MEETING REPORT

PLANT HORMONES: METABOLISM AND INTERACTION

A report of the FEBS Summer School no. 12 held in Halle (Saale), German Democratic Republic, on 14–18 November, 1969

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Received 22 March 1971

1. Introduction

This Summer School was organized by the Biochemical Society of the German Democratic Republic and the Institute of Plant Biochemistry in Halle of the German Academy of Sciences at Berlin. There were 81 participants from 13 countries, including 13 invited lecturers from 7 countries. The scientific programme contained 15 lectures and discussions on 3 main topics: (1) metabolism, (2) action and interaction, and (3) mode of action of plant hormones. Two general discussions were devoted to modern methods of identification and structural elucidation of plant hormones.

A comprehensive summary of the meeting was given by Professor J.P. Nitsch (Gif-sur-Yvette, France), who said in his concluding remarks:

"First of all let us see if we agree what a growth substance is. I think even if we have not defined it the following is agreed: it is an organic substance which is produced by the plant and which is active in small amounts (in the range of 10^{-7}M) and which controls growth. Especially the definition of gibberellins has been the subject of discussion. The substances which are involved in the control of plant growth can be classified into several categories (which are well known), namely the auxins, gibberellins, cytokinins, ethylene and native inhibitors. The most important native auxin generally distributed within plants is doubtless indoleacetic acid (IAA). Despite this, further research seems to be necessary to clarify whether there are other native auxins in plants. For instance, perhaps indoleacrylic acid may be a naturally occuring auxin in roots, and there are other indications that non-indolic auxins may exist. As for the gibberellins, 28 different compounds from the fungus *Gibberella fujikuroi* (Saw.) Woll. (= *Fusarium moniliforme* Sheld.) have so far been isolated and identified. More new gibberellins, especially conjugated gibberellins, will certainly be found. The native cytokinins zeatin and isopentenyl adenine are widely distributed, sometimes as ribosides or nucleotides. The important question, whether or not the nicotinamide derivative isolated from plant tissue cultures by Wood and Brown is the real cytokinin, must be reserved until its chemical structure has been elucidated. The most important native inhibitor is abscisic acid, which fulfils all the criteria of a plant hormone. Apparently every plant that has been analysed for abscisic acid has been found to contain it. It is not known yet if there are other substances like abscisic acid which perform similar functions. The chemically related phaseic acid possibly arises from abscisic acid during metabolism."

2. Metabolism of plant hormones

In the section on auxin metabolism (Chairman: J.P. Nitsch) the first lecture, Auxin Biosynthesis, was presented by E. Libbert (Rostock, GDR). He reviewed the extensive literature, showing that if tryptophan is the precursor of IAA it can be transformed into indoleacetic acid along various routes.

The most favoured pathway proceeds via indolepyruvic acid and indole-acetaldehyde with some branching off from indolepyruvic acid to indolelactic acid or from indole-acetaldehyde to indole-ethylalcohol. Another possible pathway, which certainly exists in microorganisms but may be not so common in higher plants, involves tryptamine as an intermediate. A pathway which may exist in higher plants, especially in the *Brassicaceae,* involves the rather complicated molecule of glucobrassicine, and indole-acetonitrile may be an intermediate. It is possible that IAA may not actually arise from tryptophan. There is only a little evidence for this, but it should be investigated further; for example, it is possible that anthranilic acid is converted into indoleacetic acid without going through tryptophan; indoleglycerol-phosphate could also be a precursor of indoleacetic acid. As to the site of auxin production in the plant, we know that it is produced in the meristematic tips of the plant, especially in the stem apex and also in developing seeds."

