BIOLOGIA PLANTARUM (PRAHA)

27 (2—3) : 145—153, 1985

Auxin Biosynthesis and Its Regulation on the Molecular Level

M. KUTÁČEK

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Ke dvoru 15, 166 30 Praha 6, Czechoslovakia

Abstract. IAA synthesis proceeding by indol-3-ylpyruvate (IPyA) pathway seems to be regulated in two steps. In the first the L-trp conversion into IPyA is reduced by a low affinity of L-trp to the unspecific aminotransferase, by competition of L-trp with some aminoacids (*e.g.* L-asp) and indoles (*e.g.* indol-3-ylacetylaspartate). Simultaneously, a specific L-trp-dehydrogenase in dependence on the NAD(P)/NAD(P)H ratio regulates by its reversible effect the level of IPyA, connecting photosynthesis with growth. A second more "delicate" regulation of IAA level is carried out by the indol-3-ylacetaldehyde system. In pea plants two indol-3-ylacetaldehyde oxidases with pH optima 4.5 and 7.0 were found. The oxidases are differentially inhibited by an excess of IAA, different indoles as indol-3-ylacetylaspartate and aminoacids as L-asp. GA_3 and kinetin stimulate the conversion of indol-3-ylacetaldehyde to IAA.

The peculiarity of the auxin synthesis is a big difference between the levels of its precursor L-tryptophan (L-trp) and IAA. In tobacco tissue cultures we found the free L-trp content to be 20—100 times higher than that of the free IAA. The necessity of an efficient regulation of the IAA biosynthesis is obvious.

Several pathways are leading in higher plants from L-trp to IAA (SCHNEIDER and WIGHTMAN 1978). The most widely spread pathway of IAA synthesis seems to be the "indolylpyruvate pathway" (I = indol)

$$\mathrm{I{-}CH_2{-}CH\,.\,NH_2{-}COOH} \leftrightarrows \mathrm{I{-}CH_2{-}CO{-}COOH} \rightarrow \mathrm{I{-}CH_2{-}CHO} \rightarrow \mathrm{I{-}CH_2{-}COOH}$$

$$\mathrm{I{-}CH_2{-}CHO} \rightleftarrows \mathrm{I{-}CH_2{-}CH_2\,.\,OH}$$

Corresponding enzymes were detected in a wide range of higher plants (WIGHTMAN and COHEN 1968, TRUELSEN 1973). This way consists of several steps. The first step, deamination of L-trp leading to indol-3-ylpyruvate (IPyA) could be catalysed by several enzymes, an aminotrasferase (TAT), a newly discovered L-trp dehydrogenase (TDH), by a cyclic reaction of *o*-chinones. The second step, decarboxylation of IPyA to indol-3-ylacetaldehyde (IAAld) is catalysed by a not well known decarboxylase or is assumed to proceed spontaneously (SCHNEIDER and WIGHTMAN 1978). The third step, oxidation of IAAld to IAA is catalysed by IAAld-oxidase. IAAld is also reversibly converted to the growth inactive tryptophol (TOH), a reserve product in the pathway.

TABLE 1

Kinetics of pea L-tryptophan aminotransferase

Data	Substrate	
	L-trp 6 mM	L-phe 6 mM
Optimal pH	8.5	8.5
Optimal temperature	45 °C	45 °C
Linear aerea of enzyme activity	3 h	3 h
Activation by PRP 0.13 mM	+ 62.1%	+ 59 3%
K_M [KG 3 mM]	0.41 mM	0.21 mM
K_M [KG, PRP]	0.10 mM	0.07 mM
Inhibition by L-asp 9 mM	−83.3%	−79.1%

MATERIAL AND METHODS

The plant material were 7—9 day old etiolated pea seedlings (*Pisum sativum* L., cv. Jupiter). Maize (*Zea mays* L., cv. Ta 37/71 O_2), kohlrabi (*Brassica oleracea* var. *gongylodes* L., cv. Moravia) and tomato (*Solanum lycopersicum* L., cv. Stupické) seedlings were used for comparison of the results with pea plants.

