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IAA OXIDASE PREPARATIONS FROM FRESH AND AGED *IPOMOEA BATATAS* TUBER DISCS

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Abstract—IAA oxidase preparations from fresh sweet potato tuber discs oxidized IAA only in the presence of added phenolic cofactors, and the pH optimum for enzyme activity depended on the cofactor used Ageing of tuber discs, either by aeration in distilled water or by incubation on moist filter paper, resulted in increased peroxidase and phenol-stimulated IAA oxidase activities, as well as the development of IAA oxidase activity in the absence of added cofactors High phenolase activity of fresh tuber discs decreased considerably with ageing Phenol-stimulated IAA oxidase activity reached maximal levels before IAA oxidase activity in the absence of added cofactors Enzyme preparations from aged tuber discs had double pH optima, similar to those previously described for sweet potato root IAA oxidase preparations IAA in the concentration range 10^{-4} to 10^{-2} M inhibited the increase in peroxidase and IAA oxidase activities with ageing DCP-stimulated IAA oxidase activities in preparations from both fresh and aged sweet potato tuber discs were inhibited by manganous ion

INTRODUCTION

IN PREVIOUS communications,[1-3] IAA oxidase preparations from sweet potato roots were shown to oxidize IAA in the absence of added phenolic cofactors, to have double pH optima and to be both stimulated and inhibited by scopoletin as well as caffeic and chlorogenic acids Considerable peroxidase and phenolase activities were also found to occur in these enzyme preparations Since the peroxidase activity of sweet potato tuber discs is known to increase with ageing,[4,5] and increases in IAA oxidase activities have been demonstrated in aged root[6] and etiolated seedling[7] tissues, IAA oxidase activities of fresh and aged sweet potato tuber discs were examined It is shown that IAA oxidase activity in preparations from tuber tissue also increased with ageing Enzyme preparations from fresh tuber discs oxidized IAA only in the presence of added phenolic cofactors but the capacity for enzymatic IAA destruction in the absence of added cofactors developed with ageing Increases in IAA oxidase activity were, however, inhibited when discs were aged in the presence of IAA The pH optima as well as the stimulation of enzyme activity by phenolics, in preparations from fresh and aged tuber discs are compared with these properties in sweet potato root enzyme preparations

[1] IMBERT, M P and WILSON, L A (1970) *Phytochemistry* **9**, 1787
[2] IMBERT, M P and WILSON, L A (1972) *Phytochemistry* **11**, 29
[3] IMBERT, M P and WILSON, L A (1972) *Phytochemistry* **11**, 2671
[4] STAHMAN, M A, CLARE, B G and WOODBURY, W (1966) *Plant Physiol* **41**, 1505
[5] SHANNON, L M, URITANI, I and IMASEKI, H (1971) *Plant Physiol* **47**, 493
[6] PILET, P E and GALSTON, A W (1955) *Plant Physiol* **8**, 888
[7] GALSTON, A W and DALBERG, L Y (1954) *Ann Bot* **41**, 559

RESULTS

IAA oxidase preparations from sweet potato tubers can be made either by an acetone powder procedure[8] or by the previously described acetone precipitate method [1] The latter procedure used in this investigation gave preparations with higher enzyme activity, despite the yellow–brown coloration, due to phenolic oxidation products which developed in homogenates in the course of enzyme preparation by this method Use of insoluble polyvinylpyrrolidone (PVP)[9] in the homogenizing medium did not increase enzyme activity appreciably Extraction of enzyme preparations with solid PVP resulted in only marginal (5%) increases in IAA oxidase activity However enzyme activity decreased to 46% of its original value after standing at −10°, for 6 days In the course of enzyme preparation acetone precipitates were extracted with phosphate citrate buffers of different pHs to give 4 5 6 0 and 7 0 enzymes as previously described [2] Since IAA oxidase activities of 6 0 enzyme were always higher than other enzymes, this preparation was used in all investigations except where otherwise stated