A lecture, Auxin Catabolism, was given by Th. Gaspar (Liège, Belgium). Auxins may be catabolized in plants by oxidative enzymes, but no IAA oxidase is known. IAA, together with many other substrates, is degraded by peroxidases, catalases and phenoleoxidases. There is an extensive literature on the biochemical and different physiological aspects of auxin catabolism. Generally, there seems to be a good correlation between IAA oxidizing activity, IAA content and growth. Thus it can be said that IAA oxidase systems doubtless play an important role in growth regulation. Dr. Gaspar did not review the extensive literature on this subject but discussed the problem by talking about three particular examples experimentally studied in his own laboratory. He showed that in crown-gall tissues native phenolic enzyme inhibitors, for instance 2-hydroxy-1, 3-naphthoquinone, exert an auxin sparing action by inhibiting endogenous IAA oxidases. The known adaptation of roots to exogenous auxin, namely the decrease with time of IAA-induced inhibition, seems to be correlated with an increase of IAA destruction by bacterial laccase. In lentil roots, growth-inhibition by applied kinetin may be caused by increased peroxidase activity, which leads to destruction of the native root auxin (probably indoleacrylic acid). These studies reported by Th. Gaspar, together with many other results, strongly indicate that IAA oxidase activity measured in vitro is of little significance. All constituents of this complicated system must be studied separately under optimal conditions.

The second day was devoted to gibberellin metabolism (Chairman: J. MacMillan, Bristol, Great Britain). Gibberellin biosynthesis was extensively reviewed by J.E. Graebe (Göttingen, GFR). Using the fungus *Gibberella fujikuroi* or cell-free preparations from higher plants, the general pathway of gibberellin biosynthesis has recently been elucidated. Starting from mevalonate, the main precursor of the terpenoids, the synthesis proceeds via farnesyl pyrophosphate, and from farnesyl pyrophosphate the pathway can branch into the production of steroids or into the production of geranylgeranyl pyrophosphate; this again can branch into the pathway of carotenoid synthesis or of kaurene synthesis and the synthesis of gibberellins. Some of the reported gibberellins may be steps in a sequence, for example, gibberellin A_{12} may be the precursor of gibberellin A_{14} which, in turn, may be the precursor of other C_{20}-gibberellins (GA_{13} etc.) as well as of the C_{19}-gibberellins. The final stage in *Gibberella* cultures is GA_3 which arises from GA_7 by hydroxylation or possibly also from GA_1 by dehydrogenation. Most of the sequences before the "kaurenoic step" and the biogenetic relations between the different endogenous gibberellins are rather unclear, especially in higher plants. Some data have been obtained on the enzyme systems involved. Certain inhibitors are important for biogenetic studies as well as for practical purposes, for instance, CCC and AMO-1618 which inhibit cyclization of *trans*-geranylgeranyl pyrophosphate to copalyl pyrophosphate.

The metabolism of gibberellins excluding biosynthesis was the topic of the next lecture by H. Kende (East Lansing, USA). He reported data obtained in extensive studies in his laboratory and by some other groups in the institute of Prof. A. Lang, on the fate of radioactive gibberellins in plants. He pointed out that these studies are of a physiological rather than a chemical nature and were subordinated to a main problem, that is to find out the site of action of gibberellins and to get information on their mode of action. The first requirement for metabolic studies is a source of radioactive gibberellins of high specific radioactivity and high biological potency. The methods elaborated by Kende for preparing ^{3}H-GA_1 with a specific activity up to 1 Ci/mmole by partial hydrogenation of GA_3 and its chemical transformation to

^{3}H-GA_5 are superior to methods formerly used, namely Wilzbach-tritiation or biosynthetic labelling in *Gibberella fujikuroi* cultures by feeding ^{14}C-mevalonate or ^{14}C-acetate. Studies on the fate of these radioactive gibberellins in growing dwarf pea seedlings showed that their metabolism is not very fast and reaches a maximum 25% in 24 hr. Despite their different biological activity in light- and dark-grown peas, GA_1 and GA_5 had a similar metabolism under both conditions. The results suggest that the gibberellins A_1 and A_5 are active per se; further conversion is unnecessary.

In ripening pea seeds exogenously applied ^{3}H-GA_1 and ^{3}H-GA_5 are laid down in water-soluble forms which, in the case of bound GA_1, are hydrolyzed in seeds during germination giving a less polar compound soluble in ethyl acetate. One question is still open: Are the gibberellins catabolized in plants and how are they degraded by microorganisms? Studies on the cellular fate of gibberellins have so far given no positive indication of their site of action. H. Kende found that the radioactivity of ^{3}H-GA_1 or ^{3}H-GA_5, applied to growing peas, is associated only with compounds of low molecular weight. There is no evidence of a strong attachment of gibberellins to anything that is larger than molecular weight 700 and there is also no evidence of any sizeable amount of radioactivity in subcellular particles.

