THE INFLUENCE OF SOME PHENOLIC COFACTORS ON THE PH OPTIMUM OF INDOLEACETIC ACID OXIDASE FROM PEA ROOTS

M. G. H. JANSSEN

Botanisch Laboratorium, Utrecht

SUMMARY

The pH optimum of IAA-oxidase of pea roots is determined both by the nature of the cofactor and by its concentration.

1. INTRODUCTION

In a previous paper (JANSSEN 1969b) it was demonstrated that differences in the pH optima of crude IAA-oxidase preparations from roots of pea and cucumber were caused by the different low-molecular thermostable cofactors of IAA-oxidase present in these crude extracts. An influence of the cofactors used on the pH optima was also shown by STUTZ (1957), who found that the optimum pH value for the destruction of IAA by the crude IAA-oxidase from lupine was 6.5, but after purification and addition of 2,4-dichlorophenol (DCP) the optimum was at pH 5. Similar results were obtained for IAA-oxidase preparations from pea roots by KONINGS (1964). GASPAR (1966) demonstrated that the optimum pH value for IAA-oxidase activity of extracts from *Lens* roots was at pH 6 with p-hydroxybenzoic acid, but at pH 5.5 when resorcinol was used as a cofactor.

According to ENGELSMA & MEIJER (1965) the pH optimum of IAA-oxidase of gherkin hypocotyls was influenced by the concentration of the cofactor used (p-coumaric acid). In a previous paper it was demonstrated that dilution of the boiled crude cucumber root extract, which contained cofactor activity, also caused a shift of the pH optimum of the purified IAA-oxidase from cucumber roots (JANSSEN 1969b).

As many mono- and meta-diphenols are known to be cofactors of IAA-oxidase activity, the influence of a number of synthetic cofactors on the pH optimum of IAA-oxidase from pea roots and also the effect of the concentration of some of these cofactors on the pH optimum was investigated. From the results presented in this paper it is clear that the pH optimum is influenced both by the nature of the cofactor and by its concentration.

2. MATERIAL AND METHODS

Roots of *Pisum sativum* cv. "Vlijmsche Gele Krombek" were grown as described before (JANSSEN 1969a). After 65 hours roots of 3–6 cm length were cut off and collected in ice-cold phosphate-citrate buffer solution according to Mc.

Ilvaine pH 5. The roots were ground with sand and centrifuged during 30 minutes at 27000 g. The supernatant was partially purified by precipitation of the proteins with acetone at –20 °C. To each ml supernatant about 10 ml acetone was added. The acetone was decanted and replaced by fresh acetone. This procedure was repeated 4 to 5 times. Finally the precipitate was vacuum dried and an almost white powder was obtained, which was stored at 4 °C.

From the acetone powder an enzyme solution was prepared in distilled water (10 mg/ml), which was centrifuged during 10 minutes at 1000 g to remove undissolved parts. Only after addition of a cofactor the enzyme solution was able to destroy IAA.

The IAA-oxidase activity was determined at different pH values ranging from 3 to 7 in a reaction mixture consisting of 0.2 ml IAA 10^{-3} g/ml (in some experiments an IAA solution of 1.5×10^{-3} g/ml was used), 3.3 ml buffer solution, 0.5 ml of the substance to be tested and 1 ml of the enzyme solution. The residual IAA was measured with Salkowski reagent (15 ml 0.5 M $FeCl_3$, 500 ml distilled water and 300 ml H_2SO_4 s.w. 1.84). The tests were performed in dim red light at 22 °C. During the incubation time the reaction mixtures were carefully shaken.

3. RESULTS

3.1. The influence of monophenols and meta-diphenols on the pH optimum of IAA-oxidase

The results obtained with p-, m- and o-cresol are presented in *fig. 1* and the results obtained with the other substances are presented in *table 1*. It is clear that the pH optimum was determined by the cofactor used. The substances tested in a high concentration (10^{-4} g/ml) only caused a weak or moderate stimulation of IAA-oxidase activity, the substances used in a low concentration (10^{-6} g/ml), however, were very active cofactors. An exception on this rule was 4-(methylthio) phenol of which the maximal promotion was found at a concentration of 10^{-6} g/ml, whereas the IAA destruction proceeded only very slowly with this concentration.

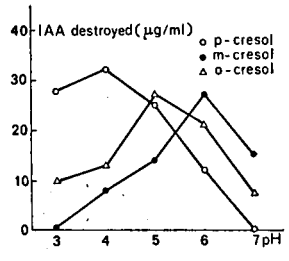

Fig. 1. The pH optimum of IAA-oxidase from pea roots with p-, m- or o-cresol as a cofactor. Incubation time 10 minutes.

