SEPARATION OF IAA DEGRADING ENZYMES FROM PEA ROOTS ON COLUMNS OF POLYVINYLPYRROLIDONE. II

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SUMMARY

Membrane-bound and free IAA degrading enzymes from pea roots both consist of a peroxidative and a non-peroxidative moiety. Retention of the latter protein is probably not due to the constituent amino acids.

Polyvinylpyrrolidone shows gel-chromatographic properties comparable with Sephadex G-100, but with a lower exclusion molecular weight and with a higher degree of adsorption.

1. INTRODUCTION

In a previous paper (VAN DER MAST 1969) evidence was presented for the existence of two types of enzymes capable of degrading IAA in homogenates of pea roots. The first type showed peroxidase activity, was not denaturated by 3 M urea and was not retained by polyvinylpyrrolidone (PVP). The second type did not possess peroxidase activity, was denaturated by 3 M urea and showed adsorption to PVP.

JANSSEN (1969) obtained two fractions of IAA oxidase after gel chromatography of pea root homogenates on Sephadex G-100, one fraction of high and one of lower molecular weight. Conversion of IAA oxidase of high molecular weight to a smaller, active IAA oxidase could take place by certain treatments.

Evidence, which will be communicated later by the present author, indicates that the larger part of IAA degrading enzymes is bound to membranes and that another part is present as virtually free enzyme molecules. The molecular weight in the bound state ranges up to a value larger than 30×10^6, while in the free state it is about 35000.

The bound and the free enzyme fraction might each represent one of the types of proteins found after chromatography on PVP, instead of being aggregated as was presumed in the previous paper. It was also possible that spontaneous release from the membranes to the free state was only effected for one type of protein. As PVP breaks the bond between all IAA degrading enzymes and the membranes, both types of enzyme molecules could then behave as reported in the previous paper (VAN DER MAST 1969).

It was therefore decided to repeat part of the experiments with the bound and the free enzyme fraction separately and also to elucidate further the nature of the peaks found in the elution pattern from the PVP columns.

2. MATERIAL AND METHODS

Roots of *Pisum sativum* cv. "Vlijmsche Gele Krombek" were used. The method of growing the seeds has been described earlier (VAN DER MAST 1969). The roots were homogenized in either Tris-HCl buffer, pH 7,4, which contained 10 mM $MgCl_2$ or in Mc Ilvaine's buffer, pH 5,0. Usually 20 ml of the 27000 g supernatant was layered on a column of Sephadex G-100, dimensions 5 × 21 cm, and eluted with the appropriate elution buffer. Fractions of 15 ml were obtained. Several fractions were pooled to give three separate batches. Their volume was reduced by adding dry Sephadex G-25 or, in the case of the low molecular weight peak, by Sephadex G-10. Imbibition was complete after one minute. The concentrated solution was recovered by centrifuging the Sephadex in a basket centrifuge at 700 g during 5 minutes.

PVP (Polyclar AT powder) was purchased from the General Aniline & Film Corporation, New York. Chromatography on this polymer was performed under a pressure of 3 atm. N_2. Column dimensions were 3 × 15,8 cm. Usually 7 ml concentrated solution was layered on the gel bed and chromatographed. The flow rate was 5–10 ml per hour. Fractions of 5 ml were obtained.

Degradation of IAA and peroxidase activity were determined as described previously.

The presence of NaCl was detected with a conductivity meter.

Beef hemoglobin, type 1, was purchased from Sigma, hog pepsin from Mann Research Laboratories.

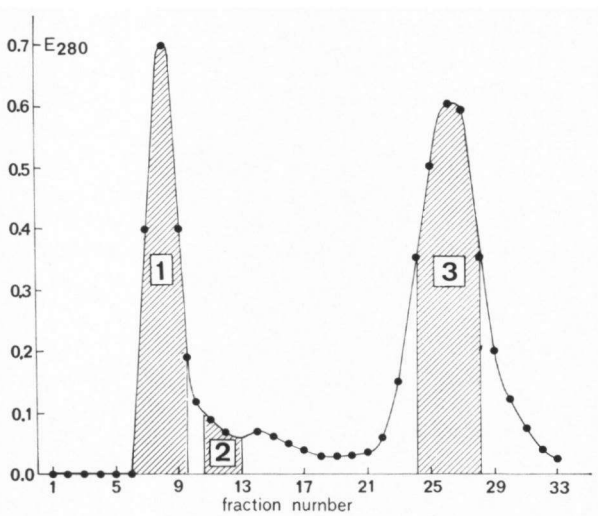

Fig. 1. Elution pattern of 20 ml pea root supernatant after gel chromatography over Sephadex G-100. Isolation and elution were performed with Tris-HCl buffer, pH 7,4, or in Mc Ilvaine's buffer, pH 5,0. The hatched areas represent the three batches that were further analysed on PVP.

