THE FORM OF INDOLEACETIC ACID OXIDASE OF PEA ROOTS

M. G. H. JANSSEN

Botanisch Laboratorium, Utrecht

SUMMARY

Indoleacetic acid oxidase can be present in extracts of pea roots either as a high molecular form or as a lower molecular one or both. The smaller units can separate from the high molecular unit by certain treatments of the enzyme preparation.

1. INTRODUCTION

The destruction of indoleacetic acid (IAA) by IAA-oxidase of various origin has been ascribed to the activity of a single enzyme by some investigators, while others found that two enzymes were involved. SEQUEIRA & MINEO (1966) demonstrated the presence of two distinct fractions with high IAA-oxidase activity after purification of extracts from tobacco roots by means of column chromato graphy. CRONENBERGER *c.s.* (1966), however, found only one IAA-oxidising fraction in extracts from pea roots. We also purified extracts from pea roots and found sometimes one sometimes two fractions capable of destroying IAA. When only one IAA-oxidising fraction was present, the elution pattern differed from that obtained by Cronenberger c.s., although in both cases the extracts were filtered through Sephadex G 100 columns. In our experiments the IAA-oxidase activity coincided with the high molecular protein fraction, while Cronenberger c.s. found the activity in fractions of lower molecular weight.

Evidence will be presented that the different results are caused by variations in the methods used in preparing the extracts. It will also be demonstrated that the high molecular form of IAA-oxidase from pea roots can be split into smaller units.

2. MATERIAL AND METHODS

Roots of *Pisum sativum* c.v. "Vlijmsche gele krombek" were grown as described before (JANSSEN 1969). After 65 hours root tips were cut off at 5 mm length. To every 60 tips was added either 1 ml of ice-cold phosphate-citrate buffer solution according to Mc. Ilvaine of pH 5 or 1 ml of ice-cold destilled water. The tips were ground with sand and centrifuged at 27000 g during 30 minutes. The supernatant (the crude enzyme preparation) was used immediately or after stoage at – 20° C. An extract, which had been kept at – 20° C was centrifuged for a second time at 27000 g during 10 minutes before use.

The crude enzyme preparation was purified by filtration of 3 ml extract through a Sephadex G 100 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 30 ml had passed, 12 fractions of 2.5 ml each and 16 fractions 5 ml each were collected.

The IAA-oxidase activity of the individual fractions was determined in a reaction mixture consisting of 3.3 ml phosphate-citrate buffer of pH 5, 0.5 ml p-coumaric acid 10^{-5} g/ml, 0.2 ml IAA 10^{-3} g/ml and 1 ml of the fraction. During the incubation the reaction mixtures were carefully shaken. 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. 1.84). The tests were performed in dim red light at 22° C.

The protein content of the various fractions was determined by the method of LOWRY *c.s.* (1951).

3. RESULTS

3.1. The IAA-oxidase activity of the various fractions after filtration of extracts prepared in buffer solution of pH 5 through a Sephadex G100 column

The experiments described in this section were performed with extracts prepared in buffer solution of pH 5, which had been stored for some time at –20° C. The results from one typical experiment are presented in *fig. 1. 1.*

From *fig. 1.1* it is clear that there are two distinct fractions which exhibit IAA-oxidase activity, a high molecular fraction and a lower molecular one. The second IAA-oxidising fraction corresponds with that found by Cronenberger *c.s.* (1966).

The Lowry measurements seem to indicate that the fractions 23 to 26 contained much protein. With the precipitation method with tannic acid according to MEJBAUM-KATZENELLENBOGEN & DOBRYSZYCKA (1959), however, no protein could be demonstrated in these fractions. The positive reaction in the Lowry measurements must therefore be caused by low molecular substances such as some amino acids or phenols.

In freshly prepared extracts the second IAA-oxidising fraction was less pronounced than in extracts which had been stored at –20° C. A possible explanation is that the phosphate of the buffer solution crystallized faster during freezing than the citric acid. This would result in a decrease of the pH value of the solution, which could have led to some transformation of the enzyme molecules.

It could be expected that, when extracts were prepared in destilled water, this change of pH value during freezing would not occur. This idea is checked in the next section.

3.2. The IAA-oxidase activity of the various fractions after filtration of extracts prepared in destilled water through a Sephadex G100 column

