AN EXPLANATION OF THE DIFFERENT pH OPTIMA FOR INDOLEACETIC ACID OXIDASE ACTIVITY OF EXTRACTS FROM ROOTS OF PEA AND CUCUMBER

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SUMMARY

Crude extracts from roots of pea and cucumber have different pH optima for IAA-oxidase activity, which are caused by the different natural thermostable cofactors present in the two extracts.

1. INTRODUCTION

Indoleacetic acid oxidase preparations of various origin have different pH optima for the destruction of indoleacetic acid (IAA). The optimal pH value for IAA-oxidase activity of extracts from pea epicotyls (TANG & BONNER 1947) and bean roots (WAGENKNECHT & BURRIS 1950) was about 6.5. GORTNER & KENT (1953), however, demonstrated that an extract from pineapple had its maximum IAA-oxidase activity at pH 3.5. Mainly based on the different pH optima Gortner & Kent concluded that the IAA-oxidase of pineapple is distinct from the IAA-oxidase of peas and beans. MUDD, JOHNSON, BURRIS & BUCHHOLTZ (1959) found that the buffer used influenced the pH optimum of IAA-oxidase from quackgrass (*Agropyron repens*) rhizomes and they concluded that "chelating effects of the buffers used may explain some of the differences in pH optima found for IAA-oxidase preparations from different sources". Similar results were found by GOLDSCHMIDT, GOREN & MONSELISE (1967) for IAA-oxidase of citrus roots. We found that the optimum pH value for the destruction of IAA by a crude extract from pea roots was at pH 6 to 7 but for the destruction of IAA by a crude extract from cucumber roots a pH optimum of 4 was found.

From the experiments described in this paper it follows that the different pH optima are caused by the different natural cofactors of IAA-oxidase present in the two extracts.

2. MATERIAL AND METHODS

A crude enzyme preparation from roots (65 hours old) of *Pisum sativum* cv. "Vlijmsche Gele Krombek" was prepared in phosphate-citrate buffer solution according to Mc. Ilvaine of pH 6.5. as described before (JANSSEN 1969). For these experiments 60 root tips of 5 mm were collected per ml.

A crude enzyme preparation from roots of cucumber was obtained in the following way: 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 complete root systems were collected in ice-cold phosphate-citrate buffer solution of pH 5. To every 0.75 g of fresh weight 1.0 ml buffer was added. The roots were ground with sand and centrifuged at 27,000 g during 30 minutes. The supernatant was used as the crude enzyme preparation.

The crude extracts were stored at –20° C and were centifuged for a second time during 10 minutes before use.

A purified enzyme preparation was obtained by filtration of 5 ml of the crude extract through a Sephadex G 25 column in daylight at room temperature. The length of the column was about 35 cm and the diameter about 2 cm. The elution buffer was 10 × diluted phosphate-citrate buffer solution of pH 5. After a void volume of about 48 ml had passed 12 ml were collected and used as the purified enzyme preparation.

The IAA-oxidase activity was determined at different pH values ranging from 3 to 7 in a reaction mixture consisting either of 0.2 ml IAA 10^{-3} g/ml, 4.3 ml buffer solution and 0.5 ml of the crude extract or of 0.2 ml IAA 10^{-3} g/ml, 3.3 ml buffer solution, 0.5 ml of the boiled crude extract and 1.0 ml of the purified enzyme preparation. 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 daylight at room temperature.

3. RESULTS

3.1. The pH optima of crude IAA-oxidase preparations from roots of pea and cucumber

First we investigated the effect of the pH value on the destruction of IAA by crude extracts from roots of pea and cucumber.