TAT was extracted and its activity determinated by a method derived from the procedure of TRUELSEN (1972) and DIMOVA and KUTÁČEK (1985). L-trp and L-phenylalanine (L-phe) were used as substrates for transamination, α-ketoglutarate served as amino group acceptor. The activity of TAT was determined spectrophotometrically from the formed IPyA, stabilized in borate complex.

TDH was extracted in a similar way as TAT (fraction of 60—80 % ammonium sulphate saturation). The activity was determined in both directions, using L-trp with NAD(P) and IPyA, NH_4Cl with NAD(P)H as substrates and

TABLE 2

Inhibition of pea L-tryptophan aminotransferase (TAT) and L-phenylalanine aminotransferase (PAT) by amino acids

Amino acid 9 mM	Inhibition [%]		Substrate activity of aminoacid (L-trp = 100%) (25)
	TAT	PAT	
L-aspartic acid	−83.3	−79.1	+95
L-lysine	−79.2	−70.4	−288
L-methionine	−65.2	−81.0	+216
L-alanine	−54.1	−52.0	+202
L-asparagine	−39.2	−42.7	+213
L-histidine	−28.2	−16.1	+82
L-valine	−15.0	−15.1	+62
L-cysteine	−14.5	−17.6	+4
L-glutamic acid	−9.2	−8.4	−
L-tyrosine	−6.0	−8.3	+131
D-phenylalanine	−5.2	−11.8	−
D-tryptophan	−4.8	−5.9	−

coenzymes (KUTÁČEK and DIMOVA 1985a). The method was derived from glutamate dehydrogenase (GDH) activity determination.

IAAld oxidase was extracted by the method of SUZUKI *et al.* (1981). As product of enzymatic activity indol-3-ylcarboxylic acid (ICOOH) was determined arising from indol-3-ylaldehyde (IAld) as substrate (KUTÁČEK and DIMOVA 1985b).

Proteins were determined by the Coomassie brilliant blue G-250 method (BRADFORD 1976).

RESULTS AND DISCUSSION

L-tryptophan Aminotransferase

Enzyme specificity:

TAT is an enzyme with a group specificity for amino acids and L-trp represents only one of the substrates belonging to this group. This could be clearly seen when comparing L-trp and L-phe as substrates of the aminotransferase (Table 1). The identity of the enzyme catalysing the transamination of both amino acids, L-trp and L-phe, is evident from identical pH-, temperature-optima, similar duration of the linear phase of activity, similar increase of activity with added coenzyme PRP and similar inhibition of activ-

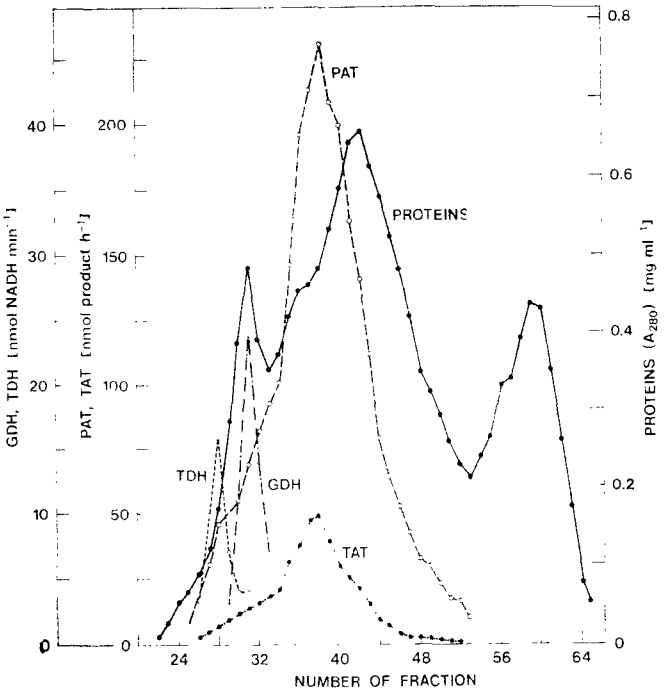

Fig. 1. Separation of pea L-tryptophan dehydrogenase (TDH) from L-glutamate dehydrogenase (GDH), L-tryptophan aminotransferase (TAT) and L-phenylalanine aminotransferase (PAT) by gel chromatography on Sephadex G-200 column (50 mM TRIS-HCl buffer pH 8.5; 2.5 × 120 cm; 6 cm^3 h^{-1}).

