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INVERTASE AND AUXIN-INDUCED ELONGATION IN INTERNODAL SEGMENTS OF *PHASEOLUS VULGARIS*

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Abstract—A close positive correlation was observed between segment elongation and the specific activity of soluble acid invertase in stem segments of *P vulgaris* incubated for 21 hr in the presence of IAA or of several synthetic auxins and auxin analogues Optimum concentrations for the stimulation of growth and invertase activity were similar and varied from 10^{-6} M (2,4-D) through 10^{-5} M (IAA, IBA, α-NAA, β-NAA) to greater than 10^{-4} (IPA, PoAA, *trans*-cinnamic acid) The weak activity of *trans*-cinnamic acid, a competitive inhibitor of auxin action, may have resulted from *cis–trans* isomerization during incubation The concentration of hexose sugars in the segments fell during incubation in the presence of auxin, the greatest decline in hexose concentration occurring in the presence of compounds exhibiting the greatest stimulation of growth

INTRODUCTION

Correlated changes in the activity of acid invertase (β-fructofuranosidase, EC 3 2 1 26) and rates of cell expansion growth have been reported in the stems, roots and leaves of several species in which sucrose is the principal phloem-mobile carbohydrate The occurrence and significance of this association has recently been reviewed by Morris [1, 2] Tissues undergoing rapid cell expansion growth characteristically contain high concentrations of hexose sugars and low concentrations of sucrose, and the hydrolysis of imported sucrose by a vacuolar acid invertase is probably the principal means by which hexose sugars are made available for metabolism during cell growth [3, 4] Furthermore, by reducing sucrose concentrations in the apoplast or symplast of sink tissues, acid invertases located in the cell wall, the cytoplasm and/or the cell vacuoles may play a major role in maintaining the source-to-sink gradients in sucrose concentration and hydrostatic pressure which drive phloem transport [2, 5]

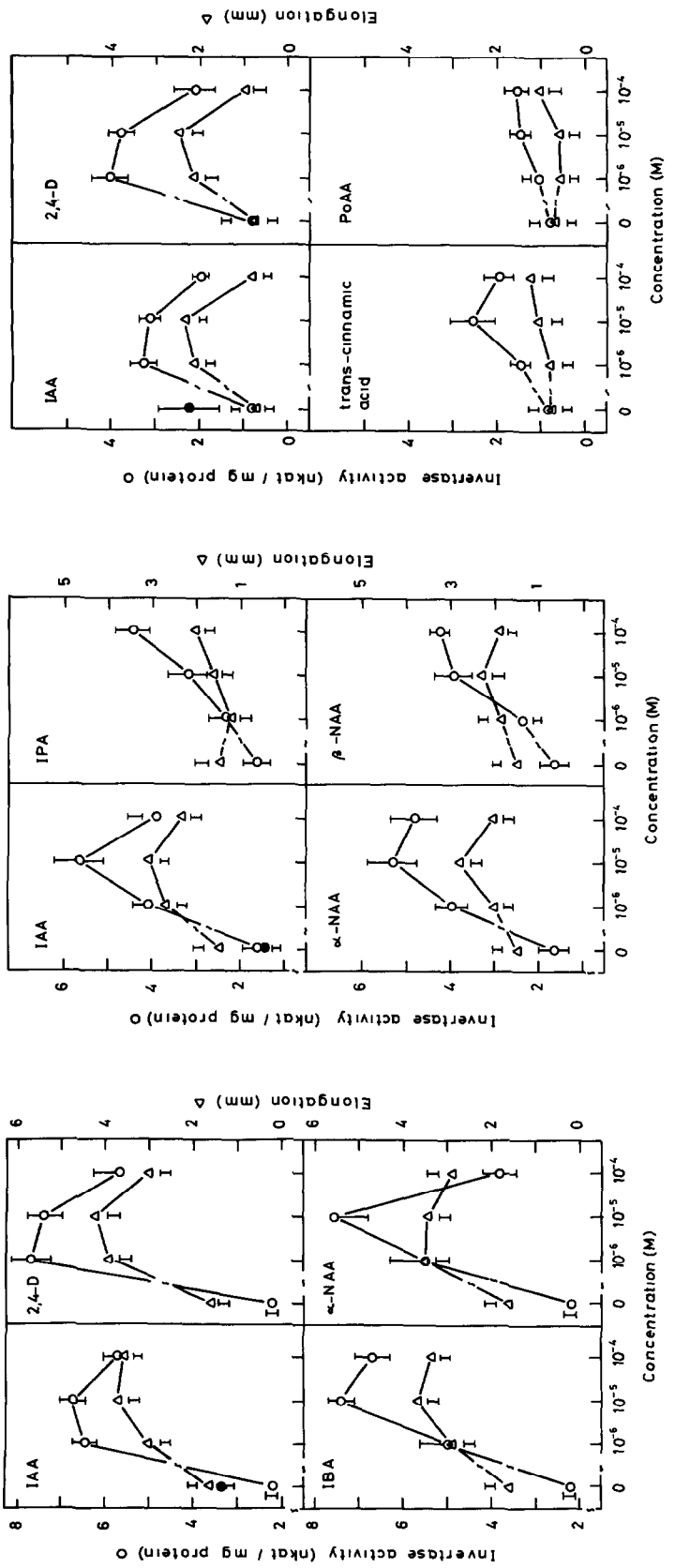
There is now much evidence to indicate that the synthesis and/or activity of acid invertase may be regulated by hormones produced in, or accumulated by, sink tissues [1, 2] In leaves [6] and stems [7] of bean (*Phaseolus vulgaris* L) highest specific activities of the enzyme and maximum rates of cell expansion occur at the time of peak free indol-3-yl-acetic acid (IAA) concentration, and applications of exogenous IAA can substitute for the shoot apical bud both in promoting an increase in acid invertase activity in the treated, decapitated internode, and in polarizing the flow of ^{14}C-labelled assimilate towards the treated internode [1] When incubated in water, segments cut from young expanding internodes of *P vulgaris* show a gradual loss of acid invertase activity (half-time for the decay about 16 hr) In the presence of 10^{-5} M IAA, however, a substantial rise in the specific activity of the enzyme occurs with a lag of 3–6 hr, and elongation of the internode segments is promoted [8]

