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MOLECULAR MECHANISMS OF THE REGULATORY ACTION OF PHYTOHORMONES AND DEFOLIANTS OF THE CYTOKININ TYPE

O. N. Veshkurova, Yu. S. Mangutova, N. Zh. Sagdiev, V. V. Uzbekov, and Sh. I. Salikhov

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A comparative study has been made of the interaction of a number of phytohormones and defoliants with a cytokinin-binding protein from cotton shoots by the method of a competitive receptor test. Methodological approaches to the molecular modeling of processes involved in the interaction of a fragment of the structure of a cytokinin-binding protein with the compounds under investigation have been developed.

As is known, cytokinins possess a broad action spectrum, regulating many physiological processes in plants [1]. Particular interest is presented by investigations of the regulatory action of cytokinins on leaf shedding in the cotton plant. Thidiazuron (Dropp) [N-phenyl-N-(1,2,3-thiadiazol-5-yl)urea] — one of the most effective defoliants — exhibits a high cytokinin activity, exceeding that of benzylaminopurine (BAP). With respect to cytokinin activity, determined in various biotests, the phytohormones and defoliants that have been investigated may be placed in the following order: thidiazuron > BAP > isopentyladenine > zeatin > kinetin [2].

Using the competitive receptor test method, we have made a comparative study of the interaction of a number of phytohormones and defoliants with a cytokinin-binding protein (CBP) isolated from cottonplant shoots [3]. In the test system used we determined the binding of ^{3}H-BAP with the CBP by filtration on nitrocellulose filters, and investigated in parallel the competitive influence on binding of a number of other compounds exhibiting cytokinin activity (Table 1). The order of inhibition of the binding of ^{3}H-BAP with the CBP is as follows: BAP > thidiazuron > cytodef > isopentyladenine (IPA) > serotonin > kinetin. It can be seen from the results obtained that one of the best inhibitors of the binding of ^{3}H-BAP with the CBP is thidiazuron. An analysis of the structures of these compounds showed that a common feature was the —N—C—N— grouping, which apparently determines their physiological activity.

There has been a simultaneous development of methodological approaches to the molecular modeling of the processes involved in the interaction of a fragment of the structure of the cytokinin-binding protein with low-molecular-mass ligands, using the method of fixing the partners with the optimum structure according to energetic parameters. For molecular modeling we used the structure of the peptide BAP — the binding site of the wheat CBP isolated by Brinegar [4], which showed that a histidine residue close to the C-end was modified by a photoaffinity reagent:

Ala-Phe-Leu-Gln-Pro-Ser-His-**His**-Asp-Ala-Asp-Glu.

The question arises why only one of the two histidine residues adjacent to one another is bound selectively with the BAP derivative. Calculation by the Chou-Fasman method [5] of the α- an β-helices formed by this polypeptidehas shown that only **His** (8) is energetically accessible for modification by 2-azido-^{14}C-BAP (Fig. 1).

In the calculation we used the conformational parameters P_α, P_β, and P_c given by the authors, which characterize the tendency of amino acid residues for being included in α-helix and β-structure.

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (371) 162 70 71. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 507—509, July-August, 1999. Original article submitted March 15, 1999.

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TABLE 1. Inhibition of the Specific Binding of ^{3}H-BAP (10^{-8} M) with the CBP, Phytohormones, and other Compounds (10^{-4} M), and Energies of their Interaction with the Active Center of the Receptor

Compound	Inhibition, %	E of the complex, kcal/mole
BAP	93.5±3.4	24.97
Thidiazuron	77.2±2.6	-
Cytodef	69.4±3.8	21.64
Isopentyladenine (IPA)	18.3±2.8	22.80
Serotonin	28.3±2.4	27.88
Kinetin	11.4±2.0	37.80
Adenine	4.6±1.1	-
Naphthylacetic acid (NAA)	3.4±1.4	-
Abscisic acid (ABA)	1.2±0.4	-
Gibberellinic acid (GBA)	0.6±0.1	-