In these experiments we compared the distribution of the IAA-oxidase activity

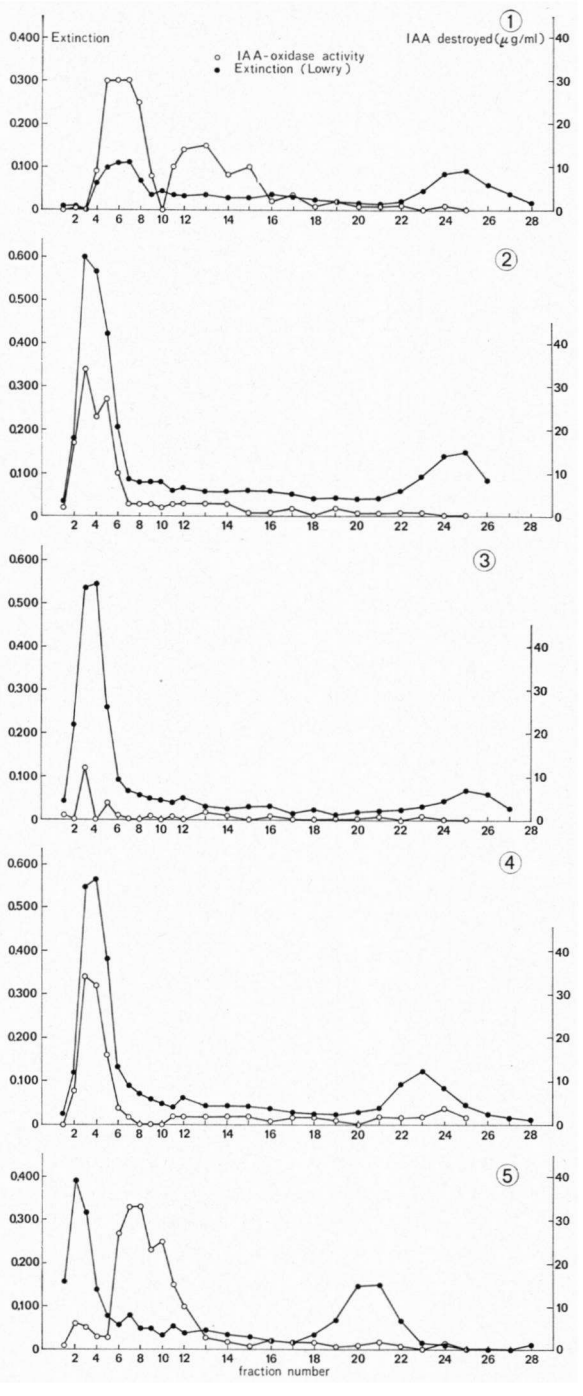

Fig. 1. The IAA-oxidase activity and the Lowry measurements of the various fractions after filtration of 3 ml extract from pea roots through a Sephadex G100 column. After a void volume of 30 ml had passed, 12 fractions of 2.5 ml each and 16 fractions of 5 ml each were collected. 1. The extract was prepared in buffer solution of pH 5 and was used after storage at −20 °C; 2. the extract was prepared in destilled water and used freshly; 3. the extract was prepared in destilled water and used after storage at −20 °C; 4. the extract was prepared in destilled water. After being centrifuged, 250 mg NaCl was added to 5 ml of the supernatant. It was used freshly; 5. the extract was prepared in destilled water. After centrifuging, 250 mg NaCl was added to 5 ml of the supernatant. It was used after storage at −20 °C;

in the fractions of extracts prepared in destilled water, which were used either freshly or after storage at –20° C. The results are presented in *figs. 1. 2 and 1. 3.*

From these figures it is clear that the second IAA-oxidising fraction (of *fig. 1.1*) is absent in extracts prepared in destilled water, even after storage at –20° C. This freezing, however, caused a great loss of activity.

The different distribution of IAA-oxidase activity of extracts prepared in buffer solution of pH 5 and of extracts prepared in destilled water possibly could be explained by an insolubility of the lower molecular enzyme in destilled water. We therefore investigated the influence of NaCl on extracts prepared in destilled water.

3.3. The IAA-oxidase activity of the various fractions after filtration of extracts prepared in destilled water to which NaCl was added through a Sephadex G100 column

In these experiments extracts were prepared in destilled water. After being centrifuged, NaCl (1–10%) was added to the supernatant. One part of an extract was used freshly, the other part was stored at –20° C before use. The results are presented in *figs. 1. 4* and *1.5.*

From *fig. 1. 4* it is clear that the addition of NaCl had no effect on the distribution of the IAA-oxidase activity. After addition of NaCl and storage at –20° C however almost all the IAA-oxidase activity was shifted to fractions of lower molecular weight *(fig. 1. 5)*. The absence of this lower molecular enzyme in the experiments with extracts prepared in destilled water can not be caused by its insolubility in destilled water. The low molecular fraction, capable of destroying IAA, present in *fig. 1. 5* originated from the high molecular IAA-oxidase from *fig. 1. 4.*

4. DISCUSSION

From the results presented in this paper it is clear that IAA-oxidase in extracts of pea roots can be present either as a large or as a small form or both.

HEIDRICH & HANNIG (1968) demonstrated that, depending on the isolation procedure, either one or five active fractions were present in beaf liver catalase preparations after electrophoresis.

A similar situation apparently also exists for IAA-oxidase of pea roots. After grinding the root tips in buffer solution of pH 5, two distinct IAA-oxidising fractions were found, but after grinding the roots in destilled water, only the high molecular fraction was present. The absence of the low molecular fraction in this case was not caused by its insolubility in destilled water, for after addition of NaCl and storage at –20° C it was produced from the high molecular fraction. We must conclude therefore that the low molecular form of IAA-oxidase from pea roots can separate from the high molecular one by certain treatments of the enzyme preparation. Possibly the high molecular form of the enzyme is the native form of IAA-oxidase from pea roots. Therefore, the fact that CRONENBERGER *c.s.* (1966) only found the lower molecular enzyme could be the result of their purification procedure.

From our results it is not clear wether the high molecular IAA-destroying fraction, obtained after filtration through a Sephadex G 100 column, is in fact only a high molecular protein, or that the enzyme is bound to larger bodies such as ribosomes or membranes.

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