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A Role of the Molecular Structure of Phytoregulators in Chemical Signal Perception by Receptors of Plant Hormonal Systems

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Abstract—The role of the molecular structure of phytoregulators in chemical signal perception by receptors of plant hormonal systems and the character of complementarity of a phytoregulator to a receptor have been considered on the basis of (1) the mechanism of signal perception and transduction in a plant cell and (2) the postulates of the physiological paradigm, which lies at the heart of the strategy of chemical design of phytoregulators with specified properties. For regulatory interaction, as distinct from interactions of enzymes with their substrates, topochemical, rather than geometric (a key in a lock), complementarity of a bioregulator to a biotarget is of crucial importance. The action of a bioregulator on a receptor is assumed to be cooperative and quantized. It is shown that molecular parameters of quaternary ammonium salts that determine their antigibberellin (retardant) activity can be used as a measure of topochemical complementarity to a receptor if physiological activities are compared for compounds of the same series (cluster). Submolecular consideration of the physiological activity of a molecule as the sum of the activities of its constituting effector moieties, with taking into account the effect of the moieties that determine polar and hydrophobic binding to a receptor, is suggested as a possible means for developing the QSAR method to make it heuristic.

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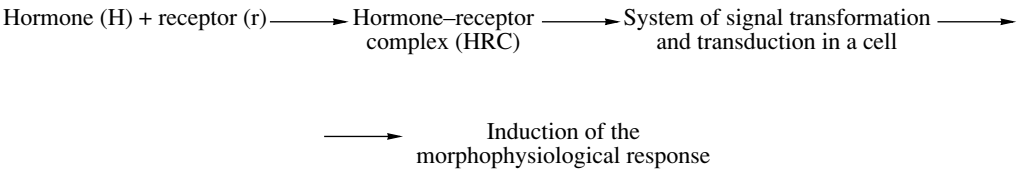
According to [1], hormone signal perception and transduction in a plant cell proceed as shown in Scheme 1. A receptor should meet the criteria [2, 3] common for the hormonal systems of plants and animals. It should uniquely recognize the structure of the hormone molecule and should reversibly bind it, with a high affinity and saturation of the binding sites. In a series of compounds with hormonal activity (phytohormone analogues), it is suggested that their affinity for the receptor is related to their physiological activity. The hormone–receptor interaction should initiate the physiological response typical of a given type of cell.

The approaches developed in [1, 4] make it possible to consider, from a general standpoint, the role of the molecular structure of a bioregulator in perception by receptors of chemical signals from natural phytohormones and their agonists, biomimetics; from antagonists that inhibit the biosynthesis of natural phytohormones; and from blockers of regulatory systems, such as enzyme poisons, e.g., herbicides.

How can the above requirements for a receptor be related to the structure of physiologically active substances interacting with it? Here, similar interactions are exemplified by some phytohormones and their biomimetics and antagonists, as well as by some herbicides and fungicides. To have definite activity, phytoregulator molecules must contain effector moieties [4–7], which



Scheme 1. Hormonal signal perception and transduction in a plant cell.

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can initiate or inhibit the physiological response typical of a given type of cell upon interaction with a receptor.

For auxins, such a moiety is a system of fused or separate aromatic rings binding to a polar group (carboxyl, amide group, nitrile, or hydroxyl) through a methylene, dimethylene, trimethylene, or oxyalkylene bridge [8]. It was recently shown that the minimal (simplest) topochemical analogue of such a structure is the benzyl group binding to the same polar substituents through a heteroatom, attached to the methylene group, and a chain of carbon atoms [9, 10].

For retardants, the effector group is an onium group that inhibits conversion of linear geranylgeranyl pyrophosphate to copalyl diphosphate, which precedes the formation of tetracyclic kaurene, a gibberellin precursor [8]. The antigibberellin activity is manifested by onium salts with a quaternary ammonium, ternary sulfinium, or quaternary phosphonium groups [11]. The 1,2,4-triazolyl and imidazolyl groups and some related groups can also act as the effector moiety that blocks gibberellin biosynthesis. However, they have a different mechanism of antigibberellin activity. Azolyl derivatives inhibit the oxidation of the kaurene C18± methyl group into carboxyl to produce kaurenoic acid, which is further converted to gibberellic acid [8]. Other types of phytohormones and their biomimetics and antagonists also feature their characteristic effector groups.

