

## Indole-3-butyric acid in plants: occurrence, synthesis, metabolism and transport

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Indole-3-butyric acid (IBA) was recently identified by GC/MS analysis as an endogenous constituent of various plants. Plant tissues contained 9 ng g<sup>-1</sup> fresh weight of free IBA and 37 ng g<sup>-1</sup> fresh weight of total IBA, compared to 26 ng g<sup>-1</sup> and 52 ng g<sup>-1</sup> fresh weight of free and total indole-3-acetic acid (IAA), respectively. IBA level was found to increase during plant development, but never reached the level of IAA. It is generally assumed that the greater ability of IBA as compared with IAA to promote rooting is due to its relatively higher stability. Indeed, the concentrations of IAA and IBA in autoclaved medium were reduced by 40% and 20%, respectively, compared with filter sterilized controls. In liquid medium, IAA was more sensitive than IBA to non-biological degradation. However, in all plant tissues tested, both auxins were found to be metabolized rapidly and conjugated at the same rate with amino acids or sugar.

Studies of auxin transport showed that IAA was transported faster than IBA. The velocities of some of the auxins tested were 7.5 mm h<sup>-1</sup> for IAA, 6.7 mm h<sup>-1</sup> for naphthaleneacetic acid (NAA) and only 3.2 mm h<sup>-1</sup> for IBA. Like IAA, IBA was transported predominantly in a basipetal direction (polar transport). After application of <sup>3</sup>H-IBA to cuttings of various plants, most of the label remained in the bases of the cuttings. Easy-to-root cultivars were found to absorb more of the auxin and transport more of it to the leaves.

It has been postulated that easy-to-root, as opposed to the difficult-to-root cultivars, have the ability to hydrolyze auxin conjugates at the appropriate time to release free auxin which may promote root initiation. This theory is supported by reports on increased levels of free auxin in the bases of cuttings prior to rooting. The auxin conjugate probably acts as a 'slow-release' hormone in the tissues. Easy-to-root cultivars were also able to convert IBA to IAA which accumulated in the cutting bases prior to rooting. IAA conjugates, but not IBA conjugates, were subject to oxidation, and thus deactivation. The efficiency of the two auxins in root induction therefore seems to depend on the stability of their conjugates. The higher rooting promotion of IBA was also ascribed to the fact that its level remained elevated longer than that of IAA, even though IBA was metabolized in the tissue.

IAA was converted to IBA by seedlings of corn and *Arabidopsis*. The K<sub>m</sub> value for IBA formation was low (approximately 20 μM), indicating high affinity for the substrate. That means that small amounts of IAA (only a fraction of the total IAA in the plant tissues) can be converted to IBA. It was suggested that IBA is formed by the acetylation of IAA with acetyl-CoA in the carboxyl position via a biosynthetic pathway analogous to the primary steps of fatty acid biosynthesis, where acetyl moieties are transferred to an acceptor molecule. Incubation of the soluble enzyme fraction from *Arabidopsis* with <sup>3</sup>H-IBA, IBA and UDP-glucose resulted in a product that was identified tentatively as IBA glucose (IBGlc). IBGlc was detected only during the first 30 min of incubation, showing that it might be converted rapidly to another conjugate.

**Key words** – Auxin, auxin conjugates, auxin transport, IAA, IAA aspartic acid, IAA glucose, IBA aspartic acid, IBA glucose, rooting.

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## Introduction

The auxin indole-3-acetic acid (IAA) was the first plant hormone to be used in rooting (Cooper 1935). In the same year, Zimmerman and Wilcoxon (1935) discovered that several new synthetic auxins, among them indole-3-butyric acid (IBA), also promoted rooting. It was demonstrated that IBA is very effective in promoting rooting of a wide variety of plants, and it is used commercially to root many plant species world-wide (Hartmann et al. 1990). Since its introduction more than 50 years ago, IBA has been the subject of hundreds of experiments and articles. Many of the experiments involved trial and error studies of different concentrations, formulas, additives and treatment durations. Today one can still find varieties and cultivars in almost every species that do not root even after treatment with this auxin, respond to treatment during only part of the growing season, or produce roots only in a fraction of the treated cuttings. In recent years, several attempts were made to understand the role of IBA in the rooting process in plants at the metabolic level. For recent reviews on rooting see Gaspar and Hofinger (1988) and Blakesley et al. (1991). In this minireview we will concentrate on the occurrence, biosynthesis, metabolism and transport of IBA in plants.