Another aspect of gibberellin metabolism was reported by G. Sembdner (Halle, GDR), who talked about Conjugated Gibberellins. This term is proposed for gibberellins bound to other molecules of low molecular weight by covalent binding whereas bound gibberellins sensu strictu should be defined as gibberellins bound to macromolecules, for example proteins, or attached to cell structures. So far, 5 native conjugated gibberellins have been isolated and structurally elucidated: *O*(2)-acetyl GA_3, from *Fusarium*, GA_3 *O*(2)-β-D-glucopyranoside from *Pharbitis*, GA_8 *O*(3)-β-D-glucopyranoside from *Phaseolus* and *Pharbitis*, as well as GA_{26} and GA_{27} *O*(3)-β-D-glucopyranosides from *Pharbitis*. Probably gibberellin glycosides are widely distributed in plants. Exogenously applied gibberellins are also converted into glycosides, at least in part. Therefore glycosylation seems to be a common and important reaction in gibberellin metabolism of plants. The glycosylation leads to reversible inactivation of gibberellins. The biological potency of gibberellin glycosides depends on their enzymatic hydrolysis in plants. They may have a depot function, as could be shown with beans, and they may be favoured translocation forms, for example in the bleeding sap of trees.

The next lecture was given by V.F. Kucherov (Moscow, USSR), on Chemical Transformation of Gibberellins and Related Systems. Prof. Kucherov described some photochemical reactions of the 2-ketone of gibberellic acid methyl ester, performed in his own laboratory. By ultraviolet radiation this compound gives derivatives with an aromatic ring A, some of which possess phenolic character. Thus, using this method it seems quite possible to obtain derivatives and degradation products of gibberellins of biological interest, perhaps at least as models for natural catabolic pathways in plants.

Under H. Kende's chairmanship the cytokinin field was represented by lectures from K. Mothes (Halle, GDR) on the Chemistry and Biosynthesis of Cytokinins and L. Engelbrecht (Halle, GDR) on Physiological Activity and Natural Occurrence of Cytokinins. Prof. Mothes discussed the chemical structures of naturally occurring cytokinins such as zeatin, isopentenyl adenine and a new adenine derivative found in bacteria with an isoprenoid side chain in 6-position and a thiomethyl group in 2-position. Some alkylated adenines have been identified in tRNAs. Furthermore, an extensive survey was given on the structures and biological activities of different types of artificial cytokinins (adenine and pyrimidine derivatives and some other non-purines, for instance diphenyl urea, benzimidazole derivatives and phenyl urethanes). It is known that the isoprenoid side chain of natural cytokinins is synthesized from mevalonic acid. The question whether the native free cytokinins are products obtained only by destruction of tRNA cannot be answered exactly, but there are some indications that this pathway cannot be the main source of soluble cytokinins in the higher plants. For instance, there are some differences with regard to the side chain stereochemistry e.g. of zeatin, depending on the source of this cytokinin: free zeatin shows *trans*-configuration at the side chain double bond and that from tRNA *cis*-configuration, although an isomerisation step has not been excluded.

Dr. Engelbrecht dealt with important physiological aspects concerning the action and natural occurrence of cytokinins in plants. Thus, she gave instruc-

tive examples of how cytokinins induce cell division in different tissues, prevent chlorophyll degradation and yellowing in senescent leaves and act as attractants to several metabolites. Another characteristic effect of cytokinins is the promotion of buds by overcoming their dormancy or by releasing them from apical dominance. Cytokinins are active in influencing the resistance of plants against all manner of stresses, e.g. dryness, heat, high salt concentration and ultraviolet radiation. Most of these cytokinin effects, and others, have been the basis for the development of bioassays. Little is known about the distribution of cytokinins within plant organs and tissues and nothing at all is known about distribution within the cell. Furthermore, it is not yet known whether a high cytokinin level is necessarily of great physiological significance. In this connection, Dr. Engelbrecht presented interesting studies on the cytokinin contents of bean plant organs at different stages, and of green islands of autumn leaves from several trees and their causal agents (caterpillars and fungi).