An important attribute of the interaction of the hormone or its biomimetic with the receptor is saturability of the binding sites. As a rule, the properties and effect of a single molecule are examined when considering bioregulator–receptor interactions. At the same time, it is known that each bioregulatory system has its threshold sensitivity to a chemical or biochemical impact. Signals whose intensity is lower than this threshold are not perceived, and the system does not respond to them. It is reasonable to assume that we are, most likely, dealing with cooperative and quantized action of bioregulators. It is thereby necessary that the receptor simultaneously have several occupied binding sites (cooperativeness) and the number of occupied binding sites be no lower than a definite number (characteristic of a given system) required for initiating the response (quantization).

Another important criterion is the reversibility of hormone–receptor interaction. Binding saturation and reversibility of interaction ensure the more/less operation mode for the signal perception and transduction process, which is crucial for regulation. The regulatory impact should depend not only on the number of occupied sites of the receptor but also, due to the reversibility of the process, on the lifetime of the hormone–receptor complex, i.e., on the residence time of bioregulator molecules sitting on the receptor. Let us define the product of the distribution density on the receptor by the mean residence time of molecules at binding sites as bioregulator moment.

As distinct from the hormone–receptor interaction, the enzyme–substrate interaction occurs in the yes/no mode. To attain the required selectivity, the substrate should bind irreversibly to the active site of the enzyme and should fit into the active site like a key in a lock (geometric complementarity) [10]. The chemical transformation of the substrate, inherent in a given substrate–enzyme pair, gives the chemical signal to release the binding site. The substances that bind irreversibly to the active site of the enzyme and are unable to undergo chemical transformation specific for a given system are enzyme poisons. This is a common property of enzyme systems of plants, animals, and fungi.

Computer modeling of herbicides from three families inhibiting photosystem II in plants—triazines, carbamates, and benzimidazoles—shows that the effector moiety in these compounds is the –C(X)–N–R group (X is carbonyl oxygen, imine nitrogen, or a highly electronegative group (for example, $-CF_3$); R is a hydrogen atom or an alkyl group). It is precisely this group that is responsible for irreversible binding of herbicide molecules and blocks the operation of photosystem II. As a result, photosynthesis ceases, and the plant dies.

The irreversible interaction of the biotarget with the active site accounts for the fungicide activity of 1,2,4-triazole and imidazole derivatives, e.g., *RR* stereoisomer of 4,4-dimethyl-2-(1',2',4'-triazolyl-1')-1-(2",4"-dichlorophenyl)-3-pentanol [15, 16]. The NH group of the azolyl moiety binds irreversibly to the heme of cytochrome P-450. The group is substituted for oxygen, and this prevents the oxidative demethylation of the C14α carbon atom of lanosterol and its conversion to ergosterol, which is the major component of the cytoskeleton of the cell membrane of fungi.

Thus, the specific physiological response to hormone–receptor or enzyme–substrate interaction depends on the effector group in a hormone molecule, its biomimetic, antagonist, or blocker of the enzyme system. In addition, the bioregulator molecule should feature moieties that bind to the hydrophobic and polar sites of the receptor or the active site of the biotarget and ensure the selectivity and prolonged action of the bioregulator. These binding sites impose additional requirements on the structure of both the bioregulator molecule as a whole and its parts. In [17, 18], the retardant activity of peptide analogues of *SS* isomer of 4,4-dimethyl-2-(1',2',4'-triazolyl-1')-1-(4'-chlorophenyl)-3-pentanol, known as paclobutrazole [19], has been modeled. The imidazolyl group of histidine, the isopropyl group of valine, and the phenyl group of phenylalanine mimic, respectively, the 1,2,4-triazolyl, *tert*-butyl, and 4-chlorophenyl moieties of paclobutrazole. The *tert*-butyl group of di-*tert*-butyl pyrocarbonate (Boc protection of the amino group in peptide synthesis) and the benzyloxycarbonyl group (Z protection of the amino terminus of the peptide chain or of the secondary amino group in the imidazolyl moiety of histidine) also function as mimetic moieties: they can mimic, respectively,