**Abbreviations** – 2,4-D, 2,4-dichlorophenoxyacetic acid; DIAasp, 3-hydroxy-2-indolone-3-acetylaspatic acid; IAA, indole-3-acetic acid; IAGlc, indoleacetyl glucose; IAAsp, indole-3-acetylaspatic acid; IBA, indole-3-butyric acid; IBGlc, indolebutyryl glucose; IBAsp, indole-3-butyrylaspatic acid; IPA, indolepropionic acid; NAA, naphthaleneacetic acid; OxIAAsp, 2-indolone-3-acetylaspatic acid.

## Natural occurrence of IBA

Although IBA was identified as a natural product in potato peelings by paper chromatography almost 40 years ago (Blommaert 1954), it is still referred to as a synthetic auxin (Hartmann et al. 1990). During the past 20 years IBA was identified in various plants and tissues (Tab. 1), such as tobacco (Bayer 1969) and *Phaseolus vulgaris* (Brunner 1978); and only recently by GC/MS analysis in pea (Budenoch-Jones et al. 1984, Schneider

et al.1985), in cypress (Epstein et al. 1988), and maize (Epstein et al. 1989, Ludwig-Müller and Epstein 1991). An attempt was also made to correlate the infection of plants with growth-influencing bacteria and IBA content (Fallik et al. 1989, Epstein et al. 1991). Very little is known about the actual endogenous concentrations of IBA in plant tissues. Sutter and Cohen (1992) synthesized [<sup>13</sup>C]indole-[ring 2]-3-butyric acid and used it as an internal standard for the determination of IBA in tobacco leaves by isotope dilution GC/MS. They found that the tissue contained 9 ng g<sup>-1</sup> fresh weight of free IBA and 37 ng g<sup>-1</sup> fresh weight of total IBA, compared with 26 ng g<sup>-1</sup> and 52 ng g<sup>-1</sup> fresh weight of free and total IAA, respectively. The occurrence of free and conjugated IBA under different growth conditions was studied more thoroughly in plantlets of *Arabidopsis thaliana* grown in liquid medium under sterile conditions (Ludwig-Müller et al. 1993). IBA level increased during the development of the plantlets, but never reached the level of IAA. In seeds of *Arabidopsis*, IBA concentration was higher than that of IAA; both hormones were secreted into the medium. Concentrations of free IBA were between 7 and 25 ng g<sup>-1</sup> fresh weight, whereas total IBA was in the range of 100 ng g<sup>-1</sup> fresh weight.

## IBA metabolism

It is generally assumed that the greater ability of IBA compared with IAA to promote rooting is due to its relatively higher stability (Hartmann et al. 1990). Robbins et al. (1988) demonstrated that IBA can be stored for 6 months in amber or clear glass at various temperatures (22–25, 6 and 0°C) without significant change in biological activity or breakdown. Nordström et al. (1991) showed that IBA was more stable than IAA in KOH solution in black containers at room temperature. Nissen and Sutter (1990) studied the stability of IAA and IBA under various tissue culture procedures. They showed that the concentrations of IAA and IBA in autoclaved MS (Murashige and Skoog 1962) medium were reduced by 40% and 20%, respectively, compared with filter sterilized controls. Under growth chamber conditions, IAA and IBA losses from both liquid and

Tab. 1. Indole-3-butyric acid as a natural endogenous compound in plants.

