STUDIES ON THE BIOSYNTHESIS OF INDOLE-3-ACETIC ACID IN TOMATO SHOOTS

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry, Auckland University, 1989.

Abstract

The relative contributions of the three main intermediates of indole-3-acetic acid (IAA) biosynthesis from L-tryptophan (L-Trp); indole-3-pyruvate (IPyA), tryptamine (TNH_2) and indole-3-acetaldoxime (IAOX), were investigated *in vivo* in tomato shoots. Initially, L-Trp, D-Trp, IPyA, TNH_2 and IAA were purified from shoots, identified by full-scan mass spectrometry and their concentrations measured using gas chromatography with an electron capture detector. High specific activity [5-^{3}H]IAOX and [5-^{3}H]IPyA were synthesized from L-[5-^{3}H]Trp and used as internal standards. Purification of endogenous IPyA was enabled by forming a stable pentafluorobenzyl oxime derivative in the crude plant extract. The respective endogenous concentrations of L-Trp, D-Trp, TNH_2, IPyA and IAA were found to be 2,520, 103, 146.3, 5.9 and 8.5 ng g^{-1} f. wt. However, IAOX could not be identified as a natural constituent of tomato shoots by full-scan GC-MS. Secondly, incubation of tomato shoots for 6, 10 and 21 h in 30% ^2H_2O was used as a means of labelling IAA and its putative precursors *in vivo*. L-Trp, D-Trp, TNH_2, IPyA and IAA were then extracted and purified and the ^{2}H content measured by combined gas chromatography-mass spectrometry. These indole compounds were labelled rapidly with up to four ^{2}H atoms. Direct comparison of the number and the amount of ^{2}H atoms incorporated (pattern) was obtained from the mass spectral data on the common m/z 130 ion and its isotope peaks. IAA and L-Trp demonstrated an increase in ^{2}H label with up to 17% and 21% of their molecules labelled at 10 h respectively. This was followed by a significant decrease in ^{2}H label at 21 h to 12% for both L-Trp and IAA. This decrease in ^{2}H label was attributed to an increase in protein catabolism, following shoot excision, resulting in the dilution of free L-Trp pool(s) with unlabelled L-Trp from which IAA is biosynthesized. This is reflected in the observed 1.6 to 1.8 fold increase of free L-Trp from 10 to 21 h. In contrast, tryptamine demonstrated a continual increase in ^{2}H label with an average of 8, 20 and 28% of the molecules labelled at 6, 10 and 21 h respectively, suggesting that TNH_2 and IAA were synthesized from separate Trp pools. In addition, the relatively slow rate at which ^{2}H is incorporated into tryptamine would not be sufficient to account for the rate at which IAA becomes labelled. However, IPyA demonstrated a rapid increase in ^{2}H with 22% and 37% of its molecules labelled at 6 and 10 h respectively. From the rate at which IPyA was labelled with ^{2}H and the concentration of IPyA in tomato shoots a rate of synthesis for IPyA in tomato shoots was estimated which was sufficient to provide most of the shoot IAA requirements. Furthermore, the extent to which IAA and IPyA were labelled relative to that of total L-Trp would imply that a smaller more rapidly metabolised pool(s) of L-Trp was the precursor of these compounds. The rate and extent that D-Trp was labelled was consistently less than that of IAA precluding it as a possible precursor of IAA.

These results indicate that in tomato shoots IAA is biosynthesized from a rapidly metabolised sub-pool(s) of L-trptophan predominantly via IPyA.

ACKNOWLEDGEMENTS

My most sincere gratitude is extended to my supervisor Dr Heather Nonhebel for her invaluble advice and guidance during the course of the studies presented in this thesis. Her unending patience, encouragement and dedication provided me with the support necessary to complete this degree.

Thanks also to Professor Alistair Renwick for his support and assistance during my time with the Biochemistry Department and to Associate Professor Paul Woodgate, Brian Dobson, Sue Alford and Dr Harry Young for their assistance in obtaining the mass spectral data.

Finally, my deepest thanks to my wife Julie, parents Des and Ann Cooney and parents-in-law Ron and Helen Howes for their love, support and encouragement throughout the past three and a half years. Lastly to my baby son Thomas, for making it all worth while.

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ABBREVIATIONS

BHT	2,6-Di-*tert*-butyl-4-methylphenol
Bq	Bequerels
CPM	Counts per minute
DPM	Disintegrations per minute
EI	Electron impact
GA_3	Gibberellin A_3
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GC-MS/SIM	Gas-chromatography-mass spectrometry using selected ion monitoring
g^{-1}.f.wt	Per gram fresh weight
h	Hour
HPLC	High performance liquid chromatography
IAA	Indole-3-acetic acid
IAAld	Indole-3-acetaldehyde
IAN	Indole-3-acetonitrile
IAOX	Indole-3-acetaldoxime
IEt	Indole-3-ethanol
IPyA	Indole-3-pyruvic acid
KBq	Kilo bequerels
MBq	Mega bequerels
min	Minutes
MS	Mass spectrometry
m/z	Mass-to-charge ratio
NAA	Naphthalene-1-acetic acid
NZIG	New Zealand Industrial Gases
PFB	Pentafluorobenzyl
PFBHA	Pentafluorobenzyl hydroxylamine HCl
PFB-IAA	Pentafluorobenzyl derivative of indole-3-acetic acid
PFB-IPyA	Pentafluorobenzyl oxime derivative of indole-3-pyruvate
PFB-MeIPyA	Pentafluorobenzyl oxime derivative of methylindole-3- pyruvate
$(PFB)_2$-TNH_2	Pentafluorobenzyl derivative of tryptamine

$(PFB)_2$-L-Trp	Pentafluorobenzyl derivative of L-tryptophan
$(PFB)_2$-D-Trp	Pentafluorobenzyl derivative of D-tryptophan
PPO	2,5-diphenyloxazole
QIP	Quench indicating parameter
TNH_2	Tryptamine
T_R	Retention time
TIC	Total ion current
Trp	Tryptophan
$t_{1/2}$	Half-life
UV	Ultra violet
2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid