ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 56, nr 2: 325-336
1987

Studies of fluorophenylalanine induced *Avena* coleoptile elongation: Possible modulation of auxin oxidase activity

ANDREW B. MAKSYMOWYCH[1], JOSEPH A. J. ORKWISZEWSKI[2]

[1] Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010, U.S.A.
[2] Department of Biology, Villanova University, Villanova, Pennsylvania 19085, U.S.A.

(Received: October 18, 1986. Accepted: November 5, 1986)

Abstract

Previous studies have established that the amino acid analogue p-fluorophenylalanine promotes the elongation of etiolated *Avena* coleoptile segments and lead us to suggest that the elongation response induced by this isostere is due to a depression of L-phenylalanine ammonia-lyase activity resulting in a decreased level of low molecular weight phenols which, directly or indirectly, modify *Avena* auxin oxidase activity and result in altered levels of growth substance in coleoptile segments. Further elucidation of the nature of the growth response necessitated additional studies on analogue specificity. To date only fluorinated analogues have been used to modify L-phenylalanine ammonia-lyase activity, therefore, data on the effects of other halide substituted phenylalanines are presented and comapred to the results of fluorophenylalanine treatment. Data indicate that the p-fluorophenylalanine response is isomer and substituent specific for no other halide substituted analogue elicits a positive elongation response. Treatment of *Avena* coleoptiles with p-fluorophenylalanine results in a modified auxin oxidase activity. A decrease in oxidase specific activity has been observed throughout a series of temperature and pH studies performed in this laboratory.

Key words: Avena coleoptile, auxin oxidase, fluorophenylalanine, L-phenylalanine ammonia-lyase, phenolic metabolism

INTRODUCTION

Hopkins and Bonnell (1969) reported that extension growth of excised *Avena* coleoptile apices in darkness was significantly enhanced by the amino

[1] This work represents a portion of the dissertation of A.B.M. in partial fulfillment of the Ph.D. degree at Bryn Mawr College.

[2] To whom reprint requests should be sent.

acid analogue DL-p-fluorophenylalanine (p-FPA). This effect, which contrasts with the normally inhibitory action of p-FPA (Miller and Ross 1966, Nooden and Thimann 1966, Richmond 1962) appears to be specific for the para isomer (Hopkins and Orkwiszewski 1971), since DL-o-fluorophenylalanine (o-FPA) is less effective and DL-m-fluorophenylalanine (m-FPA) has no effect on segment elongation. This growth enhancement was correlated with the finding that treatment of coleoptile segments with p-FPA or o-FPA results in the depression of extractable L-phenylalanine ammonia-lyase (PAL, E.C. 4.3.1.5) activity and the reduction of in vivo chlorogenic acid levels (Hopkins and Orkwiszewski 1971, Orkwiszewski and Hopkins 1974, Orkwiszewski et al. 1976). These authors suggested that p-FPA and o-FPA may promote coleoptile elongation by lowering PAL activity which in turn would decrease the synthesis of potentially inhibitory low molecular weight phenols.

To date studies have been limited to effects of the fluorinated phenylalanine (PA) analogues on PAL activity and growth. The effects on extension growth of other halogen substituted PA analogues have not been investigated. Therefore, in this paper, in order to establish p-FPA specificity as an effector of our system, the effects on elongation of the other halogen substituted PA's are compared. This is done with the intent of using p-FPA to induce changes in growth and examining extracts from treated coleoptiles for concomitant changes in auxin (indole-3-acetic acid, IAA) oxidase activity. It is for this reason that we studied the effect of p-FPA pretreatment on auxin oxidase activity. Our results are presented and discussed within the context of initial investigations.

MATERIALS AND METHODS

Unhusked seeds of *Avena sativa* L. cv. Clintland 60 (The Stanford Seed Co., Denver, Pennsylvania) were grown as described by Hopkins and Bonnell (1969). Plant material used throughout this study was 8 mm apical coleoptile segments minus primary leaves, which were excised by hand from 4-day-old etiolated seedlings. The segments were collected, randomized in 20 mM phosphate buffer (pH 6.3) and distributed into 50 cm^3 beakers containing 5 cm^3 of incubation medium. The incubation medium for control segments consisted of the above phosphate buffer plus 1.5% w/v sucrose (BSM). Treated segments were incubated on BSM containing various concentrations of p-fluorophenylalanine (p-FPA), p-chlorophenylalanine (p-CPA), p-bromophenylalanine (p-BPA), and p-iodophenylalanine (p-IPA). Coleoptile segments were incubated for 18 hr at $26 \pm 1°C$ (Orkwiszewski et al. 1976). A group of segments was removed prior to incubation and considered

to be the 0 hr time point for all studies. All operations involving living tissue were conducted either in darkness or under a dim green safelight (Hopkins and Bonnell 1969).

For growth studies, the length of each segment was measured with a binocular microsocpe and millimeter rule. Elongation is defined as the increase in coleoptile length during the period of incubation.

Deamination of halide substituted PA's was assayed with enzyme powders prepared from untreated coleoptiles, according to the procedure of Orkwiszewski et al. (1976).

Chlorogenic acid analyses were performed by a modified Hoepfner procedure detailed by Hopkins and Orkwiszewski (1971).

Auxin oxidase was prepared by making acetone powders (Hopkins and Orkwiszewski 1971) from coleptiles grown in BSM (control enzyme) or BSM and 5 X 10^{-3} M p-FPA for 18 hr. The resulting powders were suspended in 30 mM phosphate buffer (pH 5.9) at 10 mg powder per cm^3 of buffer, and extracted at 3°C for 1/2 hr. The resultant suspension was clarified by centrifugation at 20,000 × g, 1/2 hr. The clear supernatent was used as the source of IAA oxidizig activity. A 1 cm^3 aliquot of the supernatent was removed for protein determination (Lowry et al. 1951).

Enzyme activity was assayed at 261 nm (Ray 1956) and expressed as ΔA_{261} mg protein $^{-1}$ hr $^{-1}$. The reaction mixture consisted of 0.1 cm^3 each of dichlorophenol (1 mM) and $MnCl_2$ (1 mM), 0.2 cm^3 IAA (1 mM), 2.1 cm^3 20 mM phosphate buffer (pH 5.9) and 0.5 cm^3 enzyme. The reaction was initiated by addition of enzyme and was monitored at 26°C. The reaction rate was stable throughout the period of study.

The temperature profile was determined using the assay described above. An individual extract from control and p-FPA pretreated coleoptiles was assayed for 1 hr at each of the respective temperatures.

A pH vs activity profile was constructed using the same assay, with reaction mixtures made up in 20 mM buffers of the appropriate pH. An individual (control and p-FPA) sample was assayed for each point.

In figures 1 and 2 lines connect data points and do not represent fitted curves.

RESULTS

EFFECTS OF HALIDE SUBSTITUTED PHENYLALANINES ON EXTENSION GROWTH

Table 1 shows the effects of halide substituted analogues on extension growth. These data summarize results from experiments in which coleoptiles were treated with two concentrations (1 mM and 5 mM) of the para halogenated analogues of PA. p-Fluoro-, p-chloro-, p-bromo- and p-iodo-D,L-

-phenylalanines were employed. Control coleoptiles were grown in BSM with no additives. Table 1 illustrates that only the p-fluoro substituted isomer elicits the enhanced elongation response at both concentrations. This response is also dose dependent, as evidenced by an increase in elongation

Table 1

Effect of halide substituted phenylalanines on extension growth

Treatment[a]	Elongation ±s.e.[b]	%[c]	Elongation ±s.e.[d]	%[e]
BSM	2.1±0.1	100±0	2.0±0.1	100±0
p-FPA	2.9±0.2	132±9	3.2±0.2	161±9
p-CPA	1.5±0.2	71±6	1.3±0.2	65±7
p-BPA	0.9±0.1	46±3	1.0±0.2	51±6
p-IPA	0.9±0.1	42±4	0.7±0.1	35±4

[a] Eight mm coleoptile apices were measured after an 18 hr dark incubation at 26±1°C on BSM with and without the halide substituted analogue. Experiments represent the average of several (1 mM n = 10, 5 mM n = 7) trials in which 20 segments were used per experiment for each condition.

[b] Treated with BSM or 1 mM analogue ± standard error.

[c] Data expressed in terms of %BSM ± standard error. ANOVA significant at 1% level ($F = 42.78 > 4.02$).

[d] Treated with BSM or 5 mM analogue ± standard error.

[e] Data expressed in terms of %BSM ± standard error. ANOVA significant at 1% level ($F = 50.23 > 4.22$).

(168% versus 138%) at the higher versus the lower concentration of the analogue. Treatment of coleoptiles with the other compounds does not elicit an elongation response and in fact appears to be inhibitory to extension growth at both concentrations.

DEAMINATION OF HALIDE SUBSTITUTED PHENYLALANINES

Table 2 summarizes studies in which the halide substituted analogues were used as substrates for the native PAL extracted from untreated coleoptiles. These experiments were performed in order to determine the

Table 2

Deamination of halide substituted phenylalanines by PAL

Substrate[a]	Specific activity ±s.e.[b]	%[c]
L-Phe	0.660±0.025	100±0
p-FPA	0.382±0.013	55±1
p-CPA	0.446±0.005	65±2
p-BPA	0.296±0.009	43±1
p-IPA	0.088±0.003	13±0

[a] The concentration of L-Phe, p-FPA, p-CPA, p-BPA is 30 mM. The concentration of p-IPA is 12 mM. All concentrations are at saturation.

[b] Specific activity is defined as $\Delta A_{290} \cdot h^{-1} \cdot mg\ protein^{-1}$.

[c] Data are expressed in terms of % L-Phe ± standard error.

extent to which each of the analogues would serve as a substrate for PAL. The concentrations of each analogue substrate in their respective reaction mixtures were at saturation with respect to the enzyme protein (Lee 1978). Deamination of each analogue by PAL is compared to the native substrate of the enzyme, L-phenylalanine (L-Phe). Our data illustrate that only L-Phe is efficiently deaminated by PAL. The halogenated substrates cause approximatly a 50% decrease in enzyme specific activity in vitro. The low specific activity listed for p-IPA may be related to the size of the halide substituent causing steric hindrance and influencing the enzyme substrate complex. The charge of the substituent and orbital interactions may also play a role. Therefore, in vitro deamination data do not correlate with the growth experiments. This aspect of our study may warrant further investigation.

THE EFFECT OF HALIDE SUBSTITUTED PHENYLALANINES ON CHLOROGENIC ACID LEVELS

Table 3 describes the effects of halide substituted phenylalanines on in vivo chlorogenic acid levels. Orkwiszewski and Hopkins (1974) demonstrated

Table 3

Effects of 5 mM halide substituted phenylalanines in in vivo chlorogenic acid levels

Treatment[a]	Chlorogenic acid levels	
	nmoles ±s.e.[b]	%[d]
Initial	38.1±3.0	100±0
18 hr control	17.3±3.0	44±5
p-FPA	10.0±3.8	23±8
Difference	−7.3±2.2[c]	−21±8
Initial	38.1±4.1	100±0
18 hr control	13.1±4.3	31±9
p-CPA	22.5±2.6	60±1
Difference	+9.4±2.6[c]	+29±9
Initial	34.0±4.3	100±0
18 hr control	9.0±3.6	24±8
p-BPA	15.6±4.2	42±7
Difference	+6.6±2.6[c]	+18±5
Initial	41.7±1.0	100±0
18 hr control	14.2±3.8	33±8
p-IPA	26.7±4.3	63±9
Difference	+12.5±1.7[c]	+30±4

[a] Initial values determined at time of excision. The remainder of the segments were analyzed after an 18 hr dark incubation at 26±1°C on BSM with and without the halide substituted analogue (5 separate paired experiments for each condition).

[b] nMoles per 60 segments ± standard error.

[c] Significant at the 2.5% level.

[d] Data expressed in terms of % initial level. ANOVA significant at 1.0% ($F = 10.03 > 5.95$).

that in vivo chlorogenic acid levels in coleoptile tissue are directly related to PAL activity. These data are paired results which represent the difference in chlorogenic acid levels as compared to the controls. Clearly only p-FPA elicited the response consistent with the previously reported decrease of chlorogenic acid levels (see Summary table 5, Maksymowych and Orkwiszewski 1983). The response elicited by the other halogenated analogues is opposite to the p-FPA induced response and may correlate with elongation data (Table 1).

SPECIFIC ACTIVITY OF AUXIN OXIDASE

The effect of p-FPA on the specific activity of auxin oxidase (Table 4), illustrates data which suggest that treatment of coleoptiles with p-FPA results in a reduction of auxin oxidase activity. Table 4 supports our extended

Table 4

Specific activity of auxin oxidase

Treatment[a]	$\Delta A_{261} \cdot$ mg protein$^{-1} \cdot$ hr^{-1}[b]
BSM	0.225 ± 0.034
p-FPA	0.109 ± 0.019

[a] Segments were incubated on BSM with and without 5×10^{-3} p-FPA for 18 hr.
[b] The average of 7 experiments ± standard error. T-test BSM vs p-FPA treated segments is significant at the 5% level.

hypothesis that reduced levels of low molecular weight phenols, some of which may be cofactors of auxin oxidase, decrease clearance of endogenous IAA resulting in enhanced elongation. These data are also consistent with the previously reported effect of p-FPA pretreatment reducing extractable PAL activity, decreasing chlorogenic acid levels and enhancing elongation of treated *Avena* coleoptile apices (Hopkins and Orkwiszewski 1971, Orkwiszewski and Hopkins 1974, Orkwiszewski et al. 1976, Maksymowych and Orkwiszewski 1983).

TEMPERATURE EFFECT ON OXIDASE SPECIFIC ACTIVITY

Figure 1 represents the effect of temperature on *Avena* auxin oxidase. This prelimiary characterization of the enzyme was undertaken to determine if temperature changes would affect the apparent depression in oxidase specific activity (Table 4) from p-FPA pretreated coleoptiles. The data points in Figure 1 illustrate that both enzyme extracts exhibit a similar temperature coefficient (p-FPA $Q_{10} = 2.19$; control $Q_{10} = 2.12$). Also, in this

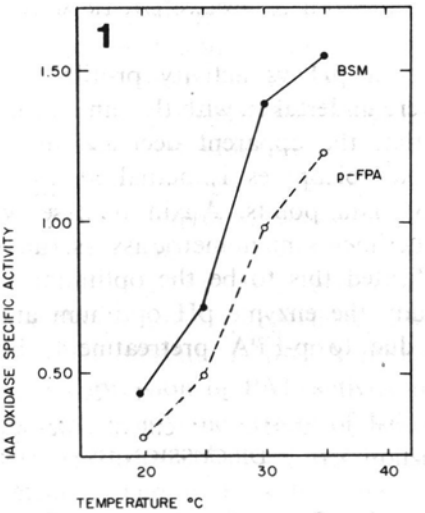

Fig. 1. Temperature vs activity profile for *Avena* auxin (IAA) oxidase. Enzyme specific activity (ΔA mg protein $^{-1}$ hr $^{-1}$) was plotted versus the temperature at which enzyme activity was assayed. Oxidation of IAA was determined with enzyme preparations from *Avena* coleoptiles preincubated in BSM (control) and p-FPA for 18 hr in the dark

study the depression of specific activity of the enzyme extracted from p-FPA pretreated coleoptiles becomes more apparent ($[\text{control} - \text{p-FPA}]_{20} = 0.134$; $[\text{control} - \text{p-FPA}]_{35} = 0.317$) with increased assay temperature.

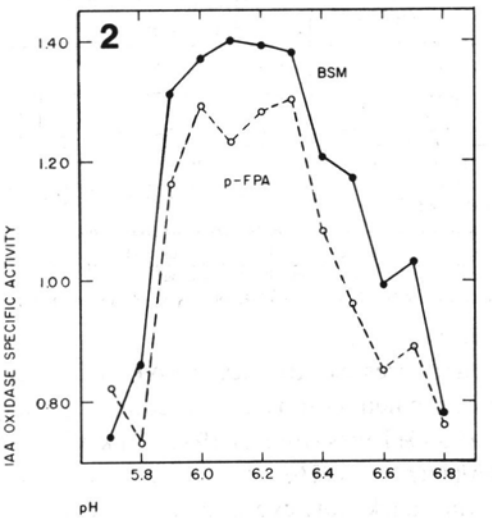

Fig. 2. pH vs activity profile for *Avena* auxin (IAA) oxidase. Enzyme specific activity (ΔA mg protein $^{-1}$ hr $^{-1}$) was plotted versus the pH at which reaction mixtures were assayed. Oxidation of IAA was determined with enzyme preparations from *Avena* coleoptiles preincubated in BSM (control) and p-FPA for 18 hr in the dark