Plant material	Detection method	Reference
Potato peelings	Paper chromatography	Blommaert et al. 1954
Tobacco leaves	Gas chromatography	Bayer 1969
<i>Phaseolus</i> hypocotyls	TLC and bioassay	Brunner 1978
Pea root nodules	GC/MS	Budenoch-Jones 1984
Pea root, epicotyl & cotyledons	GC/MS	Schneider et al. 1985
Cypress leaves	GC/MS	Epstein et al. 1988
Maize leaves & kernels	GC/MS	Epstein et al. 1989
Maize roots	GC/MS	Fallik et al. 1989
Maize roots, leaves & coleoptiles	GC/MS	Ludwig-Müller and Epstein 1991
Carrot	GC/MS	Epstein et al. 1991
<i>Arabidopsis thaliana</i>	GC/MS	Ludwig-Müller and Epstein 1992
Tobacco leaves	GC/MS	Sutter and Cohen 1992

agar-solidified MS were significant. In liquid medium, IAA was more sensitive than IBA to non-biological degradation. In the light, IAA and IBA concentrations were reduced by more than 97% and 60%, respectively, compared to a decline by 70% and 30%, respectively, in the dark. In agar-solidified MS, IAA and IBA concentrations were reduced by 45% and 38%, respectively, after 30 days in darkness, and ca 95% and 80%, respectively, after only 3 days in the light. Similar results were obtained after application of auxins to plant tissue. Wiesman et al. (1988) found that labeled IBA and IAA were metabolized rapidly by cuttings of mung bean (*Vigna radiata* L.), and 24 h after application only a small fraction of the radioactivity of both auxins corresponded to the free auxin. Similarly, Pythoud and Buchala (1989) found that only 17% of the  $^{14}\text{C}$ -IBA which was applied to the bases of cuttings of trembling aspen (*Populus tremula* L.) was still present as unmodified IBA after 24 h. IAA was metabolized more quickly than IBA by green cuttings (leaves and apex excised) of *P. tremula* L. (Merckelbach et al. 1991). Baraldi et al. (1993) also observed a rapid disappearance of IBA in pear (*Pyrus communis* L.) plantlets propagated in vitro. Only a small fraction of the total extractable radioactivity could be identified as free IBA after 12 h of incubation. In apple shoots cultured in vitro only 5% of IBA and 1% of IAA were found in the free form (van der Krieken et al. 1992a,b).

#### IBA conjugates

It has been established that natural plant hormones such as IAA, gibberellin, cytokinin and ABA are present in plant material both as free acid and in the form of conjugates (Cohen and Bandurski 1982). All plants that have been studied had most of their IAA in conjugated form, and conjugates may account for over 90% of the IAA in some tissues. Evidence has emerged over the last several years which indicates that conjugates play an important role in auxin physiology and metabolism. They are thought to be involved in transport of the hormone, the storage and subsequent usage of the hormone, protection from enzymatic destruction, and the homeostatic control of the concentration of the hormone within the plant (Bandurski 1980, Cohen and Bandurski 1982). Andrea and Good (1955, 1957) reported that pea sections converted IBA to compounds which had chromatographic properties similar to those of IBA-aspartic acid (IBAsp), IAA-aspartic acid (IAAsp) and IAA. They concluded that IBA was converted by  $\beta$ -oxidation to IAA and that both IBA and IAA (formed from the applied IBA) were conjugated to aspartic acid by pea sections.

Wiesman et al. (1988, 1989) studied the metabolism of IAA and IBA by mung bean cuttings. The cuttings were incubated with  $^{14}\text{C}$ -IAA and  $^3\text{H}$ -IBA and samples were extracted after various periods of incubation and the radioactive compounds were identified. The major