TABLE 3

Comparison of L-tryptophan aminotransferase (TAT) and L-phenylalanine aminotransferase (PAT) activities in different plants (K_M values; PRP 50 $\mu g\ cm^{-3}$)

Plant	TAT ($K_M \cdot 10^{-4}$ M)		PAT ($K_M \cdot 10^{-4}$ M)	
	− PRP	+ PRP	− PRP	+ PRP
Pisum sativum (*Fabaceae*)	4.16	1.04	2.10	0.72
Zea mays (*Poaceae*)	6.82	3.04	4.96	1.97
Lycopersicum esculentum (*Solanaceae*)	9.07	7.45	7.11	4.25
Brassica oleracea var. *gongylodes* (*Brassicaceae*)	0	0	0	0

ity with L-asp. Gel chromatography on a Sephadex G-200 column showed an identical fraction of proteins to transaminate L-trp and L-phe. Differences in K_M indicate a higher affinity of L-phe to the aminotransferase. In an interaction L-phe inhibits the transamination of L-trp to IPyA more strongly (−87.4 %) than in the reverse L-trp inhibits L-phe conversion to phenylpyruvate (−15.0 %). The more intense transamination of L-phe as well the interaction with TAT activity are in agreement with the higher content of phenylacetic acid (PAA) in the plants in comparison with IAA.

The regulatory properties of L-trp aminotransferase in IAA synthesis:

The transamination of L-trp is fundamentally influenced by the relatively low affinity of L-trp to the enzyme with a broad specificity ($K_M = 4.16 \times \times 10^{-4}$M). The first reaction of IAA synthesis has not a specific character and only a limited amount of L-trp from his wide pool enters into the pathway.

The activity of an aminotransferase is induced by a catalytical amount of keto acid, serving as acceptor of the amino group (BALDWIN 1949). The specificity of TAT for keto acids is still not well understood. In extracts from the whole plant a series of keto acids is active (mung bean — TRUELSEN 1972, pea — DIMOVA and KUTÁČEK 1985). In contrast to that, TAT localized in spinach peroxisomes is induced only by glyoxylate and hydroxypyruvate (NOGUCHI and HAYASHI 1980). It seems that a narrower specificity of the aminotransferase towards keto acids could be expected in a compartmented form of the enzyme which was also observed in the case of aminotransferases of other aminoacids (*e.g.* LIU and HUANG 1977).

In consequence of the relatively higher affinities of several other amino acids to the aminotransferase, an interaction of them with L-trp transamination could be expected. In the extreme case they can be transaminated in preference to L-trp. The transamination of L-trp is mainly influenced by L-asp, L-lys, L-met, L-ala and L-phe (see also FOREST and WIGHTMAN 1972, DIMOVA and KUTÁČEK 1985) (Table 2). The mentioned amino acids are active substrates of TAT, with the exception of L-asp, which influence on the whole

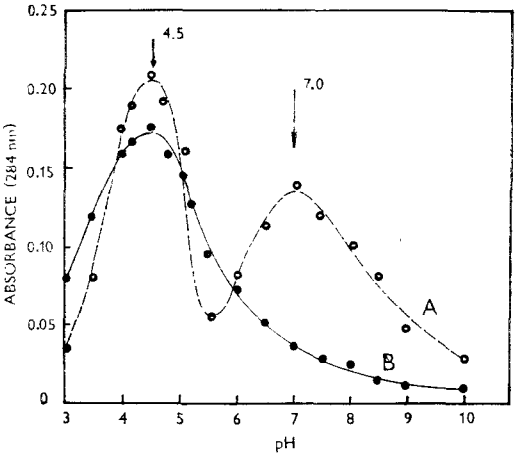

Fig. 2. Influence of pH on the pea indol-3-ylacetaldehyde oxidase activity. — A: aqueous enzyme extract of plant material; B: enzyme extract from acetone powder.

