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Auxin Structure and Activity

By

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S u m m a r y

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The dependence of auxin activity on molecular structure and conformation was studied for indole-3-acetic acid (IAA) and its derivatives bearing alkyl and halogen (F, Cl) substituents at the benzene part of the indole nucleus. In the *Avena* coleoptile section straight growth test, 4,7- and 5,7-disubstitution with bulky Cl-atoms drastically reduced biological activity while C_2 to C_4 alkyl substituents had a more gradual attenuating effect, supposedly by virtue of their flexibility. Conformational effects appear to be absent for the set of compounds evaluated here. More restrictive structural requirements were observed in a second auxin bioassay based on the developmental response of the deseeded pea pericarp.

I n t r o d u c t i o n

Interest in structure-activity relationships for auxins dates back to the very beginnings of plant hormone physiology (THIMANN 1977). Nevertheless, many essential questions on this subject have remained unanswered. When the first "auxin-binding proteins", putative receptors involved in the auxin response, were isolated and their genes cloned (VENIS & NAPIER 1995), it was hoped that the anatomy of the hormone-binding site(s) would soon be defined by unequivocal methods, such as X-ray crystallography. These expectations have so far not materialized. Comparing the structural properties of good and bad auxins has thus remained a useful backdoor approach for deducing the characteristics of the auxin receptor(s) (TOMIĆ & al. 1998). Fortunately, the experimental and computational methods which permit insight into the molecular architecture of auxins have

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significantly advanced in recent years. The results we present herein were obtained in close collaboration with a number of colleagues whose names appear among the authors of joint publications quoted below.

Materials and Methods

Detailed descriptions of experimental procedures may be found in the references cited. In brief, molecular structures in the crystalline state were determined by X-ray diffraction analysis in single crystals. The commercial molecular modeling software used included Biosym DISCOVER-CVFF, MM2, MM3, and TRIPOS; the program GAMESS was employed for ab initio conformational analysis. The oat (*Avena sativa* L.) coleoptile straight-growth test was performed as described by LARSEN 1961. Auxin effects on the development of the pea (*Pisum sativum* L.) pericarp were studied in planta, in deseeded pods (REINECKE & al. 1995, 1999).

Results and Discussion

Based on structure-activity correlations for a large number of synthetic auxin analogues, KATEKAR 1979 proposed an empirical model for the hormone-binding site of the auxin receptor mediating the stem elongation response. Its essential features include 1. an acceptor site for a carboxyl or other acidic group and 2. an electron acceptor site capable of interacting, for example, with the π electrons of aromatic rings. Auxin molecules must thus be flexible enough to interact with both (and possible further) binding sites simultaneously (KATEKAR & al. 1987). Site 2 was visualized in the shape of a shoe which loosely fits the heterocyclic nucleus of the natural substrate, indole-3-acetic acid (IAA; Fig. 1), except for two tight spots: a stretch extending from position 1 to 7, at the juncture of the pyrrole and benzene rings, and a site between positions 4 and 5 of the benzene moiety.

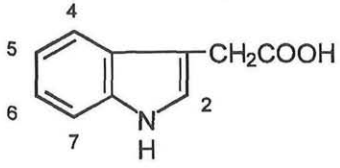

Fig. 1. Indole-3-acetic acid (IAA) including the numbering of the ring positions.

Comparing the molecular structures determined by X-ray diffraction analysis, and elongation-promoting activity in the *Avena* coleoptile section straight-growth test, we were able to provide numerical estimates for the dimensions of the "electron acceptor site", in the above sense. Most IAAs bearing one or two chlorine substituents at the benzene ring are more active than the parent compound, but 4,7- and 5,7-dichloro-IAA are weak auxins (ANTOLIĆ & al. 1999, NIGOVIĆ & al. 1996). With their large halogen substituents, these molecules appear

