THE INFLUENCE OF P- AND M-CRESOL ON THE PH OPTIMUM OF INDOLEACETIC ACID OXIDASE OF VARIOUS ORIGIN

M. G. H. JANSSEN

Botanisch Laboratorium, Utrecht

SUMMARY

The optimum pH value for the destruction of IAA by purified IAA-oxidase preparations from roots and epicotyls of pea, from roots, hypocotyls and cotyledons of cucumber and from roots and coleoptiles of oat is 1 to 2 units lower with p-cresol than with m-cresol as a cofactor.

1. INTRODUCTION

It has been demonstrated that the different pH optima for IAA-oxidase activity of crude extracts from roots of pea and cucumber are caused by the low-molecular thermostable cofactors present in the two extracts (JANSSEN 1969a). The pH optimum of partially purified IAA-oxidase from pea roots is determined by the cofactor used (JANSSEN 1969b). Especially the results obtained with p- and m-cresol seemed interesting because the pH optimum was pH 4 with p-cresol, but pH 6 with m-cresol as a cofactor (see *fig. 1*).

We now investigated the effect of p- and m-cresol on the pH optima of purified IAA-oxidase preparations from epicotyls of pea, from roots, hypocotyls and cotyledons of cucumber and from roots and coleoptiles (including the primary leaves) of oat. From the experiments described in this paper it appears that the results obtained with the various IAA-oxidase preparations are similar to the results obtained with IAA-oxidase from pea roots.

2. MATERIAL AND METHODS

Seeds of *Pisum sativum* cv. "Vlijmsche Gele Krombek" and seeds of *Cucumis sativus* cv. "Gewone Lange Groene" were put in closed plastic trays on moist filter paper and grown in darkness at 22°C. After 5 days the epicotyls of the pea seedlings and the cotyledons, the hypocotyls and the roots of the cucumber seedlings were collected in ice-cold phosphate-citrate buffer solution, according to Mc. Ilvaine, of pH 5. Seedlings of *Avena sativa* cv. "Victory C.I. 2020" were raised as described by BLAAUW & BLAAUW-JANSEN (1964). After an initial irradiation with red light for 21 hours after soaking of the seeds the seedlings were put in darkness at 22°C. After 4 days the coleoptiles (including their primary leaves) and the roots were collected in phosphate-citrate buffer of pH 5. To every 3 g of fresh weight of the pea epicotyls, 0.45 g of the cucumber

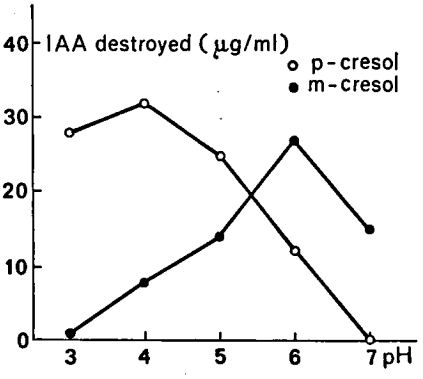

Fig. 1. The pH optimum of IAA-oxidase from pea roots with p- or m-cresol as a cofactor. (According to JANSSEN 1969b).

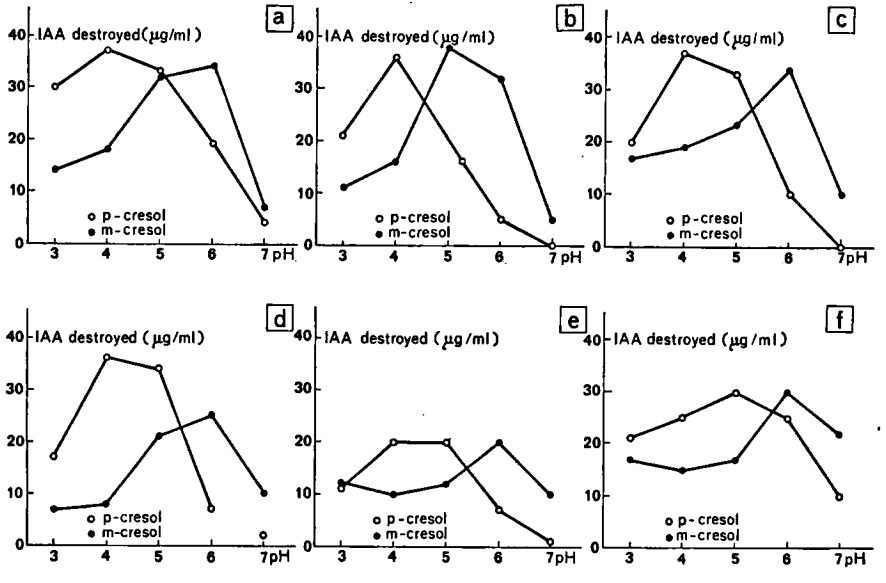

Fig. 2. The pH optimum of IAA-oxidase from a) pea epicotyls (after 30 min.), b) cucumber roots (after 10 respectively 20 min.), c) cucumber hypocotyls (after 30 resp. 60 min.), d) cucumber cotyledons (after 30 min.), e) oat roots (after 30 resp. 60 min.) and oat coleoptiles (after 120 min.) with p- or m-cresol as a cofactor.

cotyledons, 4.5 g of the cucumber hypocotyls, 0.75 g of the cucumber roots, 1.2 g of the oat coleoptiles and 0.75 g of the oat roots 1 ml buffer was added. The plant parts were ground with sand and centrifuged at 27000 g during 30 minutes. The supernatants were further purified by filtration of 5 ml of a crude extract through a Sephadex G 25 column as described before (JANSSEN 1969a).

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, 3.3 ml buffer solution, 1 ml of a purified enzyme preparation and 0.5 ml of either p- or m-cresol 10^{-4}g/ml. The residual IAA was measured with Salkowski reagent (15 ml 0.5 M $FeCl_3$, 500 ml destilled water and 300 ml H_2SO_4 s.w.l.84). The tests were performed in daylight at room temperature.

3. RESULTS

The results obtained with the various IAA-oxidase preparations are presented in *figs. 2a-f*. It is clear that the effects of p- and m-cresol are qualitatively similar to the results obtained with IAA-oxidase from pea roots *(fig. 1)*. The optimum pH value for the destruction of IAA with p-cresol as a cofactor is 1 to 2 units lower than with m-cresol as a cofactor in all cases.

4. CONCLUSION

The results presented in the present and in previous papers (JANSSEN 1969a and 1969b) clearly demonstrate that especially the nature of the cofactors determines the pH optimum of the various IAA-oxidase preparations and that the origin of the enzyme is less important in this respect.

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