pH PROFILE OF OXIDASE SPECIFIC ACTIVITY

Figure 2 presents a pH vs activity profile for *Avena* auxin oxidase. These experiments were undertaken with the same rationale as the temperature study. Data show that the apparent decrease in oxidase specific activity for p-FPA pretreated coleoptiles is actual at our assay (pH = 5.9) and treatment (pH = 6.3) data points. Auxin oxidase was assayed at pH 5.9 because our initial experiments, manometric assays, (unpublished data, Morgan and Hall 1963) indicated this to be the optimum for oxidation. However, according to this study the enzyme pH optimum and maximum depression in specific activity due to p-FPA pretreatment, both occur at pH 6.1.

DISCUSSION

Data from work with fluorophenylalanine (FPA) isomers demonstrate a consistency among the effects of p-FPA treatment (Hopkins and Orkwiszewski 1971, Orkwiszewski and Hopkins 1974, Orkwiszewski et al. 1976). Pretreatment of coleoptiles with the p-fluoro analogue of PA increases

Table 5

Effects of fluorophenylalanine

Treatment	% Increase elongation[a]	% Decrease extractable PAL activity[b]	% Alteration in vivo chlorogenic acid[c]
p-FPA	+89	−32	−14
o-FPA	+28	−17	−6
m-FPA	+5	−2	+12

[a] This is the elongation rate based on increase over control segments grown in BSM. Data are based on the average of 9 experiments for each condition.

[b] Percent is the change from segments grown in BSM. Data are based on te average of 6 experiments for each condition. PAL activity was determined on the basis of 150 segments for each condition.

[c] Percent is the change from segments grown on BSM. Chlorogenic acid levels were determined on the basis of 80 segments for each condition. Data are based on the average of 9 experiments for each condition.

coleoptiles elongation, depresses extractable PAL activity and decreases in vivo chlorogenic acid levels when compared to control values; see summary in Table 5 (Hopkins and Orkwiszewski 1971, Orkwiszewski and Hopkins 1974, Orkwiszewski et al. 1976).

Elongation, in the dark, of excised *Avena* coleoptiles is significantly enhanced by p-FPA (Hopkins and Bonnell 1969). These authors suggested that the analogue promotes elongation by lowering PAL activity, which in turn decreases the synthesis of low molecular weight phenols. We extend this hypothesis to include the suggestion that the resultant decrease of phenolic

cofactors may lower the specific activity of the endogenous auxin oxidizing enzyme resulting in net less oxidation of the growth substance (IAA). Reduced oxidation would then result in an increase of the endogenous pool of IAA in coleoptile tissues which in turn enhances elongation. The effects of p-FPA on PAL and chlorogenic acid levels, respectively, are consistent with the proposed hypothesis. Since our intent was to test this hypothesis using p-FPA to induce changes i vivo, then monitoring for a possible effect on auxin oxidase activity, it was deemed necessary to further clarify p-FPA specificity. For this reason we became interested in what effect, if any, on elongation may be elicited by the other halogen substituted PA analogues; how specific is p-FPA induction and does this isomer cause the "greatest" depression of PAL activity in vivo? Subsequent experiments were performed to study the effects of halide substituted PA's, in addition to p-FPA, on growth and phenolic metabolism. Since the effects of the ortho, para and meta isomers of FPA have been examined and para isomer specificity for the flurinated analogue established earlier (Hopkins and Orkwiszewski 1971, Orkwiszewski and Hopkins 1974), we limited this investigation only to the para isomers of the other halogenated compounds.