In this study we have investigated the ability of a range of synthetic auxins and auxin analogues, chosen for their widely different growth-promoting activities, to stimulate extension growth and the specific activity of acid invertase in isolated internode segments of *P vulgaris*

RESULTS

The results of the individual experiments are shown in Figs 1, 2 and 3 In the absence of IAA or one of the synthetic auxins very little elongation of the internode segments occurred during a 21 hr incubation period and the specific activity of acid invertase either fell substantially or did not change significantly (specific activities 65 5, 114 1 and 36 8 % of initial activities in Experiments 1, 2 and 3 respectively) Elongation growth and the specific activity of the enzyme were both strongly promoted by IAA and several of the synthetic auxins tested Most of the compounds exhibited a clear optimum concentration which, with very few exceptions, was the same for both processes (Figs 1–3) For most of the compounds examined, this optimum concentration was in the region of 10^{-5} M, but for 2,4-D which was the most active auxin tested, the optimum concentration was nearer 10^{-6} M The least active compounds, IPA, PoAA and *trans*-cinnamic acid, appeared not to have reached their optimum concentrations at 10^{-4} M

When the results for all three experiments were pooled, a close positive linear correlation was found between elongation growth over the 21 hr incubation period and the final specific activity of acid invertase (Fig 4, $y = 0\,2488 + 0\,4910\,x, r = 0\,9422\,(P = 0\,001), r^2 = 0\,8877, n = 39$) Significantly, this relationship between elongation growth and specific activity of the enzyme held both for the control (water-treated) and auxin-treated samples, and for samples treated with the same concentration of a compound in different experiments in which the



Figs 1, 2 and 3 Changes in length (Δ) and specific activity of acid invertase (○) in internode segments of *P vulgaris* incubated for 21 hr in the presence of several synthetic auxins and auxin analogues (Results of three experiments carried out at different times IAA was included in all experiments for comparison) Initial mean invertase specific activities are shown for each experiment (●)

