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Simple Phenolic Glycosides as Potential Regulators of the IAA-oxidase System

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Abstract. The effect of phenols and simple phenolic glycosides on the activity of IAA-oxidase isolated from gherkin seedlings was studied in experiments *in vitro*. Phenol stimulated the enzyme system activity, eugenol and quinol were proven as inhibitors. Simple phenolic glycosides (arbutin, gein and phenol glucoside) influenced IAA-oxidase activity only if β-glycosidase was present: rree phenols released from their bound form increased or decreased the IAA level. The potential fegulatory effect of simple phenolic glycosides on the IAA level in plants has been discussed; this effect is thought to be mediated by free phenols and by influence on the IAA-oxidase system.

The effect of phytohormones controlling plant growth is in general influenced by their optimal concentration in the cells. The maintainance of this optimal level must be an important regulatory process. Natural plant growth regulators (mostly of phenolic character) are supposed to act *via* IAA or by other mechanisms (TURECKAJA *et al.* 1969). The surplus of endogenous IAA is removed by enzymic oxidative decomposition (GALSTON 1967) or through various detoxification mechanism (TOWERS 1964). Phenolics may stimulate or inhibit IAA-oxidase activity according to their chemical character and concentration (GORTNER and KENT 1953, VARGA and KÖVES 1962, HARE 1964). In this paper we give the results of experiments in which the relationship between simple phenolic glycosides and IAA-oxidase activity mediated by β-glycosidase was studied.

Materials and Methods

Preparation of β-glycosidase

Acetone powders were prepared (PŠENÁK *et al.* 1970) from seedlings of *Geum urbanum* L. plants (17—18 days old, formation of the first leaf). Acetone powder (1g) was extracted three times with 15 ml 0·05 M acetate

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buffer (pH 4·6; 20 min; 0°C), the solution precipitated with ammonium sulphate (80% saturation), the precipitate centrifuged (20 000 g; 20 min), dissolved in 4 ml 0·005 M acetate buffer (pH 4·6) and dialysed against the same buffer for 24 hr at 0°. The solution contained β-glycosidase ("gease") activity.

Preparation of IAA-oxidase

Gherkin seedlings (*Cucumis sativus* L.) were cultivated in the dark at 25° (relative humidity 75—78%). Three day old cotyledons were homogenized in 0·05 M phosphate buffer pH 5·8 at 0° (6·6 g in 10 ml), the homogenate centrifuged (3 000 g) and the supernatant dialysed against 0·005 M phosphate buffer pH 5·8 at 0°. The solution represented a crude preparation of IAA-oxidase. A partly purified preparation was obtained according to ENGELSMA and MAIJER (1965): the homogenate was precipitated by heat (50°; 10 min), centrifuged (20 000 g; 0°), the supernatant precipitated at pH 4·0 (adjusted with acetic acid), centrifuged as given above and precipitated with ammonium sulphate (25% saturation). The supernatant was precipitated with ammonium sulphate (75% saturation), the precipitate dissolved in 5 ml 0·005 M phosphate buffer and dialysed against 0·001 M phosphate buffer pH 5·8 for 24 hr. The solution represented a purified IAA-oxidase preparation free of β-glycosidase.

Determination of IAA-oxidase Activity

IAA-oxidase activity was determined manometrically at 27° and 120 rev/ /min. Warburg vessels contained in the main compartment: 0·2 ml $MgCl_2$ (10^{-4} M), 0·5 ml IAA (5×10^{-3} M), 2·1 ml 0·05 M phosphate buffer pH 5·8: in inside well 0·2 ml 2 N potassium hydroxide, in lst side arm 0·5 ml enzyme preparation and in 2nd side arm the solution of phenol or phenolic glycoside (final concentration of free phenols was 10^{-3} M, 5×10^{-4} M and 5×10^{-5} M respectively; final concentration of glycosides was 10^{-3} M and 10^{-4} M). The IAA-oxidase activity was defined in Q_{O_2} (μl O_2 (60 min)1 mg protein). In experiments with exogenous β-glycosidase (0·5 ml in the side arm), the IAA-oxidase preparation was added to the main compartment and the enzymes mixed after 15 min of incubation.

Determination of β-glycosidase Activity

β-glycosidase activity was determined with salicin as substrate. The Warburg vessel contained 2·6 ml salicin (2% in 0·05 M phosphate buffer pH 5·8), 0·2 ml $MgCl_2$ (10^{-4} M) and 0·5 ml enzyme solution. At various intervals of incubation (27°) 0·03 ml samples were analysed by paper chromatography (Whatman No 1, solvent system: n-butanol-acetic acid-water 4:1:2). After detection with diazotized p-nitroaniline according to SWAIN (1953) the spots corresponding to saligenin were cut out and eluted twice for 15 min with 5 ml potassium hydroxide (0·2% in methanol). The solution was analysed photometrically at 485 nm and the concentration of saligenin calculated from the calibration curve (linear for 5—30 μg saligenin). The activity of β-glycosidase was defined as μg saligenin released in 60 min by 1 mg protein.