3.2. The influence of the concentration of the cofactor on the pH optimum of IAA-oxidase

Figs. 2a-c show that the pH optimum was influenced by the concentration of the cofactor used. The following cofactors have also been tested in at least two concentrations: m-cresol, o-cresol, phenol, resorcinol, m-coumaric acid and

Table 1. The influence of monophenols and meta-diphenols on the pH optimum of IAA-oxidase from pea roots.

Substance tested	concentration (g/ml)	optimum pH value
phenol	10^{-5}	6
2, 4-dichlorophenol	10^{-5}	5
resorcinol	10^{-5}	5.5
p-hydroxybenzoic acid	10^{-5}	6
m-hydroxybenzoic acid	10^{-4}	no stimulation
salicylic acid	10^{-4}	no stimulation
p-hydroxybenzoic acid methylester	10^{-4}	6
β-resorcylic acid	10^{-4}	6
γ-resorcylic acid	10^{-4}	no stimulation
α-resorcylic acid	10^{-4}	5.5
p-coumaric acid	10^{-6}	5
o-coumaric acid	10^{-4}	no stimulation
m-coumaric acid	10^{-4}	5.5
p-hydroxyhydrocinnamic acid ethylester	10^{-5}	5.5
tyrosine	10^{-4}	6
2-hydroxy-3, 5-dichlorophenylacetic acid	10^{-4}	no stimulation
4, 4′-dihydroxydiphenylmethane	10^{-6}	5.5
p-nitrophenol	10^{-4}	no stimulation
vanillic acid	10^{-5}	4
o-hydroxyhippuric acid	10^{-4}	no stimulation
3-methyl-5-hydroxybenzoic acid	10^{-4}	no stimulation
4-(ß-nitrovinyl)phenol	10^{-4}	5.5
3, 5-dimethylphenol	10^{-4}	5
4-(methylthio)phenol	10^{-6}	4
p-hydroxybenzonitrile	10^{-4}	5.5

p-hydroxyhydrocinnamic acid ethylester. An increase of the concentration always caused a shift of the pH optimum to a lower pH value.

4. DISCUSSION

The results presented in this paper demonstrate that the pH optimum of IAA-oxidase from pea roots is determined both by the nature of the cofactor and by its concentration.

The activities of the cofactors of IAA-oxidase given by GORTNER & KENT (1958) will only be valid at the pH value used in their experiments. This also holds true for the optimum concentrations of the cofactors tested by TOMASZEWSKI (1964). For a proper comparison of the activities of the various cofactors one should determine the combination of concentration and pH value at which their activities are maximal. This could not be done with our method, for at higher concentrations the cofactors interfere with the colour development of the Salkowski reaction.

One will have to take into account the results presented above, if an influence

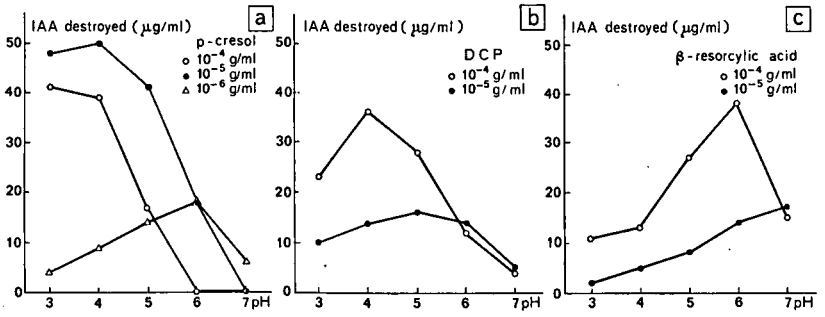

Fig. 2. The influence of the concentration of a. p-cresol (after 10 minutes), b. DCP (after 10 minutes) or c. ß-resorcylic acid (after 30 minutes) on the pH optimum of IAA-oxidase of pea roots.

of phenolic substances on e.g. growth is explained in terms of an effect via the IAA-oxidase system.

Especially monophenols having a free para-hydroxyl group are cofactors of IAA-oxidase activity (see HARE 1964). Although an exact comparison of the activities of the various cofactors is not possible with the available results, it is nevertheless clear that their activities are strongly influenced by the nature of the group *para* to the hydroxyl group. No stimulation of IAA-oxidase activity was found with 10^{-4} g/ml p-nitrophenol or a lower concentration (higher concentrations could not be tested). Tyrosine (10^{-4} g/ml) had only a small effect, p-coumaric acid, however, caused a strong stimulation at a concentration of 10^{-6} g/ml. *Fig. 2a* shows that the cofactor activity of p-cresol goes through a maximum at increasing concentrations. The highest activity was found at pH 4 at a concentration of 10^{-5} g/ml. A similar result was obtained by ENGELSMA & MEIJER (1965) with *p*-coumaric acid and IAA-oxidase from hypocotyls of gherkin seedlings.