The results in Table 1 establish the extent of p-FPA specificity. Only p-FPA stimulated elongation at both the 1 mM and 5 mM concentrations. The other halogen substituted analogues inhibited growth at both concentrations. Based on these results our hypothesis predicts that the analogues would have an effect on PAL activity. In order to gain further insight about a possible mechanism for p-FPA action we investigated the deamination of PA analogues in vitro by PAL extracted from control coleoptiles (Table 2). Since it is the only analogue which enhances elongation, one may predict that p-FPA would be the only analogue to competitively inhibit PAL activity. Data from Table 2 do not support this prediction. Only the native substrate L-Phe is efficiently metabolized by native PAL. All of the other analogues, p-FPA inclusive, caused an inhibition of PAL specific activity in vitro. Therefore, it is unlikely that the primary mechanism of p-FPA activity is simply a competitive inhibition of PAL in vivo. These data clearly invite further investigation.

The PA analogues that inhibit growth do, by some as yet unknown mechanism, increase the level of chlorogenic acid in the treated coleoptiles (Table 3). One may suggest that these analogues have similar effects with respect to levels of low molecular weight phenols in treated coleoptiles. Only p-FPA stimulates elongation and only p-FPA significantly decreases chlorogenic acid levels in vivo. Effects of PA analogues on extension growth, PAL activity and chlorogenic acid levels are therefore isomer (based on work with p-FPA isosteres) and substituent specific. In the context of our hypothesis that p-FPA enhances growth by modifying PAL activity and reducing the level of low molecular weight phenols in treated coleoptiles (lowered chloro-

genic acid levels, Tables 3 and 5), one may consider a possible mechanism by which phenols could exert their effects. As previously mentioned, an explanation may be interaction with endogenous auxin oxidase. Various phenolic compounds have been shown to influence the activity of IAA oxidase (auxin oxidase) in vitro (Goldacre et al. 1953, Gortner et al. 1958, Mumford et al. 1961, 1962, Zenk and Muller 1963, Goren and Tomer 1971, Lee 1977).

Our laboratory is currently examining IAA oxidation in coleoptiles preincubated with p-FPA. Experimental results are consistent with the predictions based on our hypothesis. Data in Table 4 show that pretreatment of coleoptile apices for 18 hours with p-FPA results in depressed auxin oxidase activity. Preliminary enzyme characterization indicates that this depression of oxidase specific activity remains actual throughout the temperature range tested (Fig. 1) and, in fact, is most pronounced at the enzyme pH optimum (Fig. 2). Currently, our laboratory is involved in assay development and enzyme characterization in an effort to gain further understanding of the mechanism through which IAA and the oxidase contribute to coleoptile elongation and ultimately their relationship to *Avena* growth and development.

Galston (1967) proposed that modification of oxidase activity may be a growth regulatory mechanism since compounds which enhance IAA oxidase (auxin oxidase) appear to inhibit growth whereas those which depress oxidase activity promote growth. It appears that we may have the tool (p-FPA) and the system (etiolated coleoptile apices) which can be used to further investigate hormone involvement in *Avena* coleoptile elongation.

We are striving toward an understanding of a possible mechanism which accounts for p-FPA induced extension growth in *Avena* coleoptiles. The data to date are consistent with the hypothesis that p-FPA effects phenolic levels in coleoptile tissue by depressing PAL activity (and chlorogenic acid levels) resulting, directly or indirectly, in modification of IAA oxidizing activity (auxin oxidase) leading to an accumulation of IAA and enhanced extension growth.

REFERENCES

Galston A. W., 1967. Regulatory systems in higher plants. Amer. Sci. 55: 144-160.

Goldacre P. L., Galston A. W., Wientraub R. L., 1953. The effect of substituted phenols on the activity of the indoleacetic acid oxidase of peas. Arch. Biochem. Biophys. 43: 358-373.

Goren R., Tomer E., 1971. Effects of seselin and coumarin on growth indoleacetic acid oxidase, and peroxidase, with special reference to cucumber (*Cucumis sativus* L.) radicles. Plant Physiol. 47: 312-316.

Gortner W. A., Kent M. J., Sutherland G. K., 1958. Ferulic and p-coumaric acids in

pineapple tissue as modifiers of pineapple indoleacetic acid oxidase. Nature 181: 630-631.

Hopkins W. G., Bonnell K. F., 1969. *Avena* coleoptile elongation: stimulation by fluorophenylalanine. Plant Physiol. 44: 281-286.