Protein Determination

Protein content was determined according to LOWRY *et al.* (1951).

Results and Discussion

Cotyledons of gherkin seedlings gave a crude IAA-oxidase containing also some β-glycosidase activity (120 μg saligenin (60 min) 1 mg protein). Purified enzyme preparation of IAA-oxidase was free of β-glycosidase activity. The Q_{O_2} values of the crude IAA-oxidase preparation were 31·7—36·6, of the purified enzyme 44·5—48·5.

Various mechanisms keep IAA at the optimal level in plants, one of them being the control of enzyme activity by oxidative decomposition of IAA. Free phenols influence this degradation, but free phenols occur in plants very rarely. Their most common form in vivo are glycosides and glucose esters. Therefore for regulation of IAA level those enzymes are of importance which release free phenols from their bound forms: glycosidases are such enzymes. A correlation between glycosidase activity, phenolic glycosides and IAA-oxidase activity might therefore represent one controlling mechanism of endogenous IAA level in plants.

TABLE 1

EFFECT OF PHENOLICS on IAA degradation (results given in Q_{O_2})

Phenolics	Concentration of phenolics			Control
	10^{-3}M	5×10^{-4}M	5×10^{-5}M	
Phenol	46·6	40·0	36·0	34·5
Quinol	33·3	12·5	25·0	33·2
Eugenol	2·5	3·8	34·8	36·6
Coumaric acid	29·1	43·0	33·5	31·7
Ferulic acid	0·0	10·0	17·2	31·7
Caffeic acid	0·0	12·0	18·1	31·7

The effect of some free phenols on IAA-oxidase activity was studied: phenol has a stimulatory effect, quinol and eugenol are inhibitors (Tab. 1). The effect of hydroxycinnamic acid derivatives on IAA-oxidase activity was reported previously (ENGELSMA and MAIJER 1965); we proved the stimulatory effect of p-coumaric acid and inhibitory effects of ferulic and caffeic acids.

TABLE 2

EFFECT OF SIMPLE PHENOLIC GLYCOSIDES on IAA degradation (purified IAA-oxidase preparation; (results given in Q_{O_2})

Phenolics	Experiments	Controls
Arbutin (10^{-3}M)	48·1	48·3
Arbutin (10^{-3}M)+	12·7	48·3
Gein (5×10^{-4}M)	48·5	48·3
Gein (5×10^{-4}M)+	7·0	48·3

+ Gease added

Simple phenolic glycosides (arbutin, gein) were ineffective upon IAA-oxidase when a purified enzyme preparation was used. If the β-glycosidase was added to the purified preparation of IAA-oxidase, inhibitory effects of arbutin and gein were demonstrated in acoordance with the effects of their aglycones, quinol and eugenol (Tab. 2). In experiments with crude IAA-oxidase preparation which contained also β-glycosidase activity, simple phenolic glycosides (arbutin, phenol glucoside) had an effect upon IAA-oxidase activity in a way corresponding to the aglycones in question: arbutin demonstrated an inhibitory effect, phenol glucoside a stimulatory effect, which were accentuated when almond emulsin was added (Tab. 3).

TABLE 3

EFFECT OF SIMPLE PHENOLIC GLYCOSIDES on IAA degradation (crude IAA-oxidase preparation; (results given in Q_{O_2})

Phenolics (10^{-3}M)	Experiments	Controls
Quinol	5·8	33·6
Arbutin	22·5	33·6
Arbutin+	10·6	33·6
Phenol	44·3	33·6
Phenol glucoside	38·8	33·6
Phenol glucoside+	41·5	33·6

+ Almond emulsin added

These experiments make it clear that simple phenolic glycosides do not influence the IAA-oxidase system in a direct way. Phenols or phenolic acids must be first set free from their bound forms due to β-glycosidase activity, and then the effects on IAA-oxidase system appear. Therefore, the β-glycosidase and IAA-oxidase system are linked through phenolic glycosides and their biochemically active aglycones. It may be presumed that such a correlation exists also *in vivo*. Phenolic glycosides are no longer considered as waste products of plant metabolism (HARBORNE 1964); one of their functions might be a regulatory effect on IAA level in plants.

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V pokusoch *in vitro* bol študovaný vplyv fenolov a jednoduchých fenolických glykozidov na aktivitu IAA-oxidázy, izolovanej z kotyledonov uhoriek. Fenol zvyšoval aktivitu enzýmového systému, eugenol a hydrochinón pôsobil inhibične. Jednoduché fenolické glykozidy (arbutín, gein a fenolglukozid) ovplyvňovali aktivitu IAA-oxidázového systému len v prítomnosti β-glykozidázy: voľné fenoly uvoľnené z viazanej formy zvyšovali alebo znižovali hladinu IAA. V diskusii sa uvažuje o možnom pôsobení jednoduchých fenolických glykozidov ako potencionálnych regulátorov hladiny IAA v rastlinách mechanizmom sprostredkovaným voľnými fenolmi a ovplyvnením systému IAA-oxidázy.