to barely squeeze in between the "tight spots" in KATEKAR'S model of the auxin receptor's electron acceptor site. The distance between those critical spots may thus be estimated from the distances between the most distant points of the van der Waals shells of the two chlorine substituents in 4,7-dichloro-IAA (6.3 Å) and 5,7-dichloro-IAA (5.4 Å). The investigated set of IAAs alkylated at the benzene ring showed near-identical optimal (~6 x 10^{-5} mol/L) and half-optimal (4 x 10^{-6} mol/L) concentrations (unpublished results from the authors' laboratories), which has been interpreted to mean that their affinities to the receptor protein are about the same. The flexible alkyl substituent thus appears to permit unrestricted interaction with the active site. What was different for the n-alkyl IAAs examined was the optimal response, supposed to reflect the efficacy (KATEKAR 1979) of the auxin-receptor complex in triggering the signal transduction cascade (BARBIER-BRYGOO 1995) which eventually results in coleoptile elongation. While the methyl-IAAs were more "efficient" than the parent compound, 4- and 6-ethyl-IAA reached only about 60% of its optimal elongation response. In the 5-position, an ethyl substituent resulted in about the same efficacy as for IAA itself while an n-propyl and n-butyl group reduced the optimal elongation by 25 and 40%.

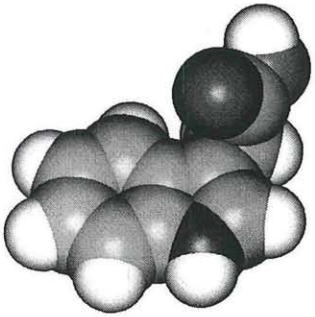

Fig. 2. Molecular structure and ("folded") conformation of indole-3-acetic acid in the crystalline state, as determined by X-ray diffraction analysis.

The folded conformation of IAA shown in Fig. 2 is found in the crystalline state and was determined experimentally (KARLE & al. 1964). In solution, the aliphatic side chain rotates about the two C-C bonds, but only a few conformations are energetically favorable ("local and global minima") and thus highly populated. Detailed insight into these relationships is obtained by computer modeling. Interestingly, a selection of commercial programs, otherwise used with confidence for molecular modeling, afforded, in the case of IAA, significantly different minimal energy conformations, separated by potential energy barriers, the height of which was of a similar order of magnitude as the level of approximation adopted in those programs. Ab initio self-consistent field conformational analysis, which is more accurate, identified the planar conformation as most stable, in addition to several local minima and intermittent energy barriers, all in the range of 10 - 15 kJ/mol (RAMEK & al. 1996). Similar results were obtained for 4-chloro-IAA (loc.

cit.). This is only a fraction of the energy (30 - 40 kJ/mol; estimated from half-optimal concentrations in bioassays) liberated when auxin binds to the receptor involved in the elongation of *Avena* coleoptiles (ANTOLIĆ & al. 1996, 1999). IAA and its derivatives bearing halogen or alkyl substituents at the benzene part of the indole ring should thus be able to turn into the conformation appropriate for binding to that receptor, regardless of their momentary conformation in solution. This is likely to be valid for a wide array of substitution patterns at the benzene ring, while the influence of substitution at the pyrrole moiety requires further study.

In addition to stem (coleoptile) elongation, auxins also affect developmental processes such as the growth of the pea pod which depends on auxin supplied by the seeds (EEUWENS & SCHWABE 1975). These contain IAA and 4-chloro-IAA (MAGNUS & al. 1997). As both auxins are highly active in stem-elongation assays, one might expect that they also act in cooperation in pea pericarps. This is, however, not the case. Treatment with 4-chloro-IAA makes deseeded pericarps grow to near-normal size and shape, application of IAA does not (REINECKE & al. 1995). Of other potent auxins in stem-elongation assays, 4-fluoro-IAA was as inactive as IAA in the pea pericarp. 4-methyl-IAA was about as active as 4-chloro-IAA, while 4-ethyl-IAA was considerably less effective (REINECKE & al. 1999). This suggests that the auxin-specific response-mediating protein in pea pericarps is more sensitive to the size of ring substituents than the "stem elongation receptor" (the van der Waals radius of fluorine exceeds that of hydrogen by a mere 35%, a methyl group is about as large as a chlorine atom). Whether this is due to the presence of "accessory binding areas" (KATEKAR & al. 1987) in the pea pericarp receptor, or to an entirely different signaling pathway, remains to be clarified.

The auxin response in bioassays may be influenced by metabolic and transport processes, a possibility which must always be borne in mind, even though concrete problems have been the exception, rather than the rule. For some of the compounds included in this study, the in vitro binding affinity to *Zea mays* auxin-binding protein 1 (ABP1) was checked and shown to correlate with their auxin activity in *Zea* coleoptile sections (RESCHER & al. 1996).

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