the *tert*-butyl and 4-chlorophenyl groups of paclobutrazole. Three peptides of this series with the primary structure Z-His-Boc, Boc-Phe-His(H), and Boc-Val-Phe-His(H), which are topochemically similar to the *SS* isomer of the prototype, show a noticeable retardant activity in standard cucumber hypocotyl bioassay [20]. The key effector moiety that determines the retardant activity is the histidine residue. The presence of this residue in the molecule containing the Boc and Z groups is sufficient for retardant activity to be manifested. The activity increases as the peptide chain is elongated with phenylalanine or the Val–Phe sequence. The transposition of valine and phenylalanine in the peptide chain eliminates retardant activity, so that the resulting tripeptide is a plant growth stimulator. The stereoisomeric structure of this tripeptide is farthest from the spatial configuration of paclobutrazole.

Thus, combining in one molecule the biomimetic effector moiety with other biomimetic moieties necessary for interaction with hydrophobic and polar binding domains at the active site of a biotarget makes it possible to obtain a new compound with a desired physiological activity (provided that the stereoisomeric structure of the biomimetic is close to that of the prototype [4–7, 18]).

It is worth noting that these peptides have retardant activity despite the fact that their molecular weights are more than twice as large as the molecular weight of paclobutrazole. Correspondingly, the molecules of the prototype and its peptide analogues differ in size. To explain this fact, we suggested that the peptide chain is coiled into a conformation suitable for exerting the retardant effect on one of the enzymes of the gibberellin cascade, while the receptor of the biotarget has a structure in which the sites binding to the peptide molecule are rather accessible if the peptide is topochemically similar to the prototype.

However, an analogous pattern (difference in structure and molecular weight and similar physiological activity) is also observed in series of phytoregulators that, as distinct from peptides, have no secondary structure. In particular, in a series of choline and *N*,*N*,*N*-triethylcholine derivatives [21, 24], the difference in molecular weight between chlorocholine chloride and *N*,*N*,*N*-triethylchlorocholine chloride, on the one hand, and the corresponding benzyl ethers of choline and *N*,*N*,*N*-triethylcholine, on the other hand, is 45 and 36%, respectively. However, all four compounds demonstrate virtually the same antigibberellin activity in the bioassay used. The entire series of ethers and halo derivatives have the antigibberellin activity of the same order of magnitude. *N*,*N*,*N*-triethyl choline is a weak retardant because of its low lipophilicity, while choline has no antigibberellin activity. Rather, it stimulates the biosynthesis of gibberellin. It is conceivable that choline fulfils a trophic function in the life cycle of the fungus. Compact molecules of halocholine halides, with high lipophilicity and the most asymmetric electron cloud, are the most active. Presumably, this is due to the lower reversibility of binding to the hydrophobic and polar sites and the higher saturation of binding sites with compact molecules, which should increase the moment, the product of the distribution density on the receptor by the mean residence time of molecules at binding sites.

It is worth noting that the molecular weights of known retardants tri-*n*-butyl-(2',4'-dichlorobenzyl)phosphonium chloride (phosphon D) and *N*-methyl-*N*-(2',4'-dichlorobenzyl)-2-(3'-pyridyl)pyrrolidinium chloride [11] exceed the molecular weight of chlorocholine chloride by a factor of 2.2 and 2.5, respectively; nevertheless, they have high antigibberellin activity.

Auxin biomimetics (N- and O-benzyl-containing compounds [9, 10]) exemplify biomimetic molecules that have considerably more compact structures and lower molecular weights than their functional natural prototype. In particular, benzyl alcohol (MW 118) is almost twice as light as the natural auxin indolyl-3-acetic acid (MW 203), whereas they show similar activities in bean rooting bioassay [12]. Conversely, 2,4-dichlorophenoxyacetic acid (herbicide 2,4-D) (MW 223) as auxin is several orders of magnitude more active than indolyl-3-acetic acid, although their molecular weights are close to each other. This can be due to a considerably lower reversibility of binding of 2,4-D to the auxin receptor and, hence, to an increase in the biomimetic moment. This is likely the physiological reason for the herbicide properties of 2,4-dichlorophenoxyacetic acid and its salts and constitutes the basis for their use.