Prof. E. Libbert was the Chair for the two subsequent reports on natural and synthetical inhibitors. Prof. P.F. Wareing (Aberystwyth, Great Britain) gave a complete survey of abscisic acid (ABA), which included most of his own fundamental work in this field, and a detailed discussion of the question: Is ABA functioning as a native plant growth regulator and how does it act? Anticipating the results, much information is available mainly from studies with exogenously applied ABA and from investigations on biosynthesis, translocation, and distribution of endogenous ABA, but no clear answer is yet available, especially as to the mode of action.

ABA is active at concentrations which normally are regarded as hormonal concentrations. ABA acts as a growth inhibitor when applied to growing tissues (by inhibiting both cell division and extension) and acts as a promotor of catabolic processes when applied to non-growing tissues, as in senescence. Another striking action of ABA is to induce and maintain dormancy; many ABA effects are also reported, which are less clear cut and less universal, for instance, accelerated or promoted flowering in some short-day plants, inhibited flowering in some long-day plants, accelerated tuberization in *Solanum* and accelerated rooting of cuttings. ABA antagonizes the action of plant growth hormones, for example the effect of auxin in abscission, that of gibberellins in dormancy and that of cytokinins in senescence. Concerning these antagonistic effects, there is usually no evidence of a specific interaction of ABA and the growth hormones and one cannot overcome the effect of ABA in most cases simply by increasing the concentration of the promotor. Thus, ABA is not acting as a competitive inhibitor of these plant hormones. Some other results suggest that ABA might be acting by inhibiting gibberellin biosynthesis (or metabolism?). It appears that ABA does have drastic effects in certain tissues upon RNA synthesis but it cannot be said how ABA inhibits this synthesis. There are two possibilities for the biosynthesis of ABA: (1) the normal terpenoid pathway from mevalonate and (2) breakdown of carotenoids, for example violaxanthin, in the light. A definite decision is not yet possible but now it seems to be more probable that ABA is formed by the terpenoid pathway. The formation of ABA glucose ester has been reported, and in some tissues ABA is rather rapidly metabolized to phaseic acid, which has only low biological potency. Finally, Prof. Wareing concluded that ABA appears to have multiple effects depending on the target tissue. ABA acts as part of the universal complex of growth regulators and there are indications that variations of endogenous ABA in association with changes in the growth promotors may well control specific stages of development.

The topics of H. Linser's (Giessen, GFR) report were native inhibitors other than ABA (some of which have been proved but none has been structurally elucidated) and the large range of artificial inhibitors (growth retardants etc.).

3. Action and interaction of plant hormones

Prof. Chailakhyan (Moscow, USSR) introduced this group of lectures the first of which was given by J.P. Nitsch on the Action and Interaction of Plant Hormones in Growth and Differentiation. Cell enlargement depends upon auxin and involves the uptake of water, extension of the cell membrane and protein synthesis. The auxin growth curve consists of two parts: promotion by low concentrations and inhibition by higher concentrations via the formation of ethylene. Cytokinins possibly may also act via

the production of ethylene. In cell enlargement, DNA synthesis is necessary, it is inhibited by light, but gibberellins may restore DNA synthesis in the light.

For cell division and multiplication two well-studied examples have been demonstrated: callus growth of tobacco pith and of tuber tissue of Jerusalem artichokes. In these systems a clear sequence of the hormones involved in cell division has been elaborated in the order: gibberellins, cytokinins, auxins.

Differentiation processes in plants selected for discussion were the formation of roots and shoots. Root formation which has been studied extensively by the use of tissue cultures and cuttings depends on high auxin concentration. It can be inhibited by gibberellin and in a similar way by cytokinin, if these hormones are applied at a very early stage. Shoot formation, for instance, in leaf discs of *Begonia rex*, is promoted by cytokinins and inhibited by gibberellins, but again the same sequence is obvious. Beside cytokinins, auxin seems to be necessary in low concentration for bud formation. The ability of *Cichorium* roots to form buds very easily could be explained by the high level of endogenous cytokinins. The endogenous factor, which inhibits bud formation in intact *Begonia* leaves attached to the plant is assumed to be a gibberellin.