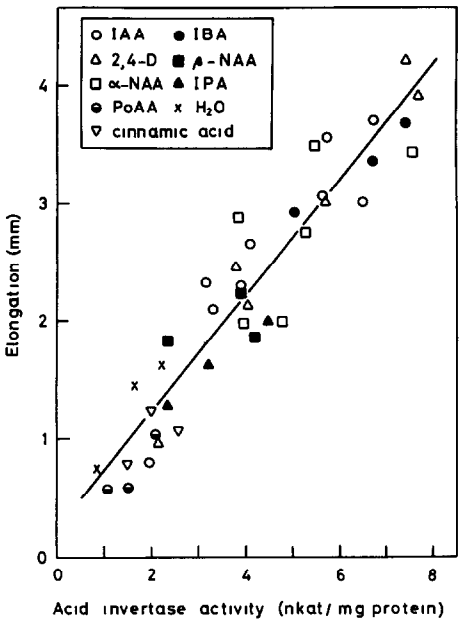


Fig 4 Regression of internode segment elongation on the specific activity of soluble acid invertase following incubation for 21 hr in the presence of IAA and several synthetic auxins Parameters for the regression analysis are given in the text

absolute response differed (for example, see the responses to 10^{-5} M IAA in experiments 1, 2 and 3, and the responses to 2,4-D in Experiments 1 and 3—Figs 1–3)

In Table 1 the compounds are listed in order of their growth-promoting activity at a concentration of 10^{-5} M Also shown in this table are the mean invertase activities of the segments at the end of the incubation period expressed as percentages of both control and initial values With the exceptions of IPA and PoAA, all compounds tested significantly increased segment elongation compared to the controls 2,4-D (the most active compound tested) increased elongation growth by almost 300% When expressed as a percentage of the control value, acid invertase activity was increased to a far greater extent by the compounds tested than was elongation However, as indicated above, control segments generally exhibited a decrease in enzyme activity during incubation When invertase activities at the end of the incubation period were expressed as percentages of initial values for each experiment, percentage stimulation of invertase activity was similar to the stimulation of elongation

Initial hexose concentrations in the segments varied considerably between the three experiments (Table 2) This variation may have resulted from small differences in the stage of internode development at the time the samples were taken [7] and from temporal variation in light intensities in the greenhouse during growth of the seedlings By the end of the 21 hr incubation periods hexose concentrations had fallen in all samples treated with synthetic auxins The decline in hexose concentration was generally greater the higher the applied auxin concentration, probably reflecting the greater stimulation of metabolism at higher auxin levels As a general rule, the greatest fall in hexose concentration occurred in the presence of the compounds causing the largest growth stimulation Levels of sucrose in the segments were below the limits of detection by the analytical methods used

DISCUSSION

The results presented here demonstrate that a wide range of synthetic auxins can stimulate both elongation growth and soluble acid invertase activity in isolated stem segments of *P vulgaris* and confirm our previous reports of a close positive correlation between expansion growth and acid invertase activity in the stem of this species [7] The responses to the different growth regulators tested were in general agreement with previous observations on the relationship between auxin structure and growth regulatory activity in typical auxin bioassay systems (cf

Table 1 Influence of IAA and several synthetic growth regulators on the stimulation of elongation in isolated internode segments of *P vulgaris* and on their specific activity of soluble acid invertase

Growth regulating compound	Elongation (% of control elongation)	Acid invertase activity (% of control value)	Acid invertase activity (% of initial activity)
2,4-D	295	397	194
IAA	251	345	246
IBA	228	334	219
α-NAA	202	334	298
β-NAA	156	241	275
trans-cinnamic acid	143	312	115
IPA	112	198	225
PoAA	79	182	67
Control (water-treated)	100 0	100 0	72

Elongation and invertase activities are expressed as percentages of the values obtained from control segments receiving no growth regulator treatment Results are for segments treated with the appropriate growth regulator at 10^{-5} M and are averages for all experiments

Table 2 Tissue concentrations of hexose sugars in isolated internodal segments of *P vulgaris* before and after incubation for 21 hr in solutions of several synthetic auxins