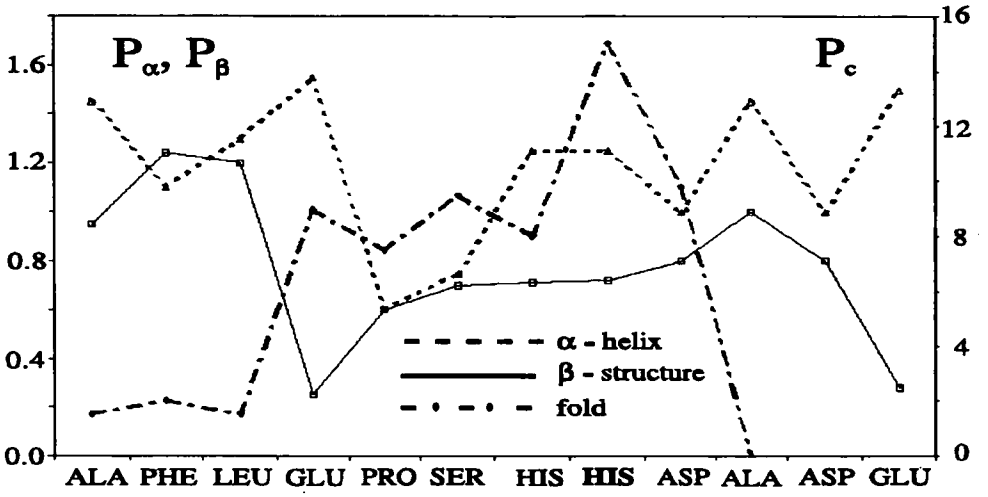

Fig. 1. Calculation by the Chou-Fasman method of the probabilities of the formation of α-helices and of a β-pleated structure of a fragment of the active center of the wheat germ CBP.

Molecular models of the interaction of the phytohormone or its analogs with a histidine residue in the active center of the receptor protein calculated by the PCM-MMX method show that the kinetin and serotonin molecules enter into interaction with the His residue through an H_2O molecule, forming a single hydrogen bond, while BAP and isopentyladenine form two, and tsitodef, three hydrogen bonds, causing a considerable stabilization of the whole structure and lowering the total energy. The energetic characteristics of the hormone-receptor complex are given in Table 2.

The results obtained agree fully with those on the degree of competitive inhibition of the binding of labeled phytohormones and their analogs with the receptor, where BAP and tsitodef are bound most actively with the receptor protein, and serotonin and kinetin least actively. It may be assumed that, on the whole, the model obtained reflects the process of interaction of phytohormones with receptors and may serve as a basis for a test system for screening growth regulators, defoliants, and herbicides of the cytokinin type.

TABLE 2. Energetic Characteristics of a Model of the Hormone-Receptor Complex

Complex	E_{mol}	E_{water}	E_{vdw}	E'_{vdw}
BAP	26.7	-4.5	-4.1	-9.9
Cytodef	25.8	-12.4	-7.4	-12.1
IPA	24.20	-7.7	-5.6	-11.3
Serotonin	25.02	-18.4	-8.3	-12.9
Kinetin	28.19	-12.1	-7.7	-13.0

E_{mol} — total energy of the molecule; E_{water} — energy of the water-solvatation shell of the complex M:H_2O:His; E_{vdw} — Van-der-Waals energy of the surface of the complex M:H_2O:His; E'_{vdw} — energy of the polar region of the Van-der-Waals surface of the complex M:H_2O:His; [E] = kcal/mole.

EXPERIMENTAL

Determination of the Specificity of the Binding of ^{3}H-BAP with Cottonplant Protein. We used the method of filtration on Synpor nitrocellulose filters (Czech Republic). A micro test-tube was charged with ^{3}H-BAP having a specific activity of 960 GBq/mmole. The concentration of the ^{3}H-BAP was chosen in such a way in each case that the final volume of 100 μl contained the required amount of labeled BAP (from 10^{-9} to 10^{-6} mole). Then to each tube was added 10 μl of water or, in the control, 10 μl of a solution of unlabeled BAP or another compounds with cytokinin activity in a concentration of 10^{-4} mole in order to determine the level of nonspecific binding. After this, 80 μl of protein preparation in the buffer for binding was added.

Binding was conducted in 25 mM tris-HCl, pH 7.6. The mixture was incubated at 20°C for 20—40 min. After the end of incubation, with switched-off vacuum, the mixture was deposited on the mat side of a filter previously moistened with distilled water and placed in a filter funnel. Then the vacuum was switched on, the mixture was sucked through, and the filter was washed with 5 ml of water and 5 ml of ethanol. The filters were dried, transferred into bottles, and covered with 3 ml of a standard toluene scintillator. Radioactivity was determined on a Beta-1 counter (Russia).

Molecular modeling was carried out in the PCM-MMX program.

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