metabolite of IAA was identified as IAAsp, whereas IBA was converted to IBAsp and at least two high molecular weight conjugates (probably conjugates with peptides). They proposed that the better rooting ability of IBA was due to the formation of IBAsp which promoted rooting better than IAA (Wiesman et al. 1989) or IAAsp (Plüss et al. 1989). Epstein and Sagee (1992) studied the metabolism of IBA in leaf midribs of citrus (*Citrus reticulata* Blanca). IBA was converted by the midribs to a metabolite that was identified tentatively as an ester conjugate with glucose (IBGlc). Pythoud and Buchala (1989) could not detect any oxidation products of IBA after feeding  $^{14}\text{C}$ -IBA to cuttings of *P. tremula*, but noticed the conversion of IBA to a glycosyl conjugate and another conjugate, probably IBA peptide. IAA, on the other hand, was both oxidized by *P. tremula* cuttings and conjugated to aspartic acid to form 2-indolone-3-acetylaspatic acid (OxIAAsp). Tsurumi and Wada (1988) found that seedlings of *Vicia faba* oxidized IAA and conjugated it with aspartic acid to form 3-hydroxy-2-indolone-3-acetylaspatic acid (DIAAsp). Plüss et al. (1989) and Merckelbach et al. (1991) identified OxIAAsp in greenwood cuttings of *P. tremula*. Neither product was biologically active (Tsurumi and Wada 1988, Plüss et al. 1989). Riov and Bangerth (1992) identified OxIAAsp in tomato fruit tissue. These reactions render IAA inactive and thereby remove it from the auxin pool. Since no such oxidation products have yet been found for IBA, this might be the cause for the better activity of IBA in rooting. However, it is also possible that IBA oxidation products have yet to be discovered.

Merckelbach et al. (1991) also showed that the IBA was conjugated mainly to IBGlc and that IAA was conjugated more slowly than IBA in cuttings of *P. tremula*, probably because IAA, and not IBA, induced its own metabolism. They ascribed the higher rootability of IBA to the fact that its level remained elevated longer than that of IAA, even though IBA was metabolized in the tissue. They also showed that following incubation with IBA, the level of IAA and IAAsp in the cuttings increased steadily.

Plantlets of easy- and difficult-to-root cultivars of pear grown in vitro converted approximately 50% of the IBA taken in to IBAsp (Baraldi et al. 1993). Baraldi et al. attributed the differences in the rooting ability of the two pear cultivars to differences in IBA uptake and metabolism and to free IAA level in the shoots. Epstein et al. (1993b) studied the metabolism of exogenously-applied IBA in easy- and difficult-to-root cultivars of sweet cherry (*Prunus avium* L.) in an aseptic system. Both cultivars rapidly metabolized the IBA to a conjugate, probably IBGlc. Autofluorography of the extracts of the plantlet bases showed that after one day no free IBA could be detected in the difficult-to-root cultivar, while the easy-to-root cultivar metabolized the IBA more slowly and free IBA could still be detected. The free IBA disappeared after 2 days of incubation, but

re-appeared after 4 days (3 days before root emergence) and disappeared again after 7 days. They postulated that the easy-to-root, as opposed to the difficult-to-root cultivar, has the ability to hydrolyze the ester conjugate at the appropriate time to release free IBA, which may promote root initiation. This theory is supported by reports on increased levels of free auxin in the bases of cuttings prior to rooting (Brunner 1978, Moncousin et al. 1989, Liu and Reid 1992, Epstein and Ackerman 1993. Epstein et al. (1993b) used the conjugate inhibitor 2,6-dihydroxyacetophenone (DHAP, Lee and Starratt 1986) in order to increase the level of free auxin in cuttings of the difficult-to-root olive cv. Uovo di Piccone. Significantly more cuttings rooted following treatment with 2 mM DHAP and 0.8% IBA than with 0.8% IBA alone (30% vs 15%, respectively).

IBA was metabolized very rapidly by *Petunia hybrida* cell suspension (Epstein 1993a). HPLC of the cell extracts demonstrated a new metabolite after only 2 min of incubation, and after 30 min 60% of the radioactivity was in the new metabolite vs 10% in the IBA. The new compound was resolved by autofluorography to two metabolites but after 24 h only one metabolite was present. One metabolite was eluted in the neutral fraction from DEAE-Sephadex column and was hydrolyzed to free IBA by both 1 M NaOH and  $\beta$ -glucosidase, proving it to be an ester conjugate of glucose with IBA (Bandurski and Schulze 1977). The second metabolite was eluted from the DEAE-Sephadex column with the acidic fraction, hydrolyzed to free IBA by 7 M NaOH, but not by 1 M NaOH or  $\beta$ -glucosidase, pointing to a peptide bond (Bandurski and Schulze 1977). In a separate experiment, it co-chromatographed with authentic IBA-aspartic acid on TLC. Autofluorography showed that IBGlc was the first to appear without a distinct lag phase simultaneously with the decrease of IBA. After 30 min, IBAsp began to show, and IBGlc decreased and disappeared after 24 h. In *A. thaliana* seedlings cultured in liquid medium, the formation of IAA and IBA conjugates was also inducible by exogenous NAA, but the amount of conjugates formed was dependent on the time of the addition of NAA to the culture medium (Ludwig-Müller and Epstein 1993).