The above facts allow us to conclude that, for regulatory interaction, as distinct from interactions of enzymes with their substrates, topochemical rather than geometric (a key in a lock) [13] complementarity of the bioregulator to the receptor of the biotarget is of crucial importance. The structure and geometry of a molecule can change but if its effector moiety, the moieties responsible for hydrophobic and polar binding, and the stereoisomeric configuration mimic those of the prototype molecule, the new compound will demonstrate the desired activity.

Topochemical complementarity of a bioregulator to a receptor is inseparably linked with the structural and physicochemical properties of a molecule. The question arises of whether molecular parameters can be a measure of this complementarity. Regression analysis [21] of the contributions of six calculated molecular parameters to the antigibberellin activity of quaternary ammonium salts, determined by a sensitive bioassay using the cell culture of fungus *Gibberella fujikuroi* [22, 23], shows that, for linear derivatives of *N*,*N*,*N*-trimethyl-*N*-(2-oxyethyl)ammonium chloride (choline) and *N*,*N*,*N*-triethyl-*N*-(2-oxyethyl)ammonium chloride (*N*,*N*,*N*-triethylcholine), the polarizability (α), proton-acceptor factor ($C_a^{\max}$), and lipophilicity ($\log P$) make the major contribution to their antigibberellin activity. In both series, the best inhibitors of gibberellin biosyn-

thesis are choline benzyl ethers and halides with maximal partial charges (q_x) on the negative end of the molecular dipole, i.e., with the most asymmetric electron cloud. The antigibberellin activity of sterically hindered *N*,*N*-dialkylpiperidinium salts is mainly determined by the steric parameter E_s, while the polarizability α, proton-acceptor factor $C_a^{\max}$, and $\log P$ are of lesser importance [24]. The scheme of hormone signal perception and transduction in a plant cell implies a relationship between the physiological activity and the bioregulator affinity for the receptor [1]. Then, in the framework of this hypothesis, molecular parameters in the series of quaternary ammonium salts under consideration can actually serve as a measure of the affinity or topochemical complementarity to a receptor if physiological activities are compared for compounds that constitute the same cluster.

In [21, 24], canonical QSAR profiles were obtained for three groups of compounds with antigibberellin activity. It is worth noting once more that benzyl ethers of choline and *N*,*N*,*N*-triethylcholine demonstrate antigibberellin activity at the same level as the chlorocholine chloride reference. At the same time, due to their auxin activity, they are efficient phytoregulators and stress protectors: they increase the resistance of industrial and food crops to meteorological and phytopathogenic stresses and considerably enhance their productivity [25–29] as compared to the known reference compounds. The classical QSAR method as a method of multiparameter correlation analysis cannot be heuristic by definition. Based on the physicochemical QSAR profile of one type of activity (for example, antigibberellin), neither a new quality of compounds (in our case, the concomitant auxin activity of quaternary ammonium salts containing O-benzyl moieties [9, 10]) nor the synergism of the concerted action of these types of activity on the regulatory systems of the entire organism can be revealed [10]. To do this, there is a need to carry out corresponding biotests, to obtain new training samples for computer programs, and to construct QSAR profiles for new types of physiological activity. A new quality of physiological activity and better technical efficiency are achieved by introducing into some molecule a new effector moiety exhibiting the activity that is complementary and compatible to the activity of this molecule. This suggests to us that all important types of activities inherent in a given compound and constituting the spectrum of its physiological activity should be revealed. This approach can be best implemented in automated bioassay (path through test) systems.

A radically new way for developing the QSAR method can be submolecular consideration of the physiological activity of a molecule as the sum of the activities of its constituting effector moieties, with taking into account the effect of the moieties that determine polar and hydrophobic binding to a receptor. In so doing, it is necessary to reveal the types of activity contributed by separate effector moieties to the spectrum of physiological activity of a given series of compounds, to consider the contributions made to a given type of activity by molecular moieties responsible for hydrophobic and polar binding, and to determine the effect of separate moieties on the molecular parameters of compounds. The relationship thus obtained between the structure, molecular parameters, and activity makes it possible to design molecules with desired properties from separate chemical moieties based on the Lego principle. Once the physiological activity spectra of the resulting compounds have been experimentally refined, the structures of compounds with optimal properties for solving a given problem can be determined using classical means of the QSAR method. The physiology of action of the regulatory system related to the problem should be rather well understood. This is a possible strategy for developing the QSAR method to make it heuristic.

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