on collaborative efforts of geneticists, physiologists, biochemists, and specialists in the field of bioorganic chemistry.

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