Epstein and Sagee (1992) showed that *Citrus* leaf midribs metabolized exogenous IBA to a compound that was tentatively identified as an ester conjugate. Zenk (1964) claimed, without supporting data, that IAGlc is formed in plants without a lag phase, and that this conjugate is the precursor of amino acid conjugates. Michalczuk and Bandurski (1982) showed that the actual substrate for the IAA-*myo*-inositol forming enzyme was IAGlc. IAA glucose is formed from UDP-glucose by a transferase, and the equilibrium of this reaction is strongly in favor of UDP-glucose by a factor of about 50:1 (R. S. Bandurski, personal communication). IAGlc is thus a highly effective acyl donor and a likely candidate for the acylation of aspartate. In the same fashion, *Petunia* cells rapidly conjugate IBA to IBGlc,

which in turn is converted to form IBAsp. It was postulated that IBAsp probably acts as a 'slow-release' hormone (Cholodny 1935). *Petunia* probably releases IBAsp into the medium, and since the cells do not absorb IBAsp readily (Shea et al. 1988), the IBA in the medium is replaced in this way by IBAsp until equilibrium is reached. Only intact cells were able to metabolize IBA and the reaction was affected by low temperature and anaerobic conditions. IAA metabolism proceeded at a slower rate, and autofluorography showed that while free IBA disappeared after 0.5 h, free IAA was still present after 1 h incubation.

Nordström et al. (1991) studied the metabolism of exogenous IAA and IBA during adventitious root formation in pea cuttings. The cuttings metabolized IAA predominantly to IAAsp. The level of free IAA in the cuttings increased considerably on the first day of incubation, but then decreased rapidly and reached control level after 4 days. IBA, on the other hand, was converted to IAA, IBAsp and IAAsp. Free IBA level remained high throughout the experimental period.

In seedlings of *A. thaliana* cultivated in liquid medium under sterile conditions, IBA was also metabolized to two major metabolites (Ludwig-Müller and Epstein 1993) that were tentatively identified as IBGlc and IBAsp. Incubation of the soluble enzyme fraction from *A. thaliana* with <sup>3</sup>H-IBA, IBA and UDP-glucose resulted in a product with an  $R_f$  value identical to that of IBGlc and which stained positive for indole. IBGlc was detected only during the first 30 min of incubation, showing that it might be rapidly converted to another conjugate, as was also demonstrated with IAA in maize (Kowalczyk and Bandurski 1991). This was the first report of an enzyme that is able to conjugate IBA in vitro.

#### Conversion of IBA to IAA

Fawcett et al. (1960) found that indolealkenecarboxylic acids with even chain lengths were converted in the plant to acetate, and those with odd chain lengths resulted primarily in propionate. They exposed wheat coleoptile and pea stem tissue to IBA solutions and subsequently identified IAA in the tissues and the residual solution. Conversion of IBA to IAA was reported in cuttings of *Pinus sylvestris* (Dunberg et al. 1981), *P. tremula* (Merckelbach et al. 1991), *P. communis* (Baraldi et al. 1993), *Malus pumila* (Alvarez et al. 1989) and *Malus* cultivar Jork (van der Krieken et al. 1992a). Epstein and Lavee (1984) used <sup>3</sup>H-IBA to demonstrate the conversion of IBA to IAA in cuttings of grapevine and olive. Noiton et al. (1992) found that the level of IAA increased markedly in apple microcuttings after treatment with IBA. Although the conversion of IBA to IAA was found to occur in so many plant species, and many authors attribute the rooting ability of IBA to its conversion to IAA, no biochemical studies

of this reaction have, to the best of our knowledge, been carried out.