The following lecture presented by Prof. P.F. Wareing on the Action and Interaction of Plant Hormones in Apical Dominance and Related Responses was based mainly on very recent, unpublished work of himself and some of his students. After demonstrating various phenomena and examples of apical dominance he described many experiments which had been done to study the role of plant hormones in these processes. Investigations with labelled cytokinins showed that bud growth in different plants, which could be affected by gravity, depends on cytokinin movement and unequal distribution within different buds. Cytokinin distribution preceeds and clearly reflects the future growth of the buds. Unlike the cytokinins, the distribution of radioactive labelled sucrose and phosphate does not depend on the orientation of the shoot. Studies on regulation by apical dominance of the stolon development in *Solanum andigenum* showed a rather complicated antagonism between IAA and gibberellin on the one hand and cytokinins on the other. From the experiments described and others, the following questions arose: Do cytokinins show polarity in transport and does auxin affect the movement of cytokinins? Prof. Wareing both gave some evidence of a polar, acropetal movement of cytokinins, apparently on a cell to cell basis, and demonstrated an inhibitory effect of IAA on the accumulation or movement of cytokinins. Therefore, in apical dominance, IAA coming from the buds in some way may inhibit the movement of cytokinins into these buds.

Prof. Wareing was in the Chair for the last two lectures of this group concerning flowering and senescence.

M.Kh. Chailakhyan reported on the Hormonal Regulation of Plant Flowering and reviewed this important chapter of plant development on the basis of his well-known anthesin theory. Early in the history of flowering hormones the term florigen was introduced for the flowering stimulus, the existence of which was proved in various ways; but its chemical identity still remains unknown. A new period was introduced by the discovery of the outstanding effects of gibberellins in flower induction, especially in long-day plants. Without a doubt these plant hormones are involved in flowering but, in fact, additional hormonal factors do exist. According to Chailakhyan, the 'florigen complex' consists of two groups of hormones: (1) the gibberellins, causing the formation and growth of flower stems and (2) the anthesins, causing the formation of the flowers. Gibberellins and anthesins are not precursors of each other but act in combination or in a sequence. Prof. Chailakhyan gave physiological evidence for the existence of anthesins in plants, but the isolation and characterization of any such compound is lacking.

The title of the following lecture given by K. Mothes was Action and Interaction of Plant Hormones in Senescence. Though it leads to death of cells, tissues, organs or the whole plant, senescence does not mean a pathological but a physiological process, which has been developed in the course of phylogeny and is incorporated in the harmony of plant development. Professor Mothes, after giving a general survey, restricted himself mainly to a discussion of senescence in green leaves; fundamental research in this field has been done by himself and some of his coworkers. In senescence of green leaves deportation of structural and metabolic substances from the leaves plays an important role. This deportation can be compensated by supplementary nitrogen nutrition and it

can be delayed by applying cytokinins and some other regulating substances, respectively. Furthermore, a senescent, yellowing leaf can be rejuvenated either by sufficient nitrogen nutrition, by the function of a healthy root system or by plant hormones. During rejuvenation, the regeneration of chloroplasts is possible providing thylakoids are still present and vacuoles have not yet appeared. Contrary to earlier views, it could be shown that the potency of protein synthesis is not markedly diminished in senescent leaves attached to the plant. Compared to young leaves the older ones lose soluble nitrogen but the remainder of the protein is still active. As to the mode of action of plant hormones in delaying senescence it is not yet known whether their influence on the trasnport phenomena mentioned above represents a primary action or whether there are primary effects on protein syntesis.

4. Mode of action of plant hormones

H. Kende presented a lecture entitled "Mode of Action of Plant Hormones on the Cellular and Molecular Level". Much attention has been given in recent years to the so-called dogma of the Jacob-Monod approach to the regulation of genetic activity. Clearly, we must think along these lines, but there are still only a few experiments that have been helpful in this direction. Any evidence for genetic activation by hormones based on work with inhibitors means only that the effect of the hormone requires the synthesis of nucleic acids and proteins. At present, apparently, there is no theory of the mode of action of plant hormones which lends itself to experimental testing. Some basic facts are known, however, that must be incorporated into any hypothesis on hormonal action. These facts were summarized as follows by H. Kende, combining unpublished work done in his own laboratory and data from the literature:

(1) From peculiarities of the hormonal dose-response relations (the dynamic range covers 3 to 5 orders of magnitude; existence of curves containing a promotion and an inhibition part etc.) it may be concluded that the hormone response includes some kind of amplification, possibly some kind of adaptation mechanism. In some cases the plant regulates growth and other hormonal responses by regulating the internal dose of the hormones, but in other cases the physiological response may be controlled by regulation of the sensitivity of the plant to the particular hormone rather than by the level of the particular hormone. This means that the endogenous dose of the hormone is not necessarily correlated with the physiological response that we want to observe.