TABLE 1 STIMULATION OF IAA OXIDASE ACTIVITY IN FRESH TUBER PREPARATIONS BY PHENOLIC COFACTORS

Cofactor	pH reaction mixture*	IAA oxidase activity μmol IAA dest hr ml enzyme
No cofactor	2 5-7 0	0 000
p-Coumaric acid	4 5	0 540
Scopoletin	4 4	0 124
Chlorogenic acid	4 4	0 029
2 4-Dichlorophenol	6 0	0 285
4-Methylumbelliferone	6 5	0 208
Resorcinol	6 0	0 138
γ-Resorcylic acid	6 0	0 080

Reaction mixtures were incubated at 33° and contained 0 125 μmol IAA $3\,2 \times 10^{-2}$ mmol cofactor (except chlorogenic acid and resorcinol, $3\,2 \times 10^{-3}$ mmol) 500 μmol phosphate citrate buffer at pH's as shown and 1 0 ml enzyme in a total volume of 8 0 ml

* pH s are optimal for cofactor stimulation

IAA oxidase preparations from fresh tuber discs

Enzyme preparations made from homogenates both in the presence and absence of insoluble PVP, by acetone precipitation and subsequent solubilization of enzyme with phosphate-citrate buffers in the range pH 4 5-7 0, showed no IAA oxidase activity in the absence of added cofactors, in reaction mixtures of pH 2 5–7 0 However, preparations normally contained considerably higher peroxidase (ΔA/min/mg protein = 4 0) and phenolase (ΔA/min/mg protein = 8 0) activities than root enzyme preparations [2] Data (Table 1) showed that considerable IAA oxidase activity could only be demonstrated in enzyme preparations in the presence of phenolic cofactors Specific activities of such preparations were highest with 2,4-dichlorophenol (DCP) (1 7 μmol IAA destroyed/hr/mg protein) and lowest in the presence of chlorogenic and resorcylic acids The pH optima for IAA oxidase activity differed with the cofactor used (Table 1) Thus DCP resorcinol, resorcylic acid and 4-methylumbelliferone gave optima at *ca* pH 6 0 6 5 and scopoletin, chlorogenic acid and *p*-coumaric acid showed optima at pH 4 4 4 5

[8] KONISHI M and GALSTON A W (1964) *Phytochemistry* **3**, 559
[9] LOOMIS W D and BATTAILLE J (1966) *Phytochemistry* **5**, 423

Effects of ageing on IAA oxidase activity of sweet potato tuber disc preparations

When sweet potato tuber discs were aged either by aeration in distilled water[10] or by incubation on moist filter paper in a desiccator,[5] enzyme preparations from discs showed IAA oxidase activity in the absence of added cofactors Such enzyme activity increased to a maximum level after 96 hr and decreased thereafter (Fig 1) Peroxidase activity continued to increase up to 120 hr, and at that time activity was some 50-fold that in preparations from fresh tubers Phenolase activities of fresh tuber preparations decreased to 10% of their original value after 96 hr ageing The time to peak IAA oxidase activity varied with the tubers used and in some experiments peak activity was attained after 72-hr ageing Development of maximal phenol-stimulated IAA oxidase activity preceded the realization of peak enzyme activity in the absence of added cofactors (Fig 2) and activities differed according to the cofactor used Thus, enzyme activities after 72-hr ageing were highest in the presence of DCP and *p*-coumaric acid and peak activity in the presence of scopoletin and resorcinol was greater than that with 4-methylumbelliferone In contrast to the cofactors DCP and *p*-coumaric acid, rapid increase in the rate of scopoletin, 4-methylumbelliferone and resorcinol-stimulated IAA oxidase activity commenced only after 24-hr ageing

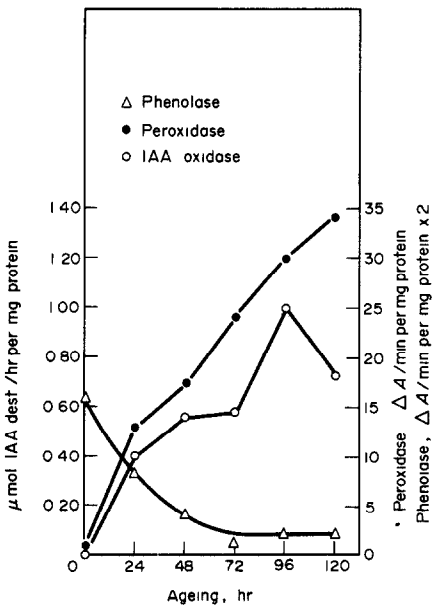

FIG 1 EFFECT OF AGEING ON COFACTOR INDEPENDENT IAA OXIDASE, PEROXIDASE AND PHENOLASE ACTIVITIES OF TUBER DISC PREPARATIONS

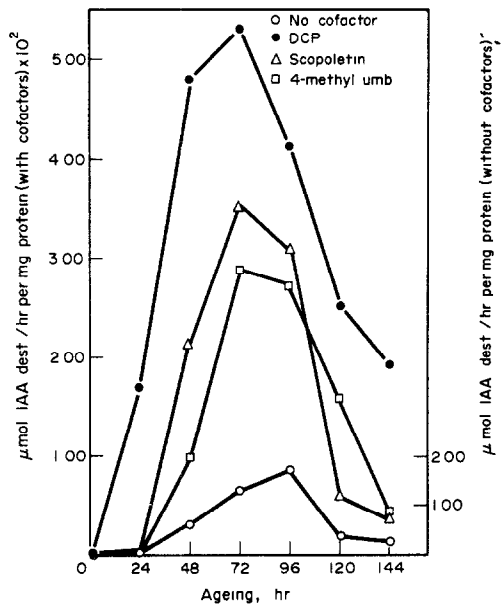

FIG 2 EFFECT OF AGEING ON IAA OXIDASE ACTIVITIES OF TUBER DISC PREPARATIONS IN THE PRESENCE AND ABSENCE OF PHENOLICS

Changes in IAA oxidase activities were similar whether ageing was done in diffuse daylight in the laboratory or in the dark Storage of tubers at 20° and 27° for up to 5 weeks did not result in the development of IAA oxidase activity in the absence of added cofactors Data (Fig 3) showed, however, that the increase in IAA oxidase activity in the absence of added cofactors, on subsequent ageing of discs from stored tubers for 72 hr was higher than that previously recorded for fresh tuber preparations (Fig 1) Also, enzyme activity was

[10] RICARDO, C P P and APREES, T (1970) *Phytochemistry* **9**, 239

ca 40% greater after 72 hr in preparations from tubers stored at the higher temperature

Ageing of sweet potato tuber discs in the presence of IAA inhibited the development of both peroxidase and IAA oxidase activity and there was progressively increased IAA inhibition of enzyme activities measured after 72 hr with increased IAA concentration, in the range 10^{-4} to 10^{-2} M (Fig 4)

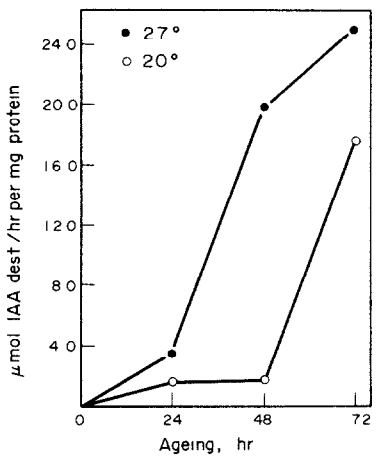

FIG 3 EFFECT OF PRE-STORAGE OF TUBERS AT 20° AND 27° AND SUBSEQUENT AGEING ON DEVELOPMENT OF COFACTOR INDEPENDENT IAA OXIDASE ACTIVITIES IN TUBER DISC PREPARATIONS

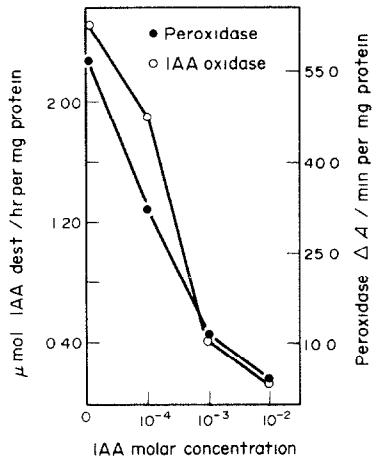

FIG 4 COFACTOR INDEPENDENT IAA OXIDASE AND PEROXIDASE ACTIVITIES OF PREPARATIONS FROM TUBER DISCS AGED FOR 72 hr IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF IAA