Hopkins W. G., Orkwiszewski J. A. J., 1971. Inhibition of phenylalanine ammonia lyase and enhancement of *Avena* coleoptile segment elongation by fluorophenylalanine. Can. J. Bot. 129-135.

Lee Eileen, 1978. Masters Thesis. Villanova University.

Lee T. T., 1977. Role of phenolic inhibitors in peroxidase-mediated degradation of indole-3-acetic acid. Plant Physiol. 59: 372-375.

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Maksymowych A. B., Orkwiszewski J. A. J., 1983. Specificity of fluorophenylalanine induced *Avena* coleoptile elongation. Amer. J. Bot. 70 (part 2): 88.

Miller J., Ross C., 1966. Inhibition of leaf processes by p-fluorophenylalanine during induction of flowering in the cocklebur. Plant Physiol. 41: 1185-1192.

Morgan P. W., Hall W. C., 1963. Indoleacetic acid oxidizing enzyme and inhibitors for light-grown cotton. Plant Physiol. 38(4): 365-370.

Mumford F. E., Smith D. H., Castle J. E., 1961. An inhibitor of indoleacetic acid oxidase from pea tips. Plant Physiol. 36: 752-756.

Mumford F. E., Stark H. M. Smith D. H., 1962. A naturally occurring cofactor for indoleacetic acid oxidase. Plant Physiol. 37: suppl. xiv.

Nooden L. D., Thimann K. V., 1966. Action of inhibitors of RNA and protein synthesis on cell enlargement. Plant Physiol. 41: 157-164.

Orkwiszewski J. A. J., Hopkins W. G., 1974. Effect of fluorophenylalanine on L-phenylalanine lyase activity in *Avena* coleoptiles. Amer. J. Bot. 61: 433-436.

Orkwiszewski J. A. J., Koroly M. J., DiMichele L., Weber D. M., 1976. Similarities between L-phenylalanine and m-fluorophenylalanine as substrates for L-phenylalanine ammonia lyase. Physiol. Plant. 37(4): 288-292.

Ray P. M., 1956. The destruction of indoleacetic acid. II. Spectrophotometric study of the enzymatic reaction. Arch. Biochem. Biophys. 64: 193-216.

Richmond M. H., 1962. The effect of amino acid analogues on growth and protein synthesis in microorganisms. Bacteriol. Rev. 26: 398-420.

Zenk M. H., Muller G., 1963. In vivo destruction of exogenously applied indolyl-3-acetic acid as influenced by naturally occurring phenolic acids. Nature 200: 761-763.

Badania nad indukcją wydłużania koleoptyli owsa przez fluorofenyloalaninę: Możliwość modulacji aktywności oksydazy auksynowej

Streszczenie

Wcześniejsze badania wykazały, że p-fluorofenyloalanina, analog aminokwasu, powoduje wydłużanie etiolowanych segmentów koleoptyli owsa. Skłoniło nas to do przypuszczenia, że reakcja wydłużania, wywołana przez ten izoster, jest spowodowana depresją aktywności amoniako-liazy L-fenyloalaniny, co powoduje spadek poziomu fenoli o małej masie cząsteczkowej, które, bezpośrednio lub pośrednio, modyfikują aktywność oksydazy auksynowej u owsa i, w efekcie, zmieniają poziom substancji wzrostowej w segmentach koleoptyli. Dalsze wyjaśnienie natury reakcji wzrostowej spowodowało potrzebę dodatkowych badań nad specyficznością

analogów. Jak dotąd używano tylko fluorowych pochodnych do modyfikowania aktywności amoniako-liazy L-fenyloalaniny, dlatego przedstawiano dane dotyczące wpływu innych fenyloalanin połączonych z haloidkami i porównywano z wynikami doświadczeń z fluorofenyloalaniną. Dane wskazują, że reakcja na p-fluorofenyloalaninę jest izomeryczna i specyficzna, ponieważ żaden inny analog z podstawionym haloidkiem nie wywołuje pozytywnej reakcji wydłużania. Traktowanie koleoptyli owsa p-fluorofenyloalaniną powoduje zmianę aktywności oksydazy auksynowej. Stwierdzono spadek specyficznej aktywności oksydazy podczas serii doświadczeń z wpływem temperatury i pH, przeprowadzonych w naszym laboratorium.