Growth regulator concentration (M)	Hexose sugar concentration (mg/g fr wt)			
Experiment 1	IAA	2,4-D	IBA	α-NAA
0	3 68 ± 0 27	—	—	—
10^{-6}	2 30 ± 0 17	2 77 ± 0 26	3 21 ± 0 32	2 96 ± 0 29
10^{-5}	2 76 ± 0 31	2 49 ± 0 16	2 98 ± 0 26	2 63 ± 0 22
10^{-4}	2 22 ± 0 14	2 81 ± 0 11	2 59 ± 0 22	2 35 ± 0 22
	(Initial concentration = 3 48 ± 0 37 mg/g)			
Experiment 2	IAA	α-NAA	β-NAA	IPA
0	6 58 ± 0 37	—	—	—
10^{-6}	5 62 ± 0 49	6 28 ± 0 38	6 57 ± 0 22	7 04 ± 0 64
10^{-5}	5 20 ± 0 42	5 87 ± 0 33	6 19 ± 0 17	5 57 ± 0 49
10^{-4}	5 52 ± 0 52	5 01 ± 0 70	6 03 ± 0 57	5 65 ± 0 44
	(Initial concentration = 7 20 ± 0 44 mg/g)			
Experiment 3	IAA	2,4-D	*trans*-cinnamic acid	PoAA
0	4 74 ± 0 36	—	—	—
10^{-6}	3 91 ± 0 56	2 75 ± 0 45	4 27 ± 0 41	2 94 ± 0 52
10^{-5}	2 55 ± 0 37	2 47 ± 0 52	3 44 ± 0 14	1 97 ± 0 77
10^{-4}	2 65 ± 0 18	1 71 ± 0 35	2 57 ± 0 29	2 12 ± 0 22
	(Initial concentration = 4 40 ± 0 59 mg/g)			

Table 1) PoAA showed little or no activity whilst 2,4-D was the most stimulatory of all the compounds tested [9, 10], α-NAA was more active than β-NAA [11, 12], and IAA and IBA were more active than the corresponding propionic acid derivative [13] The behaviour of *trans*-cinnamic acid, however, was unexpected Veldstra [14, 15] found that whilst the *cis*-isomer exhibits considerable activity in auxin bioassays, the *trans*-isomer of cinnamic acid is inactive Indeed, the *trans*-isomer is regarded as a true 'anti-auxin' which competitively inhibits the growth-promoting activity of IAA and other auxins in typical bioassay systems [16–18] In the present experiments *trans*-cinnamic acid exhibited weak but significant growth activity and, at a concentration of 10^{-5} M or greater, significant stimulation of acid invertase specific activity (Fig 3, Table 1) A possible explanation for this apparent activity is that exposure to continuous fluorescent light during incubation of the segments may have led to some conversion of the *trans*-isomer to the *cis*-isomer *Cis–trans* isomerization of related hydroxycinnamic acids by UV and blue light has been reported (see [18])

Several previous reports have shown that IAA and other compounds possessing auxin-like growth-stimulatory activity can bring about closely correlated changes in elongation growth and acid invertase specific activity in isolated tissue segments [1, 2, 8, 19–21] The present study extends the range of synthetic auxins tested and demonstrates that compounds of widely different growth-stimulating activities bring about proportional increases in elongation and enzyme activity The mechanism by which IAA and related compounds leads to an increase in soluble acid invertase activity, however, remains obscure and it is not yet clear whether the observed increase in activity is a cause or a consequence of auxin-induced growth In immature internodal tissue of sugar cane (*Saccharum officinarum* L) end product (hexose) repression of the synthesis of a vacuolar acid invertase has been demonstrated [22] By stimulating growth and hexose consumption (Table 2) IAA and synthetic auxins might remove such repression If so, the observed increase in enzyme level would be a consequence of growth Elsewhere, however, we discuss evidence which suggests that in *P vulgaris* internodes such a possibility is unlikely [8] Firstly, we have found that a substantial IAA-induced increase in acid invertase activity can occur in the presence of growth-inhibitory concentrations of Ca^{2+} ions and mannitol The increase in invertase activity, therefore, is not a consequence of cell growth Secondly, in segments prepared and incubated using methods identical to those employed here, IAA-induced stimulation of acid invertase activity is frequently increased rather than decreased by the inclusion of glucose in the incubation medium, suggesting that normal tissue concentrations of hexose are too low to suppress invertase synthesis and, indeed, may limit its synthesis (cf [23]) Thirdly, when incubated in the presence of sucrose, the hexose concentration of segments rises to a level substantially greater than the *initial* concentration In spite of this, IAA-induced increases in acid invertase activity can still occur Fourthly, in the stems of intact, light-grown plants of *P vulgaris*, cell expansion is accompanied by appreciable increases in both the specific activity of acid invertase and the concentration of hexose sugars [7] It is unlikely, therefore, that normal tissue concentrations of hexose are sufficient to suppress synthesis of the enzyme