IAA oxidase preparations from aged sweet potato tuber discs

Enzyme preparations from aged tuber discs were similar to preparations from sweet potato roots,[1-3] in their capacity to catalyse the destruction of IAA in the absence of added cofactors However, the specific activity of the aged tuber enzyme extracted from discs after 96 hr (Fig 1) was more than 300 times greater than that of the root enzyme [2] The aged tuber enzyme was also capable of stimulation and inhibition by scopoletin and chlorogenic acid Scopoletin stimulation occurred in the concentration range 2×10^{-4} to 2×10^{-2} nmol/ml with peak enzyme activity at *ca* 2×10^{-3} nmol/ml At higher concentrations, inhibition of enzyme activity occurred both in the presence of scopoletin and chlorogenic acid Enzyme activities in the presence of these and other cofactors (Fig 2) were also higher than those reported for the root enzyme

Data (Fig 5) indicated that IAA oxidase preparations from aged sweet potato tuber discs had double pH optima similar to those in the root enzyme [2] Accordingly typical 4 5 enzyme preparations had a major optimum at around pH 4 0 and lower enzyme activities at pH 4 5–6 0 Typical 6 0 and 7 0 enzymes showed major and minor peaks at pH 5 5–6 0 and pH 2 5 respectively IAA oxidase activities of 6 0 enzyme preparations were, however, approximately three times those of 4 5 and 7 0 enzymes The pH optima of preparations from aged tuber discs, in the presence of added phenolic cofactors were similar to those in preparations from fresh discs (Table 1)

Manganous ion in the pH range 3 0–7 0, at concentrations ranging from 2×10^{-4} to 2×10^{-1} nmol/ml did not stimulate IAA oxidase activity and induced inhibition at higher

concentrations Also, increasing concentrations of manganous ion at pH 6 0 resulted in progressively increased inhibition of DCP-stimulated IAA oxidase activity in preparations from aged tuber discs (Fig 6) Similar inhibition of DCP-stimulated enzyme activity was also observed in fresh tuber discs preparations

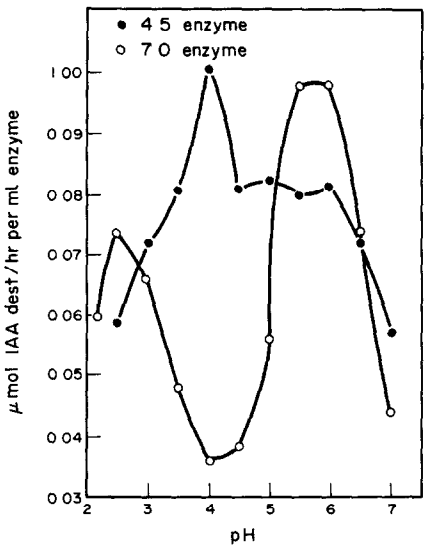

FIG 5 COFACTOR INDEPENDENT IAA OXIDASE PH OPTIMA OF 4 5 AND 7 0 ENZYME PREPARATIONS FROM AGED TUBER DISCS

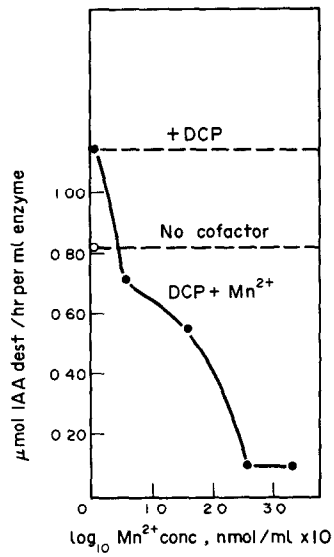

FIG 6 EFFECT OF MANGANOUS ION CONCENTRATION ON DCP-STIMULATED IAA OXIDASE ACTIVITY OF TUBER DISC PREPARATIONS

DISCUSSION

IAA oxidase preparations from fresh sweet potato tuber discs differed from root preparations in their inability to catalyse the destruction of IAA in the absence of added cofactors Enzyme activity could, however, be demonstrated in these preparations with added phenolic cofactors The pH optimum for IAA oxidase activity depended on the phenolic used, and optima for scopoletin[2] and chlorogenic acid[3] were similar to those found in the root enzyme, at around pH 4 Dichlorophenol, resorcinol and 4-methylumbelliferone showed optima at around pH 6 IAA oxidase in the presence of scopoletin and chlorogenic acid, peroxidase, and particularly phenolase activities in fresh tuber preparations were also greater than those in root preparations [2,3]