IAA synthetic pathway seems to be of a more specific character. L-glu is nearly without influence.

An inhibitory effect was also detected with a series of naturally occurring indoles (DIMOVA and KUTÁČEK 1985). Most efficient as inhibitors of TAT activity were TOH (—83 % at 9 mmol l^{-1} TOH), indol-3-ylacetylasparate (IAAsp) (—88 % at 9 mmol l^{-1} IAAsp), both indoles show a competitive character of inhibition; IAA was without feed back effect.

Both, amino acids and indoles, influenced TAT activity in relatively high concentrations. However, it may be assumed that these substances could regulate TAT activity in the cell as a group of naturally occurring compounds.

TAT activity was proved in pea, maize and tomato seedlings (Table 3). Aromatic aminoacids L-trp and L-phe served not as substrates for transamination with enzyme extracts from kohlrabi seedlings. It corroborates the

TABLE 4

Regulation of pea indol-3-ylacetaldehyde oxidases (pH 4.5 and 7.0) by auxins, indole compounds, amino acids, and growth substances

Inhibitor [10^{-3} M]	% inhibition IAAldx. pH 4.5	pH 7.0	Inhibitor [10^{-3} M]	% inhibition IAAldx. pH 4.5	pH 7.0
Auxins			Amino acids		
IAA	—88.9	—34.0	Aspartic acid	—81.6	—31.0
NAA	—63.9	—77.5	Asparagine	+12.0	—71.0
2,4-D	—50.0	—30.0	Glutamic acid	+ 5.0	+24.0
Indole compounds			Glutamine	0	—15.0
IAAsp	—67.8	—31.0	L-Trp	—5.6	0
TOH	—75.0	—31.0	D-Trp	—1.1	0
IAN	—30.9	—20.0	L-Phe	+2.2	+10.0
IAA	—44.5	—55.0	D-Phe	0	+ 2.0
I-COOH	—74.6	—26.0	Growth substances		
			GA_3	+16.7	+40.0
			Kinetin	+52.4	+21.3

presence of a specific pathway of auxin synthesis in plants of the *Brassicacea* family (KUTÁČEK and KEFELI 1968, MAHADEVAN and STOWE 1972).

L-tryptophan Dehydrogenase Activity

Some characteristics of the enzyme:

Earlier no pyridine nucleotide dehydrogenase catalysing the transformation of an aromatic amino acid was known; it was supposed that only aliphatic amino acids could serve as substrates for dehydrogenases (*e.g.* glutamate

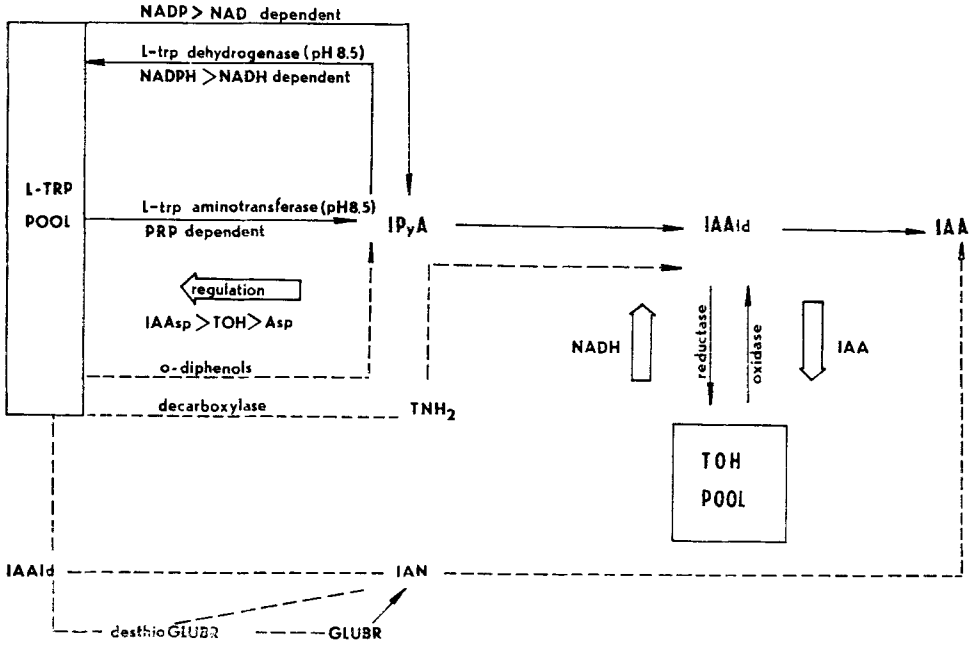

Fig. 3. Pathways of auxin biosynthesis. Regulation of indol-3-ylpyruvate biosynthesis.

dehydrogenase — GDH). L-trp dehydrogenase (TDH) is the first specific dehydrogenase with an aromatic amino acid as substrate; L-phe is enzymatically not transformed (KUTÁČEK and DIMOVA 1985a).

The new L-trp specific enzyme has (at room temperature) a pH optimum 8.5. In the presence of both coenzymes NAD and NADP it catalyses a reversible deamination of L-trp and amination of IPyA. The latter reaction proceeds more intensively. On a Sephadex G-200 column TDH is well separated from TAT as well from GDH (Fig. 1). Using specific inhibitors, the presence of a carbonyl group in the active centre as well as the absence of a heavy metal could be demonstrated. Pea TDH inhibited by EDTA could be reactivated by Ca^{2+} and Mn^{2+} ions. TDH differs from GDH by less sensitivity to SH-inhibitors and by a higher activity with NADP as coenzyme. In case of GDH it is the coenzyme NAD which activates the enzyme more intensely. TDH activity was proved in pea, maize and tomato seedlings, but not in kohlrabi, where only GDH activity could be demonstrated (EBEID *et al.* 1985).

Indol-3-ylacetaldehyde Oxidase System in Pea Plants

Some characteristics of the system:

Two oxidases differing in their pH optima were isolated from pea plants (Fig. 2). The oxidase pH 4.5 is twice as active as the oxidase with pH optimum 7.0. In the molecule of the oxidase pH 7.0 is evidently built in a lipidic com-

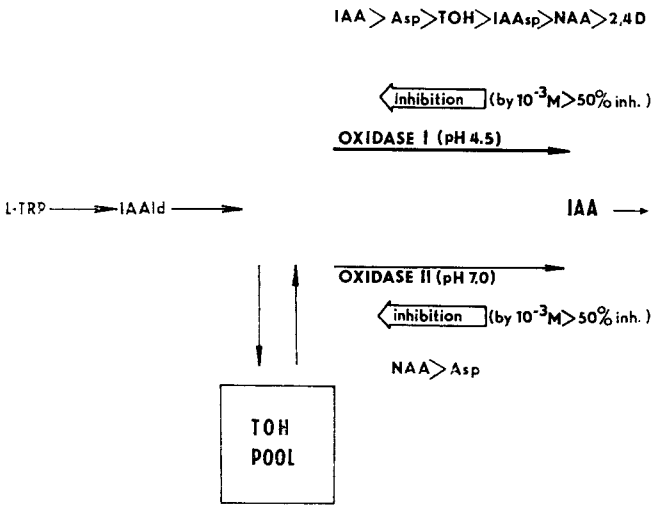

Fig. 4. Regulation of pea indol-3-ylacetaldehyde oxidases activity.

ponent essential for its activity, which can be split off by acetone. The pea enzymes have not a character of dismutase and their activity is not stimulated by addition of pyridine nucleotides (KUTÁČEK and DIMOVA 1985b). It seems that IAld-oxidases in single plants could be of a different character (SCHNEIDER and WIGHTMAN 1978).

Some regulatory properties of the oxidases:

Higher concentrations of IAA (mM), IAAsp and of synthetic auxins inhibit the single oxidases to different degrees (Table 4).