Kinetic studies using the *in vitro* system described here have indicated that measurable change in acid invertase specific activity can occur between 3 and 6 hr after the addition of IAA to the segments, whilst measurable change in length takes place within 1–2 hr [8] Evidence has been presented to show that the auxin-induced growth of isolated tissue segments of several species involves two

separable responses [24] The first, very rapid, response is an acid-induced wall loosening which allows a short-lived turgor driven expansion of cells This response (which can be mimicked by H^+) is insensitive to inhibitors of nucleic acid and protein synthesis [25] This response is followed by a second auxin-inducible response which leads to steady-state cell growth Unlike the first response, the second cannot be induced by H^+ ions, is prevented by inhibitors of RNA and protein synthesis, and may involve the synthesis of compounds essential for wall growth [24] A rapid increase in the incorporation of label from [^{14}C]glucose into wall polysaccharides occurs following IAA treatment of pea stem segments and there is an increase in enzymes concerned with cell wall synthesis [26–28] We have found that both auxin-induced elongation and auxin promotion of invertase activity in isolated *P vulgaris* stem segments is prevented by inhibitors of protein synthesis [8], and similar observations have been reported for the gibberellin-induced promotion of stem elongation growth and invertase activity in other species [23, 29] Protein synthesis appears necessary for these hormone-induced effects We propose that by releasing hexose for the synthesis of cell-wall and cytoplasmic components in the expanding cell, the stimulation of acid invertase synthesis may be a necessary part of the second, long-term component of auxin-induced cell expansion

EXPERIMENTAL

Plants of *Phaseolus vulgaris* L cv Masterpiece were grown in 22 × 37 cm seed trays containing John Innes seedling compost (80 seeds per tray) under natural daylight in a warm greenhouse Plants were used 4 or 5 days after emergence (8–9 days from sowing) when internode I was between 10 and 12 mm long Segments (9 75 mm) were cut from internode I using a double-bladed cutter, surface sterilized for 30 sec in EtOH and thoroughly rinsed in a large excess of sterile distilled H_2O Batches of 5–8 segments were transferred aseptically to sterile 50 ml conical flasks containing 5 ml of test soln which had been sterilized by passing through a Sartorius cellulose nitrate membrane filter All transfers were carried out in a laminar cabinet The plugged flasks were transferred to a shaking incubator and gently agitated at 25° for 21 hr under continuous weak illumination (100 μM/m^2/sec) from daylight fluorescent lamps

At the end of the incubation period each batch of segments was blotted dry and weighed The final length of each segment in each batch was determined by arranging them on a glass microscope slide previously marked with a scale of known length, and measuring the lengths of images of the segments scale projected onto a horizontal surface by a conventional photographic enlarger Batches of measured segments were frozen and stored overnight at −20° prior to biochemical analysis

Thawed segments were homogenized in 25 ml ice-cold acetate buffer (0 1 M, pH 5) and the homogenates were centrifuged at 2500 *g* for 20 min at < 4° Soluble acid invertase was assayed by incubating 0 3 ml aliquots of the supernatant with 0 7 ml 100 mM sucrose for 1 hr at 30° Invertase activity was determined as the difference in hexose released [30] by active and heat-inactivated samples and results were expressed per unit of soluble protein [31] Hexose concn in the supernatants was determined [30] and sucrose was assayed by measuring the change in hexose concn after hydrolysis by 0 3 M HCl (100°, 1 hr) Hydrolysed samples were neutralized with 0 3 M KOH Full details of the techniques and their validation appear elsewhere [6]

The following growth regulating compounds were tested in unbuffered aq solns at concns of 10^{-6}, 10^{-5} and 10^{-4} M, indol-3-yl-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D) and naphth-1-yl-acetic acid (α-NAA) (Sigma), γ-(indol-3-yl-butyric) acid (IBA), indol-3-yl-propionic acid (IPA), phenoxy-acetic acid (PoAA), naphth-2-yl-acetic acid (β-NAA) and *trans*-cinnamic acid (Aldrich)

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