There was a 50-fold increase in peroxidase activity with ageing, which compared favourably with 10-fold[4] and 100-fold[5] increases in activity of this enzyme previously demonstrated in sweet potato tuber discs, aged in the absence and in the presence of ethylene, respectively Ageing also resulted in the development of IAA oxidase activity in the absence of added cofactors Such activity reached peak levels after 96 hr However, peroxidase continued to increase up to 120 hr and phenolase activity decreased considerably with ageing Shannon *et al* [5] demonstrated *de novo* synthesis of peroxidase isozymes in sweet potato discs aged in the presence of ethylene Such *de novo* synthesis of peroxidase could explain the increase in IAA oxidase activity with ageing in our preparations However, the decrease in IAA oxidase activity after 96-hr ageing, despite further increases in peroxidase activity is interpreted to mean that factors other than peroxidase synthesis were responsible for the observed IAA oxidase activity in the present experiments

The development of IAA oxidase activity in the absence of cofactors with ageing suggested that either synthesis of cofactor(s) or disappearance of inhibitor(s) might be involved in the expression of enzyme activity in aged tuber discs The specific activity for IAA oxidase from such discs was some 300-fold that observed in sweet potato root enzyme preparations Comparisons of phenol-stimulated IAA oxidase activity in fresh and aged tubers also indicated that increases in scopoletin *p*-coumaric acid and DCP-stimulated enzyme activity with ageing were from 350 to 430 times the activities in fresh tuber preparations and were therefore of a much higher order than the 50-fold increase in peroxidase activity which occurred with ageing Linear relationships between peroxidase and IAA oxidase activities recorded in cotton leaves[11] were not evident in the sweet potato tuber disc system The inverse relationship between phenolase and IAA oxidase activities recorded (Fig 1) indicated that phenolase enzymes[2,12] were probably not involved in the IAA oxidase activity of sweet potato tuber preparations However the possibility of participation of a specific protein other than peroxidase in IAA destruction[13] cannot be excluded

IAA oxidase preparations from fresh and aged sweet potato tuber discs were stimulated by a range of phenolics previously shown to be cofactors for IAA oxidase systems The low level of stimulation by γ-resorcylic acid with optimal activity at around pH 6 was similar to data reported by Mato[14] Scopoletin and chlorogenic acid stimulations occurred at lower concentrations than those used with the root enzyme[1,3] Stimulatory concentrations of scopoletin were also of a lower order than those recorded for peroxidase isozymes by Schafer *et al*[15] The changing pH optimum with different cofactors agreed with data presented by Janssen[16,17] Inhibition of IAA oxidase activity by a wide concentration range of manganous ion in the presence of DCP[18,19] was not due to the presence of high concentrations of manganous ion in the enzyme preparations used

Storage of tubers prior to ageing increased IAA oxidase activity in the absence of cofactors which developed in aged tuber disc preparations Tubers prestored at 27° germinated during storage and developed higher enzyme activities than tubers stored at 20° Germination and growth of tubers in the presence of light was shown by Akita *et al*[20] to increase IAA oxidase activity and to reduce IAA content of "mother" tubers These changes were associated with enhancement of lignification and suppression of cell proliferation, cell expansion and carbohydrate storage compared with tubers growing in the dark Our results showed that IAA restricted the development of both peroxidase and cofactor independent IAA oxidase activity with ageing Similar suppression of a peroxidase isozyme by IAA during ageing of pea stem tissue was recorded by Okerse *et al*[21] Since peroxidase is known to function as a lignin polymerase[22] and lignin precursors e g *p*-coumaric acid[23] are known