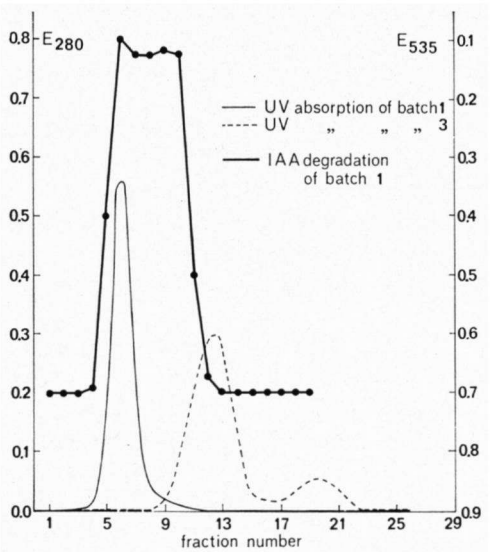

Fig. 2. Elution pattern and IAA degrading capacity of batch 1 after chromatography over PVP. The elution pattern of batch 3 over PVP is also given. This last batch normally contains no IAA degrading activity. Isolation and elution was carried out with Tris-HCl buffer in both cases.

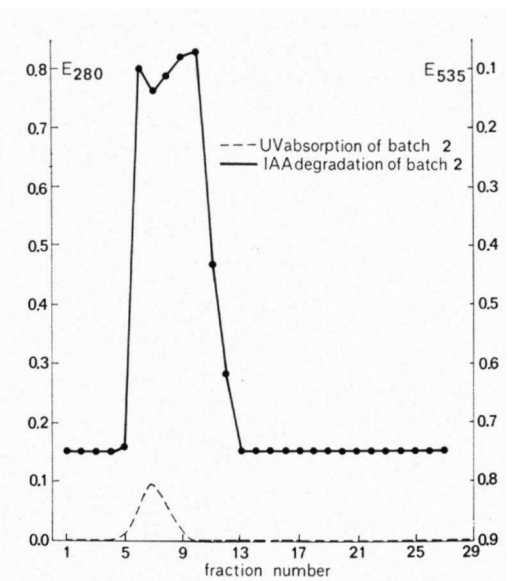

Fig. 3. Elution pattern and IAA degrading activity of batch 2 after chromatography on PVP. Isolation and elution was carried out with Tris-HCl buffer. Batch 2 contains less IAA degrading enzymes than batch 1. This difference is not apparent in *fig.* 2 and 3 because of lack of the substrate IAA.

3. RESULTS

The three batches that were isolated from the Sephadex column are shown in *fig. 1.*

Batch 1 contains predominantly ribosomes and membranes with bound to them part of the IAA degrading enzymes. Its volume of 45 ml was reduced to 12 ml prior to chromatography on PVP. The result is given in *fig. 2.*

Batch 2 consists of proteins with molecular weights varying between 35000 and 70000. Free IAA degrading enzymes are present here. This batch had the same volume as batch 1 and was treated as mentioned above. The result is shown in *fig. 3.*

Both batches elute from the PVP column after passage of the void volume. Due to the high pH the IAA degrading enzymes show a relatively poor adsorption.

Batch 3 comprises the low molecular weight compounds as amino acids and other small molecules. Its volume of 60 ml was reduced to 18 ml prior to further chromatography. The results are given in *fig. 2.* This batch gives rise to the second and the third peak of a whole homogenate. Compounds present in the second peak of batch 3 apparently have been adsorbed, because small molecules like urea and NaCl eluted earlier in another experiment as shown by *fig. 5.* This peak is lower than reported in the previous paper, presumably due to loss of material in the concentration procedure.