(2) The kinetics of the hormone response must be taken into account when deciding any experiment, especially if we are interested in the primary response. The first very fast response to auxin was observed with protoplasm streaming, and recently, it has also become possible to measure growth response to auxin within two minutes. In these experiments auxin action showed practically no lag phase. Therefore, we have to think of mechanisms other than gene derepression, if we want to explain the effect of IAA on growth, since under the most optimistic estimate in plants it takes more than 10 minutes to start making a messenger RNA and to start and finish making a protein from this RNA. The fastest response to gibberellins observed till now is a growth reaction measurable after 15 min. In the barley aleuron layer system there is a lag phase of 6–10 hr until release of α-amylase takes place; however, structural changes can be observed by electron microscopy at much earlier stages after applying GA_3. Besides the very fast hormone reactions another point in the kinetics of hormone responses is of great importance: the hormone must be present not only for induction, but also for a longer critical period. This means that once hormone responses occur, they are reversible by withdrawal of the hormone.

(3) Site of action of hormones: oestrogens, the most studied of steroid hormones, are known to combine very quickly with the so-called receptors, which transfer the hormone inside the cell from the cytoplasm to the nucleus. These receptor proteins have a very specific affinity for the hormones. It seems possible that plant hormones also act on some proteins and that their high stereospecificity may be due to the fact that the hormone structure is in some way recognized by a specific binding site on a protein. Recent work of Prof. Kende in fact strongly indicated that plant hormones interact with a protein and that this interaction is of a non-covalent nature, which can only be demonstrated by techniques where reversible binding is observable.

(4) The relations between hormones, RNA, and protein synthesis were not comprehensively reviewed but were discussed for some selected cases. Cytokinins are the only plant hormones covalently bound in a regulatory molecule, such as tRNA, and they occur at a position which is very characteristically at the end of the presumed anticodon. Does cytokinin exert its function by being there or does it exert its function independently while it is there? Various results indicate that we have to look for another action mechanism of cytokinins. Gibberellin-induced synthesis of α-amylase in barley aleuron cells is the best known example of new protein formed under the influence of a plant hormone. Evidence for a de novo synthesis is based on the density gradient experiments done by Varner and coworkers ($H_2{}^{18}O$ is used for hydrolysis of reserve protein giving new α-amylase with a higher density). But enzyme synthesis is only observed 6–10 hr after gibberellin application and many things occur in this time.

The very lively and extended discussion on the problems of the mode of action of plant hormones, which followed Prof. Kende's lecture, was introduced by R. Wollgiehn who reported the results of experiments done in recent years in Halle on the action of cytokinins on the metabolism of RNA in detached leaves. Using different techniques it could be shown that the hormonal action resulted in an increased synthesis of each RNA fraction to the same degree but there was no indication of a cytokinin induced synthesis of a specific RNA.

5. Modern methods

An introduction to a general discussion on modern methods for the identification of plant hormones was given by J. MacMillan. He described general procedures for isolation, purification and separation of gibberellins, cytokinins, auxins, and abscisic acid as well as the possibilities and limitations of identifying plant hormones by use of the chemical and physical methods currently available. This detailed and complete review culminated in a demonstration of the identification of all the known gibberellins by combined gas chromatography–mass spectrometry linked to a computer.

K. Schreiber introduced and chaired a general discussion on modern methods for the structural elucidation of plant hormones. Contributions were made by V.F. Kucherov and M. MacMillan. The usefulness and the need for physical methods were stressed, especially UV, IR, and mass spectroscopy as well as NMR, ORD, CD, and X-ray analysis. These methods were described and examples of their application mainly in the gibberellin field were discussed in detail.