[11] FOWLER M C and MORGAN P W (1972) *Plant Physiol* **49**, 555
[12] KONINGS, H (1964) *Acta Bot Neerl* **13**, 566
[13] SEQUEIRA, L and MINFO L (1966) *Plant Physiol* **41**, 1200
[14] MATO M C (1970) *Phytochemistry* **9**, 275
[15] SCHAFER P S WENDER H and SMITH, F C (1971) *Plant Physiol* **48**, 232
[16] JANSSEN M G H (1969) *Acta Bot Neerl* **18**(4)
[17] JANSSEN M G H (1969) *Acta Bot Neerl* **18**(6)
[18] GALSTON A W BONNER J and BAKER R S (1950) *Arch Biochem Biophys* **42**, 456
[19] GALSTON, A W and BAKER R S (1950) *Ann Bot* **38**, 190
[20] AKITA S YAMAMOTO T ONO F KUSUHARA, M and IKEMOTO, S (1962) *Bull Chugoku Agric Exp Stn* **8**, 75
[21] OKERSE R SIEGEL, B Z and GALSTON A W (1966) *Science* **151**, 452
[22] HIGUCHI T (1957) *Plant Physiol* **10**, 621
[23] SHIMADA M YAMASAKI T and HIGUCHI T (1970) *Phytochemistry* **9**, 1

to occur in sweet potato tubers,[24] the above results might indicate that an IAA/IAA oxidase/peroxidase system plays a critical role in the regulation of lignification versus cell proliferation and expansion during tuber development, as suggested by Wilson,[25] Wilson and Lowe [26] IAA has also been shown to stimulate peroxidase genesis in root[27] and callus[28] tissues, respectively, and stimulation/inhibition effects of 2,4-D on tobacco callus peroxidase isozymes associated with growth enhancement and reduction demonstrated [29] The regulatory effects of IAA on peroxidase genesis might, therefore, reflect the finding by Gautheret[30] that high auxin concentration inhibited vascularization of Jerusalem artichoke callus, while low concentrations enhanced it A specific peroxidase isozyme has now been linked with lignification in sweet potato tuber tissues[31] and high IAA concentrations have been shown to inhibit the lignin polymerase activity of peroxidase preparations [32]

EXPERIMENTAL

Materials Sweet potato (cv 049) tubers were harvested from the field and used for enzyme preparation or ageing as soon as possible thereafter In storage experiments tubers were washed with tap H_2O swabbed with EtOH, dried and placed in storage rooms at either 20° or 27°

Ageing of tuber discs Discs (10 × 1 5 mm) were cut from 10 mm dia cylinders with a guillotine, washed 3 × H_2O and aged by continuous aeration in a large vol of H_2O, which was changed 2 × during the first 4 hr and at 12-hr intervals thereafter Ageing was also carried out by placing washed discs on moist filter paper in Petri dishes in a 12-l desiccator, containing a saturating quantity of H_2O

Enzyme preparation and assay Enzyme preparation and assay of IAA oxidase, peroxidase (guaiacol) and phenolase (catechol) activities, including adjustment of the pH optimum of reaction mixtures were carried out as previously described [1-3] Reaction mixtures for IAA oxidase activities were incubated at 33° and contained 0 125 μmol IAA, 3 2 × 10^{-2} nmol DCP or other cofactors except where otherwise shown in tables or figures, 500 μmol phosphate–citrate buffer pH 6 0 or as indicated and 1 0 ml enzyme in a total vol of 8 0 ml Protein contents of enzyme preparations were determined by the Folin method [33]

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[24] IMBERT, M P (1968) M Sc Thesis, University of the West Indies, Trinidad Library (unpubl)
[25] WILSON, L A (1970) *Proc 2nd Int Symp Tropical Root and Tuber Crops* **1,** 24
[26] WILSON, L A and LOWE, S B (1973) *Ann Bot* in press
[27] JENSEN, W A (1955) *Plant Physiol* **30,** 426
[28] LAVEE, S and GALSTON, A W (1968) *Ann Bot* **55,** 890
[29] LEE, T T (1972) *Plant Physiol* **49,** 957
[30] GAUTHERET, R J (1961) *Compt Rend* **253,** 1381
[31] MATSUMO, H and URITANI, I (1972) *Plant Cell Physiol* **13,** 1091
[32] SIEGEL, S M , FROST, P and PORTO, F (1960) *Plant Physiol* **35,** 163
[33] LOWRY O H , ROSEBROUGH, N J , FARR A L and RANDALL R J (1951) *J Biol Chem* **193,**