L-asp strongly inhibits both oxidases. The enzymes are well differentiated by the effect of L-asparagine.

Gibberellic acid and cytokinin enhance the activity of the oxidases at a mM concentration.

Regulation of Auxin Synthesis on the Molecular Level (a Draft)

We assume two steps in regulation of the indolylpyruvate pathway of auxin synthesis (Fig. 3). The first step consists of a reduced L-trp influx into the biosynthetic pathway. The second involves a finer regulation of the IAA level in tissues in accordance with its actual physiological need, *e.g.* in biorhythms *etc.*

First part of IAA synthesis, L-trp conversion to indol-3-ylpyruvate:

The low affinity of L-trp to unspecific TAT (expressed by a relatively high value of K_M) has several consequences. A reduced amount of L-trp is entering

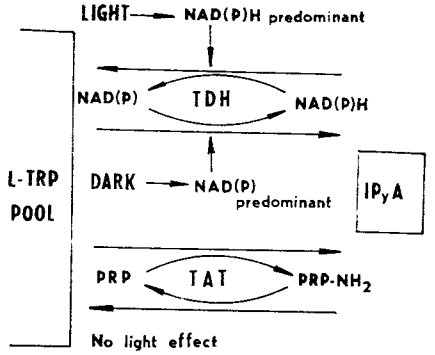

Fig. 5. Influence of light on the regulation of indol-3-ylpyruvate biosynthesis by L-tryptophan dehydrogenase and L-tryptophan aminotransferase.

the pathway of IAA synthesis. The interference of a group of amino acids and indoles showing a higher affinity to the enzyme than L-trp may influence the TAT activity. Noteworthy is the inhibiting effect of L-asp, L-phe, IAAsp.

Interrelation between the catalytic effects of the enzymatic systems of TAT and TDH will be discussed later.

Second part of the IAA synthesis, indol-3-ylacetaldehyde conversions:

The fine regulation is executed by the reversible conversion of IAAld into TOH, a reserve product without auxin activity. The conversion of IAAld into TOH is catalysed by the allosteric IAAld-reductase, the reverse reaction by TOH-oxidase. Both enzymes regulate the level of IAAld, which is a direct precursor of IAA. The conversion of IAAld to IAA is catalysed by IAAld-oxidase (Brown and Purves 1980).

In pea plants it was shown that a system of two oxidases, one, more active, of optimal pH 4.5 and the other of 7.0 convert IAAld into IAA (Fig. 4). Both oxidases are inhibited to a different degree by indoles (IAA, IAAsp) and amino acids (L-asp). We suppose an allosteric character of the oxidases, as a positive effect of gibberellic acid and cytokinin on the enzyme activities was found.

Influence and regulation by ecological factors:

The stimulatory effect of bivalent cations (Ca^{2+} and Mn^{2+}) on TDH activity can be considered as a link between plant nutrition and auxin synthesis (Vacková *et al.* 1985).

The influence of light on a decrease of the IAA content in comparison with dark grown plants (Suzuki *et al.* 1981) could be explained by the TDH reversible activity (Fig. 5). The effect of light transmitted by photosynthesis leads to an accumulation of the reduced form of pyridine nucleotide coenzymes. TDH activated by NAD(P)H reduces the size of the IPyA pool, the intermediary product of IAA synthesis. In dark grown plants accumulates the oxidized form of pyridine nucleotide coenzymes. TDH enhances the production of IPyA synergically with TAT, the plants consequently contain more IAA. The pyridine nucleotide regulated activity of TDH connects IAA synthesis with photosynthesis and by that way with the effect of light. The TDH-activity located in the cell in the chloroplast fraction is relatively high (Vacková *et al.* 1985).

We expect that the study of enzyme compartmentation and the resolution of their forms in the single compartments will afford further important information and will improve our present knowledge concerning the regulation of auxin synthesis on the molecular level, collected in this draft.

REFERENCES

BALDWIN, E.: Dynamic Aspects of Biochemistry. — Cambridge University Press, Cambridge 1949.