Fig. 1 shows that the pH optimum of IAA-oxidase of the crude extract from pea roots was 6–7, but that the crude extract from cucumber roots had maximum IAA-oxidase activity at pH 4. These different pH optima cannot be explained

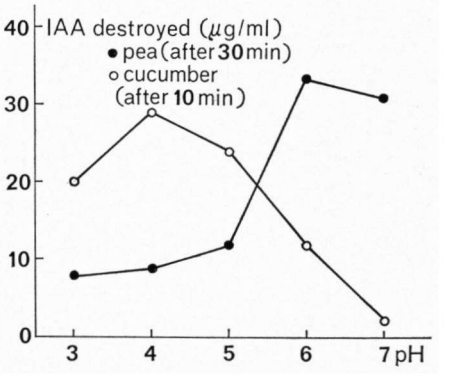

Fig. 1. The pH optima of crude IAA-oxidase preparations from roots of pea and cucumber.

by an effect of the buffer solution used, for in both cases phosphate-citrate buffer solution was used. A possible explanation could be that roots of pea and cucumber contain different IAA-oxidases or that the extracts contain different cofactors of IAA-oxidase activity.

3.2. The pH optimum of the purified IAA-oxidase preparation from pea roots to which a boiled crude pea root extract was added

After filtration of a crude extract from pea roots through a Sephadex G 25 column, the IAA-oxidase activity had disappeared almost completely. During the purification procedure a low molecular cofactor (or group of cofactors) must have been removed from the enzyme preparation, for after addition of a (synthetic) cofactor such as p-coumaric acid the activity was restored. We now investigated whether a boiled crude extract from pea roots could be used as "cofactor" for the purified IAA-oxidase preparation.

Fig. 2 shows that the addition of a boiled crude extract to the purified IAA-oxidase preparation gave results similar to those obtained with a crude extract (*fig. 1*). The crude extract from pea roots therefore contained a low-molecular thermostable cofactor of IAA-oxidase, which can be removed from the extract by filtration through Sephadex G 25 columns.

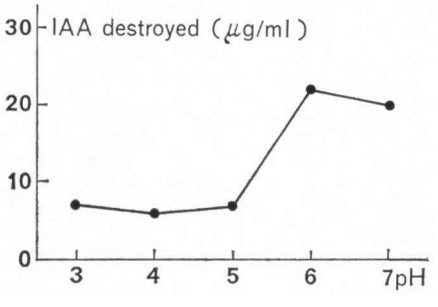

Fig. 2. The pH optimum of the purified IAA-oxidase preparation from pea roots to which a boiled crude pea root extract was added. Incubation time 60 min.

3.3 The effect of boiled crude extracts of pea and cucumber roots on the IAA-oxidase activity of purified enzyme preparations of these roots

Figs. 3 and *4* clearly demonstrate that the pH optima found in 3.1 were not determined by the purified IAA-oxidase preparations, but by thermostable cofactors of low molecular weight present in the boiled crude extracts. The boiled crude extract of cucumber roots was a more active cofactor than boiled crude pea root extract (see reaction times). According to ENGELSMA & MEIJER (1965) the pH optimum of IAA-oxidase from gherkin seedlings was influenced by the concentration of the (synthetic) cofactor used (p-coumaric acid). A possible explanation for the different results obtained with extracts from roots of pea and cucumber could be that the concentration of the natural cofactors was higher in extracts from cucumber roots than in extracts of pea roots. This was checked in the next section.

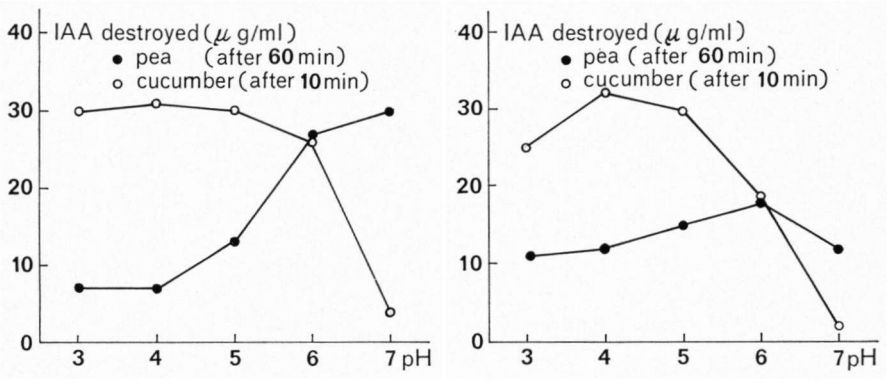

Fig. 3. and 4. The pH optima of purified IAA-oxidase preparations from roots of pea (fig. 3) and cucumber (fig. 4) to which either a boiled crude pea root extract or a boiled crude cucumber root extract was added.

3.4. The influence of the concentration of the boiled crude extract from cucumber roots on the pH optimum of purified cucumber root IAA-oxidase

The results represented in *fig. 5* show that some shift of the pH optimum occurred after dilution of the boiled crude extract. Further dilution of the cofactor, however, did cause almost no further shift of the pH optimum. It seems therefore very unlikely that the different pH optima for IAA-oxidase activity of crude extracts of pea and cucumber roots (*fig. 1*) have been caused by different concentrations of the same natural cofactor.

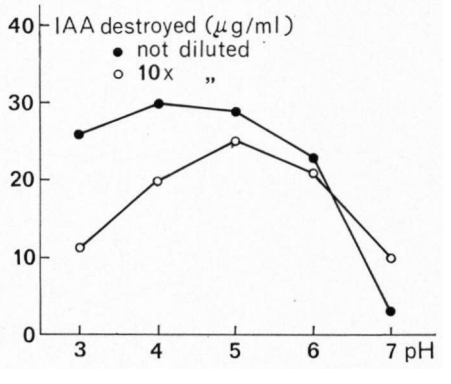

Fig. 5. The pH optimum of the purified IAA-oxidase preparation from cucumber roots to which either not diluted or 10× diluted boiled crude cucumber root extract was added. Incubation time 10 min.

3.5. Different effect of the pH value during boiling on the cofactors present in the crude extracts from roots of pea and cucumber

In preliminary experiments the extracts from pea roots were prepared in buffer solution of pH 5. The pH optimum for IAA-oxidase activity of such a crude extract was equal to that found with an extract prepared in buffer of pH 6.5. If

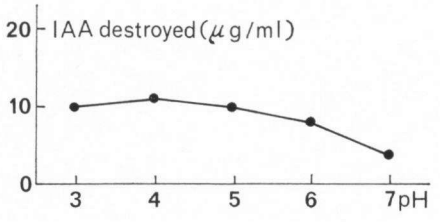

Fig. 6. The pH optimum of the purified IAA-oxidase preparation from pea roots when boiled crude pea root extract, prepared in buffer solution of pH 5, was added as cofactor. Incubation time 60 min.

an extract which was prepared in buffer solution of pH 5 was boiled and used as cofactor for a purified enzyme preparation, however, a quite distinct result was obtained (compare *figs. 2* and *6*).

It is not clear whether there was a shift in the pH optimum or that the IAA-oxidase activity at pH 6 and 7 had disappeared and only some remaining activity was present at lower pH values.

The results obtained with extracts from cucumber roots were equal if the extracts were prepared either in buffer solution of pH 5 or in buffer solution of pH 6.5.

4. DISCUSSION

The results presented in this paper demonstrate that crude extracts from pea and cucumber roots contain low molecular thermostable cofactors of IAA-oxidase. The different pH optima for IAA-oxidase activity of the crude extracts from roots of pea and cucumber can be explained by the different natural cofactors present in the two extracts.

From *figs. 3* and *4* it is evident that different pH optima cannot be accepted as an indication for a difference in IAA-oxidase, as suggested by GORTNER & KENT (1953).

The explanation of MUDD *c.s.* (1959) that differences in pH optima may be caused by the buffers used, cannot be valid here, for in all the experiments a phosphate-citrate buffer solution was used.

An influence of the cofactors used on pH optima has also been 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, after purification and addition of 2,4 dichlorophenol, however, the pH 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 with resorcinol as a cofactor.

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