The same experiments were repeated with batch 1 and 2 of roots homogenized and eluted in Mc Ilvaine's buffer. The results are given in *fig. 4.*

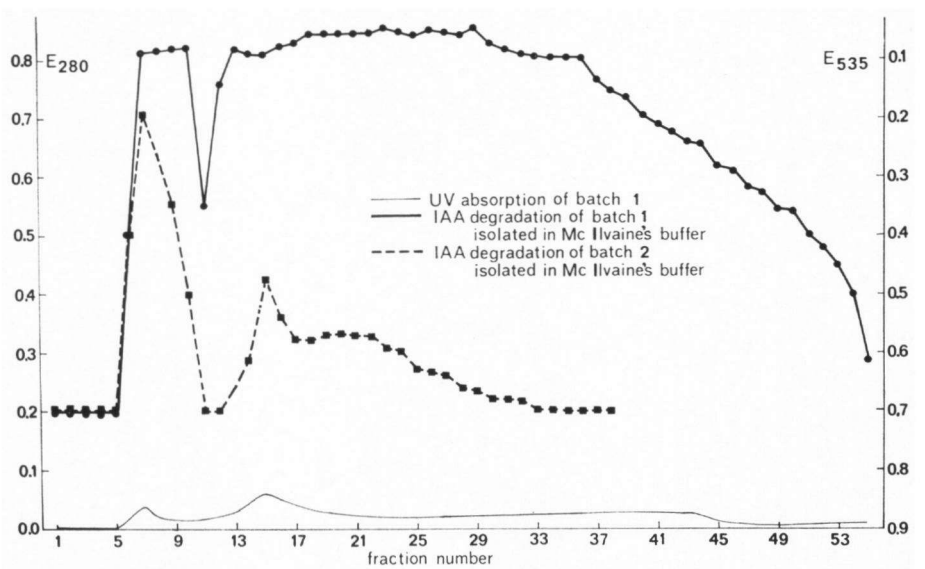

Fig. 4. Elution pattern and IAA degrading activity of batch 1 and 2 after chromatography on PVP. Isolation and elution was carried out with Mc Ilvaine's buffer. Peroxidase activity was confined to the fractions 5–12 of both batches.

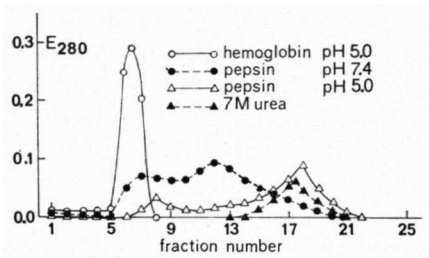

Fig. 5. Elution pattern after chromatography on PVP of hemoglobin, pepsin and urea with Mc Ilvaine's buffer. NaCl was found in the same fractions that contained urea in other experiments.

Extensive adsorption of IAA degrading enzyme molecules from both batches takes place. The high molecular weight peak, indicated by the 280 nm elution pattern, gives rise to three distinct peaks of which the second is masked by the low molecular weight peak when a whole homogenate is chromatographed. The third peak is identical with the fourth one mentioned in the previous paper (VAN DER MAST 1969).

Chromatography on PVP of pepsin and hemoglobin showed that the former protein also adsorbed dependent on pH. The results are given in *fig. 5*.

At pH 3,0 it adsorbed quantitatively to the column and could not be eluted with buffer. At pH 5,0 only part of it eluted in fractions which in other experiments contained urea or NaCl, while hemoglobin at this pH is not retained by the column. In both cases total or further removal of pepsin was effected with 6 M urea. At pH 7,4 pepsin gave rise to two peaks of which one immediately after the passage of the void volume, where according to molecular weight (35500) all pepsin should elute in case of ordinary gel chromatography. The two peaks may possibly be attributed to different states of this enzyme as pepsin shows denaturation above pH 6.

4. DISCUSSION

It is evident from *fig. 4* that both the bound and the free enzyme capable of degrading IAA consist of two moieties, i.e. a peroxidative and a non-peroxidative part. The phenomenon of adsorption of one moiety does not seem to be dependent on the presence of smaller molecules as these were removed by gel chromatography on Sephadex. This, however, does not rule out the possibility that interaction with small molecules, presumably polyphenols, is already completed before separation from the enzymes is effected. The wide range of elution of adsorbed enzyme molecules could be an indication of a nonspecific interaction between IAA degrading enzymes and various other compounds.

DOUGHERTY & SCHEPARTZ (1969) found that the aromatic amino acids tyrosine and tryptophan also adsorb to PVP. The different retention of compounds containing these amino acids is probably due to the residues. From the

non-adsorption of hemoglobin in our experiments it may be concluded that not all residues show this phenomenon, so that probably only a few amino acid species are responsible for the retention of pepsin on PVP. Adsorption of residues of a few specific amino acids then offers no explanation for the wide elution range of adsorbed IAA degrading enzymes, unless it is assumed that other compounds bound to these enzyme molecules participate in adsorption to PVP. This role could be fulfilled by polyphenols.

It is clear from the various figures that PVP shows both gel-chromatographic and adsorption-chromatographic properties.

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