BRADFORD, M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. — Anal. Biochem. **72**: 248—254, 1976.

BROWN, H. M., PURVES, W. K.: Indoleacetaldehyde reductase of *Cucumis sativum* L. Kinetic properties and role in auxin biosynthesis. — Plant Physiol. **65**: 107—113, 1980.

DIMOVA S., KUTÁČEK, M.: Enzymes of auxin biosynthesis and their regulation I. Tryptophan and phenylalanine aminotransferase. — Plant Growth Regulation, in press 1985.

EBEID, M. M., DIMOVA, S., KUTÁČEK, M.: Substrate specificity of L-tryptophan dehydrogenase and its distribution in plants. — Biol. Plant. (In press) 1985.

KUTÁČEK, M., DIMOVA, S.: Enzymes of auxin biosynthesis and their regulation II. Tryptophan dehydrogenase activity in plants, a new potential pathway of indolylpyruvate synthesis. — Plant Growth Regulation, in press, 1985a.

KUTÁČEK, M., DIMOVA, S.: Enzymes in auxin biosyntheis and their regulation III. Some properties of pea indolylacetaldehyde oxidase. — Plant Growth Regulation, in press, 1985b.

KUTÁČEK, M., KEFELI, V. I.: The present knowledge of indole compounds in plants of the *Brassicaceae* family. — In: WIGHTMAN, F., SETTERFIELD, G. (ed.): Biochemistry and Physiology of Plant Growth Substances. Pp. 127—152. The Runge Press, Ottawa 1968.

LIU, K. D. F., HUANG, A. H. C.: Subcellular localization and developmental changes of asparate-2-ketoglutarate transaminase isoenzymes in the cotyledons of cucumber seedlings. — Plant Physiol. **59**: 777—782, 1977.

MAHADEVAN, S., STOWE, B.: Conversion of 3-indole-acetaldoxime to glucobrassicin and sulphoglucobrassicin by wood (*Isatis tinctoria* L.). — In: CARR, D. (ed.): Plant Growth Substances 1970. Pp. 117—126. Springer Verlag, Heidelberg 1972.

NOGUCHI, I., HAYASHI, S.: Peroxisomal localization and properties of tryptophan aminotransferase isoenzymes. — J. biol. Chem. **255**: 2267—2269, 1980.

SCHNEIDER, E. A., WIGHTMAN, F.: Auxins. — In: LETHAM, D. S., GOODWIN, P. B., HIGGINS, T. J. V., (ed.): Phytohormones and Related Compounds. Pp. 29—105. Elsevier/North-Holland Biomedical Press, Amsterdam 1978.

SUZUKI, Y., KAMISAKA, S., YANAGISAWA, H., MIYATA, S., MASUDA, Y.: Effect of light on growth and metabolic activities in pea seedlings II. Changes in the IAA content and activities of enzymes involved in the IAA biosynthesis during growth. — Biochem. Physiol. Pflanzen **176**: 35—43, 1981.

TRUELSEN, T. A.: Indole-3-pyruvic acid as intermediate in the conversion of tryptophan to indole-3-acetic acid I. Some characterisations of tryptophan transaminase from mung bean seedlings. — Physiol. Plant. **26**: 289—295, 1972.

TRUELSEN, T. A.: Indole-3-pyruvic acid as one intermediate in the conversion of tryptophan to indole-3-acetic acid II. Distribution of tryptophan transaminase activity in plants. — Physiol. Plant. **28**: 67—70, 1973.

VACKOVÁ, K., MEHTA, A., KUTÁČEK, M.: Tryptophan aminotransferase and tryptophan dehydrogenase activities in some cell compartments of spinach leaves; the effect of calcium ions on tryptophan dehydrogenase. — Biol. Plant. **27**: 154—158, 1985.

WIGHTMAN, F., COHEN, N. D.: Intermediate steps in the enzymatic conversion of tryptophan to IAA in cell. — In: WIGHTMAN, F., SETTERFIELD, G. (ed.): Biochemistry and Physiology of Plant Growth Substances. Pp. 273—288. The Runge Press, Ottawa 1968.