### Biosynthesis of IBA

The biosynthesis of IBA can proceed according to one of the following pathways: (1) a pathway analogous to the IAA biosynthetic pathway via the tryptophan pathway (indole + serine) using glutamate- $\gamma$ -semialdehyde instead of serine; (2) by  $\beta$ -oxidation via reactions similar to those found in the biosynthesis of fatty acids; (3) a nontryptophan pathway similar to that demonstrated by Wright et al. (1991) in a mutant of the maize orange pericarp for IAA.

IAA was found to be converted to IBA by seedlings of corn (*Zea mays* L.) (Ludwig-Müller and Epstein 1991). After feeding [ $1\text{-}^{14}\text{C}$ ]-IAA to segments of corn shoots and roots, a new labeled compound was detected by TLC and HPLC which corresponded to authentic IBA. This compound was not detected in a control experiment without plant tissue. After feeding [ $^{13}\text{C}_6$ ]-IAA to the plant tissues it was possible to identify the formation of [ $^{13}\text{C}_6$ ]-IBA by GC/MS. Most of the conversion was found to occur in the leaves and some activity was found also in the roots and coleoptiles. Maximum activity was detected after 1 h incubation, and the pH optimum was 6.0 for uptake and 7.0 for IBA formation. The  $K_m$  value for IBA formation was low (approximately 20  $\mu\text{M}$ ), indicating high affinity for the substrate. That means that small amounts of IAA (only a fraction of the total IAA in the plant tissues) can be converted to IBA. It was also shown that the conversion rate was higher in a variety of corn that formed an extensive root system, than in another variety with smaller roots. It is suggested that IBA is formed by the acetylation of IAA with acetyl-CoA in the carboxyl position via a biosynthetic pathway analogous to the primary steps of fatty acid biosynthesis, where acetyl moieties are transferred to an acceptor molecule (Wakil 1989).

Several co-enzymes which might be involved in the chain elongation reaction were studied as to their effects on IBA formation in vitro (Ludwig-Müller and Epstein 1992, 1993). In the presence of the co-factors ATP,  $\text{Mg}^{2+}$ , and NAD(P)H, some IBA forming activity was detected with acetyl-CoA and propionyl-CoA, but not with malonyl-CoA or acetoacetyl-CoA. A labeled product was detected only when the 50 000 g fraction was incubated with acetyl-CoA or propionyl-CoA and its formation was increased by adding ATP or  $\text{Mg}^{2+}$ , respectively. A time course study of the formation of this product showed that it must be turning over, but it is not directly converted to IBA or IPA in this fraction. Preliminary characterization of the product indicated that it is an indole, most likely a carboxylic acid, and it is probably a conjugate. The product was collected by HPLC and incubated with the 10 000 g fraction and the supernatant. IBA-forming activity was detected in the

10 000 g fraction and found to be enhanced by NADPH. The supernatant also showed significant activity without any co-factor, but the product was unstable. In the organelle fraction, no IBA formation was detected after 1 h incubation, but after 4 h approximately 45% of the product was converted to IBA. It was therefore concluded that the biosynthesis of IBA might be a two-step reaction, but further characterization and identification of the intermediate step is necessary to confirm this hypothesis.

Incubation of axenically cultured seedlings of *Arabidopsis* with labeled IAA resulted in a labeled compound with an  $R_f$  similar to authentic IBA (Ludwig-Müller and Epstein 1993). The IBA peak decreased rapidly concurrently with the formation of a new compound which was identified as an IBA conjugate. Determination of the acidic organic phase showed that after 1 h incubation with IAA most of the IAA that was taken in (46%) was found as an IAA conjugate, a significant amount (ca 24%) was in IBA conjugates, and only ca 14% of the recovered radioactivity was present as free IBA. In contrast, van der Krieken et al. (1992a) did not find any conversion of IAA to IBA by shootlets of apple grown in vitro.