Monophenols with an additional group *meta* to the hydroxyl group cause generally only a small stimulation of the destruction of IAA (VARGA & KÖVES 1962, ZENK & MÜLLER 1963; LEE & SKOOG 1965; PILET & GASPAR 1965; GASPAR 1966). In our experiments the active stimulation by m-cresol was clearly an exception.

Monophenols with an additional group *ortho* to the hydroxyl group cause generally no or almost no stimulation of IAA-oxidase activity (GOLDACRE *c.s.* 1953; GORTNER & KENT 1958; VARGA & KÖVES 1962; ZENK & MÜLLER 1963; GASPAR *c.s.* 1964; LEE & SKOOG 1965; PILET & GASPAR 1965; GASPAR 1966). From the substances used in our experiments only *o*-cresol was an active cofactor.

As p- and m- and o-cresol are active cofactors, these substances could be important in a study of the action mechanism of the cofactors of IAA-oxidase.

ACKNOWLEDGEMENT

The author is much indebted to Miss W. van Hiele for her skillful technical assistance.

REFERENCES

ENGELSMA, G. & G. MEIJER (1965): The influence of light of different spectral regions on the synthesis of phenolic compounds in gherkin seedlings in relation to photomorphogenesis. II. Indoleacetic acid oxidase activity and growth. *Acta Bot. Neerl.* **14**: 73–92.

GASPAR, TH. (1966): Action de quelques composés phénoliques sur l'activité auxines-oxydasique, le teneur en auxines et la croissance. In: Les Phytohormones et l'Organogenèse, *Les Congrès et Colloques de l'Université de Liège* **38**: 41–53.

GASPAR, TH., M. BASTIN & C. LEYH (1964): Composés phénoliques, acide ß-indoleacétique et activité auxines-oxydasiques. *Acad. roy. Belg., Cl. Sc.* **50**: 799–815.

GOLDACRE, P. L., A. W. GALSTON & R. L. WEINTRAUB (1953): The effect of substituted phenols on the activity of indoleacetic acid oxidase of peas. *Arch. Biochem. Biophys.* **43**: 358–373.

GORTNER, W. A. & M. J. KENT (1958): The coenzyme requirement and enzyme inhibitors of pineapple indoleacetic acid oxidase. *J. Biol. Chem.* **233**: 731–735.

HARE, R. C. (1964): Indoleacetic acid oxidase. *Bot. Rev.* **30**: 129–165.

JANSSEN, M. G. H. (1969a): An investigation of the polyphenoloxidase test with catechol and proline. *Acta Bot. Neerl.* **18**: 343–346.

— (1969b): An explanation of the different pH optima for indoleacetic acid oxidase activity of extracts from roots of pea and cucumber. *Acta Bot. Neerl.* **18**: 538–543.

KONINGS, H. (1964): On the indoleacetic acid converting enzyme and its relation to geotropism, straight growth and cell wall properties. *Acta Bot. Neerl.* **13**: 566–622.

LEE, T. T. & F. SKOOG (1965): Effects of hydroxybenzoic acids on indoleacetic acid inactivation by tobacco callus extracts. *Physiol. Plant.* **18**: 577–585.

PILET, P. E. & TH. GASPAR (1965): Action des acides o-, m- et p-hydroxybenzoïques sur le catabolisme auxinique et la croissance. *Ann. Physiol. vég.* **7**: 147–155.

STUTZ, R. E. (1957): The indole-3-acetic acid oxidase of Lupinus albus. *Plant Physiol.* **32**: 31–39.

TOMASZEWSKI, M. (1964): The mechanism of synergistic effects between auxin and some natural phenolic substances. In: *Régulateurs Naturels de la Croissance Végétale.* CNRS, Paris: 335–351.

VARGA, M. & E. KÖVES (1962): Effect of phenolic compounds on the activity of indoleacetic acid oxidase. *Acta Biol. Hung.* **13**: 273–281.

ZENK, M. H. & G. MÜLLER (1963): In vivo destruction of exogenously applied indolyl-3-acetic acid as influenced by naturally occurring phenolic acids. *Nature* **200**: 761–763.