THE BIOSYNTHESIS AND CONTROL OF INDOLEACETIC ACID

by

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ABSTRACT

Attempts were made to form indoleacetic acid in cellfree extracts of mung bean (*Vigna radiata*) shoots. The extracts were incubated with radiolabelled tryptophan and other substrates and cofactors thought to be involved in indoleacetic acid biosynthesis. After incubation indolepyruvate and indoleacetic acid were separated and quantified by HPLC. There was no significant difference in the conversion of tryptophan to indolepyruvate and indoleacetic acid between the incubations and control incubations using boiled extract.

The concentrations of indolepyruvate and indoleacetic acid in mung bean hypocotyl suspension cultures were measured using GC-MS SIM over the growth of the culture, a period of 29 days. Indoleacetic acid concentrations, although scattered, mostly remained at constant low levels in the range of 6 to 9ng/g fwt of culture. The indolepyruvate levels steadily increased to a maximum level after 14 days, then remained at this level, 10 to 12 ng/g fwt, for the remainder of the culture period. This plateau in indolepyruvate concentration matched the period that the suspension culture was in the logarithmic phase of growth.

An aromatic amino acid aminotransferase was purified over 33,000 fold from the shoots and primary leaves of mung beans, as determined using a tryptophan aminotransferase activity assay. The enzyme was a monomer, with a molecular weight of about 58kDa. The pH optimum was broad, with a maximum at about 8.6. The relative activities of the aromatic amino acids were: tryptophan 100, tyrosine 83 and phenylalanine 75, and the K_ms were 0.095, 0.08 and 0.07mM respectively. The enzyme was able to use 2-oxoglutarate, oxaloacetate

and pyruvate as the oxo acid substrate at relative activities 100, 128 and 116 and K_ms 0.65, 0.25 and 0.24mM respectively

In addition to the aromatic amino acids the enzyme was able to transaminate alanine, arginine, leucine and lysine to a lesser extent, and showed slight activity with asparagine, aspartate, histidine, valine and D-tryptophan and tyrosine. Inhibition studies showed that the alanine, aspartate and histidine activities were part of the aromatic amino acid aminotransferase activity.

The enzyme was not inhibited by indoleacetic acid, although naphthaleneacetic acid did inhibit slightly. There was evidence of substrate inhibition by hydroxyphenylpyruvate at high concentrations. Addition of the cofactor pyridoxal phosphate only slightly increased the activity of the enzyme.

The enzyme was blotted onto a PVDF membrane cleaved by *in situ* trypsin digest. Three of the tryptic fragments were sequenced. These fragments had approximately 60% sequence similarity with plant aspartate aminotransferases and tyrosine aminotransferases.

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2-OG	2-Oxoglutarate
2,4-D	2,4-Dichlorophenoxyacetic acid
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
μ-	Micro- (10^{-6})
cDNA	Complemant DNA
conc.	Concentrated
fwt	Fresh weight
g	Gram
hν	Light radiation
i.d.	Internal diameter
k-	Kilo- (10^{3})
m-	Milli- (10^{-3})
m/z	Mass to charge ratio
mRNA	Messenger RNA
n-	Nano- (10^{-9})
nd	Not determined
phnj	Protein j of phn operon in *E. coli*
v	Observed enzyme rate
xg	Gravities (acceleration)
ACC	1-Aminocyclopropane-1-carboxylic acid
ADP	Adenosine dinucleotide phosphate
Ala	Alanine
Asn	Asparagine
Asp	Aspartate
BHT	2,6-Di-*tert*-butyl-4-methylphenol

Bq	Bequerel
BSA	Bovine serum albumin
CAPS	3-[Cyclohexylamino]-1-propane-sulphonic acid
C_n	n carbon compound
CPP	Copalyl pyrophosphate
D_2O	Deuterium oxide
Da	Dalton
DEAE	Diethylaminoethyl
DEPC	Diethyl pyrocarbamate
DNA	Deoxyribonucleic acid
DNP	2,4-Dinitrophenylhydrazine
DPM	Disintegrations per minute
E.C.	Enzyme convention numbering
EDTA	Ethylenediaminetetraacetic acid
EFE	Ethylene-forming enzyme
GA_n	Gibberellin A_n
GAP	Glyceraldehyde-3-phosphate
GC	Gas chromatography
GGPP	Geranylgeranyl pyrophosphate
Gln	Glutamate
Glu	Glutamine
Gly	Glycine
HPLC	High performance liquid chromatography
HR	High resolution
IAA	Indole-3-acetic acid
IAAld	Indoleacetaldehyde
IAN	Indoleacetonitrile

IAOx	Indoleacetaldoxime
Ile	Isoleucine
IPyA	Indolepyruvate
Kg	2-Oxoglutarate
K_m	Michaelis constant
$K_m^{\text{amino acid}}$	Michaelis constant for the amino acid substrate
$K_m^{\text{oxo acid}}$	Michaelis constant for the oxo acid substrate
L	Litre
Leu	Leucine
Lys	Lysine
M-	Mega- (10^6)
Mal	Malate
MC	Mesophyll cells
Me	Methyl
MS	Mass spectroscopy
MW	Molecular weight
NAA	Naphthalene-2-acetic acid
NAD^+	β-Nicotinamide adenine dinucleotide
NADH	β-Nicotinamide adenine dinucleotide, reduced form
$NADP^+$	β-Nicotinamide adenine dinucleotide phosphate
NADPH	β-Nicotinamide adenine dinucleotide phosphate, reduced form
OAA	Oxalacetate
PCR	Polymer chain reaction
PFB	Pentafluorobenzyl