### Uptake and transport of IBA

Studies of the transport of IBA using the *Avena* curvature test showed that IAA was transported faster than IBA (Went and White 1938, McCready 1963). Among the various auxins, IAA and NAA appeared to have very similar transport velocities (Hertel et al. 1969, Kaldewey 1984), while IBA was markedly slower (McCready 1963), behaving more like 2,4-D (Riov and Goren 1979). Leopold and Lam (1961) determined the rate of the polar movement of some auxins, and found that the velocity of NAA was 6.7  $\text{mm h}^{-1}$ , of IAA 7.5  $\text{mm h}^{-1}$  and of IBA only 3.2  $\text{mm h}^{-1}$ .

IBA was transported in midribs of *Citrus* leaves predominantly in a basipetal direction (polar transport), at a somewhat lower rate than IAA (Epstein and Sage 1992). Basipetal transport of IBA was approximately 60% of that of IAA and only twice the acropetal transport. After application of  $^3\text{H}$ -IBA to cuttings of *V. radiata* (Wiesman et al. 1988), *P. tremula* (Pythoud and Buchala 1989), olive and grapevine (Epstein and Lavee 1984), sweet cherry (Epstein et al. 1993b), and apple (van der Krieken et al. 1992b), most of the label remained in the bases of the cuttings. The same results were obtained using labeled IAA (Pythoud and Buchala 1989) and [ $^{13}\text{C}_6$ ]-IAA (Liu and Reid 1992). This suggests that the auxin transport is passive and is probably involved with xylem transport, which is probably due to transpiration. Pythoud and Buchala (1989) showed that IBA was associated with the cambium and phloem tissues. It was suggested (Jarvis and Booth 1981) that basal application of IBA to mung bean cuttings increased the basipetal translocation of IAA that was

applied to the leaves. Pythoud and Buchala (1989) used the agar block technique with  $^{14}\text{C}$ -IAA in the donor block at the apical end, and found no significant increase in the translocation of IAA in stem segments or petioles of *P. tremula* when  $10\text{ mg ml}^{-1}$  of IBA was present in the receiver block. However, when they used stem segments from cuttings which were pretreated with  $10\text{ mg ml}^{-1}$  of IBA for 24 h they noticed an increase in the translocation of IAA. In an experiment with *L. discolor* (Epstein and Ackerman 1993), cuttings of easy- and difficult-to-root cultivars were incubated with labeled IBA. The major difference in the transport between the two cultivars was that in the difficult-to-root cultivar the label in the leaves was almost a constant 10% of the total uptake, while in the easy-to-root leaves the value increased after 2 weeks to 30% and after 3 weeks to 45% , with a final level of 35% after 4 weeks (the same as in the shoot).

In a study of IBA uptake by easy- and difficult-to-root plantlets of sweet cherry (Epstein et al. 1993b) it was found that easy-to-root plantlets absorbed more IBA for a longer period than did difficult-to-root plantlets. The uptake of IAA and IBA was studied in suspension cell cultures of *P. hybrida* (Epstein et al. 1993a). The initial uptake of  $^3\text{H}$ -IBA was much higher than that of  $^3\text{H}$ -IAA, and after 10 min of incubation with labeled IBA and IAA ,  $4.6\text{ pM}$  vs  $0.35$ , respectively (39% vs 12% of total applied radioactivity) was found in the cell extracts. The uptake of IBA reached a plateau of  $6.0\text{ pM}$  (62%) after 2 h while that of IAA increased continuously up to  $1.5\text{ pM}$  (46%) after 24 h. Following the addition of  $40\text{ }\mu\text{M}$  of unlabeled auxin more IBA was taken in initially than IAA (39% vs 12%), but the level

almost equalized after 24 h incubation when IBA uptake reached  $890\text{ nM}$  (55%) and IAA  $840\text{ nM}$  (46%).

Uptake of IAA and IBA in *A. thaliana* was followed for 24 h and 48 h, respectively (Ludwig-Müller and Epstein 1993). There was no significant difference in the uptake of the auxins during these time periods.

The physiological events leading to root initiation may be revealed by studies of the direct genetic controls using molecular genetic techniques. Shoseyov et al. (1989) treated 20-day-old cuttings of mung bean (*V. radiata*) with IBA and checked the activity of hydrolytic enzymes. They found that the activity of endo-1,4- $\beta$ -glucanase was enhanced only in the IBA-treated cuttings, reaching a maximum 12 h after the IBA treatment. The activities of  $\beta$ -1,3-glucanase and  $\alpha$ -amylase were not affected by the treatment. Four endo-1,4- $\beta$ -glucanase genes were cloned and sequenced. In situ hybridization revealed the expression of the 4 genes in the area of adventitious root primordia formation and in the cortex, where maceration of the cells was in process, to enable emergence of roots through the hypocotyls.

### Conclusions

This review has dealt mainly with recent work on IBA in plants. There is a plethora of evidence available showing that IBA is present as an endogenous constituent in a variety of plants and tissues. Still, there is not enough information on its importance as an auxin. In all studies IBA was conjugated very rapidly, and so far no non-conjugation products of IBA have been reported. It is reasonable to assume that free auxin released from the conjugate is the major source of the free auxin

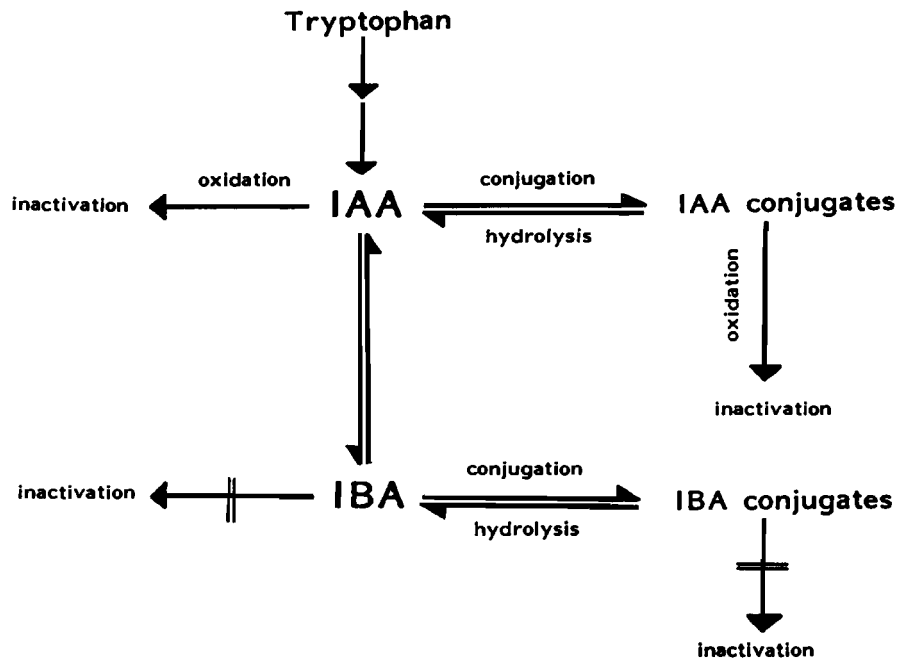


Fig. 1. Scheme for the metabolic interconversions of IAA and IBA and their conjugates.

during the rooting process. The interconversions of IAA and IBA (Fig. 1), the different conjugates in various plants, and the influence of factors such as medium, light, pH, and others on its metabolism makes it difficult to construct a hypothesis for the involvement of IBA in adventitious root initiation. There are several possible explanations for the better rooting ability of IBA than of IAA, and the failure of some plants to root even after IBA treatment: (1) IBA shows better stability in solution; (2) rooting ability depends on the formation of IAA from IBA and a certain ratio of IAA:IBA must be maintained; (3) rooting ability depends on the stability of the respective auxin conjugates; (4) rooting ability depends on the ability of the tissue to convert the auxin conjugate to the free hormone during a critical rooting phase; and (5) uptake and transport of the two auxins differ significantly. There is still not enough evidence to support any of the hypotheses, and more work is needed to elucidate the role of IBA as an auxin and rooting factor in plants.

## References

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