Peroxidase Biosynthesis in Pea Roots as Influenced by IAA Treatment

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S u m m a r y

The influence of IAA /10^{-4}M/ on the biosynthesis and activity of peroxidase in pea roots was studied. Using a starch electrophoresis method in borate buffer pH 8.5 crude extracts were purified and peroxidase was separated into three fractions : neutral /O/, anodic /A/, and cathodic /C/. Using ^{59}Fe incorporation as an index of biosynthesis, treatment with IAA was shown to increase the biosynthesis of the main fraction of peroxidase /C/. The peroxidase activity of fraction C and the total peroxidase activity of the crude extract was higher in treated tissues than in controls. Indoleacetic acid oxidase activity of crude extracts was about twice as high in the treated tissue as compared to the controls.

I n t r o d u c t i o n.

In a previous paper /Czapski and Antoszewski 1970/, it has been shown that pea roots treated with indoleacetic acid /IAA/ exhibited a greater incorporation of ^{59}Fe into the protein fraction than did the controls and that this was correlated with a higher peroxidase activity of the treated tissues. It was suggested that under the influence of IAA, peroxidase biosynthesis is increased. In order to confirm this suggestion, it would be necessary to show that

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the increased incorporation of ^{59}Fe upon treatment of tissues with IAA is the result of incorporation of the isotope into peroxidase.

This communication presents the results of investigation into the peroxidase activity and de novo biosynthesis of peroxidase in the presence of IAA.

Material and Methods.

Pea seeds /Pisum sativum L./ were germinated on moist filter paper in Petri dishes for 48 hrs in the dark at 25°C. The terminal 2 cm of the roots were removed and samples having 0.5 - 1.0 g fresh weight were vacuum infiltrated for 5 min as below:

I: Control - water + ^{59}Fe,

II: Treatment - IAA /10^{-4}M/ + ^{59}Fe.

The specific radioactivity of ^{59}Fe in the solutions was 10 uCi/ml and the iron concentration 0.6 - 3.75 ppm. The ^{59}Fe /produced by the Research Inst. of Nuclear Sci., Świerk, Poland/ was in the form of $FeCl_3$. A stock solution of IAA /10^{-3}M/ was prepared by dissolving 17.5 mg of IAA /Chemapol, Czechoslovakia/ in 100 ml water containing 0.5 ml of 1M $NaHCO_3$. After infiltration, the roots were washed in tap water for 20 sec and then surface-dried with filter paper. They were then placed on moist filter paper in Petri dishes for 48 hrs in the dark at 25°C. They were then ground in a chilled mortar with 0.02 M phosphate buffer pH 7.0 together with a small quantity of quartz sand. The volume and radioactivity of the brei were measured. The homogenate was centrifuged in the cold at 20.000 g and the supernatant tested for peroxidase and indoleacetic acid oxidase activity and for protein content.

Peroxidase activity was determined by the method of Boyarkin /1951/. Indoleacetic acid oxidase activity was determined by following loss of IAA. A mixture containing IAA /1 ml $2x10^{-3}$M/, $MnCl_2$ /1 ml 10^{-3}M/, water /1 ml/ and phosphate buffer /6 ml, 1/15M, pH 5.2/ was incubated at 25°C and 2 ml samples taken after various periods of time. To these samples were added 4 mls of Gordon and Weber reagent /1951/ and after standing 30 min the colour developed was measured at 530 nm. Total protein was estimated by the method of Lowry et al. /1951/.

Purification and fractionation of peroxidase fractions of the supernatant was achived using starch electrophoresis. Starch /hydrolized according to Smithies, 1955/ was mixed with borate buffer /pH8.5 prepared from 0.025 M H_3BO_3 and 0.01 M NaOH/ and poured into plexiglass channels /20 x 2 x 0.7 cm/. Excess buffer was removed from the starch surface by evaporation under an infrared lamp. The channels containing the starch then cooled to about +10°C and the sample applied to a slit cut in the centre of the starch strip. After filling the slit with a small quantity of the starch, the strips were subjected to electrophoresis at 600 V for 2.5 hrs at 5°C using borate buffer /0.3 M H_3BO_3 + 0.06 M NaOH; pH 8.5/.

Peroxidase isozymes were located by spraying the freshly cut surface of a starch electropherogram with a mixture of equal volumes of benzidine solution and 5% H_2O_2 /Alvarez and King 1969/.

Other electropherograms were divided into 1 cm segments and peroxidase eluted from the gel in tubes with 5 ml water for 12 hrs in the cold. ^{59}Fe-radioactivity of these eluates was determined with a low-background anticoincidence Geiger-Müller counter. Peroxidase activity was determined by the method of Boyarkin /1951/.

R e s u l t s.

The electrophoretic separation of peroxidase from extracts of pea roots treated with IAA and of controls is presented in Fig. 1.

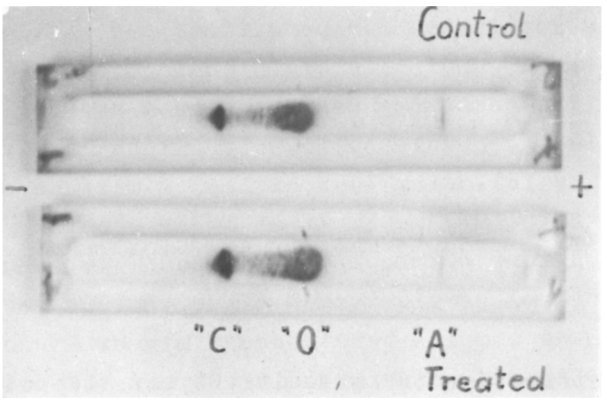

Fig. 1. Electrophoretic separation of peroxidase isozymes from crude extracts of pea roots.

Following electrophoresis, three fractions of peroxidase could be observed: neutral /"O"/, cathodic /"C"/, and anodic /"A"/. No qualitative differences could be observed between the treated and the control plants. The electrophoretical mobilities for particular fractions were respectively:

"O" - 0 $cmV^{-1}hr^{-1}$

"C" - -0.002 $cmV^{-1}hr^{-1}$

"A" - +0.004 $cmV^{-1}hr^{-1}$.

Places on the electropherograms indicating peroxidase activity have always indicated ^{59}Fe radioactivity and vice versa. Fraction "C" had the highest peroxidase activity.

The distribution of ^{59}Fe radioactivity on the electropherograms is presented in Fig. 2. Fraction "C" had a higher activity than fraction "O" or "A". The incorporation of ^{59}Fe into fraction "C" was higher for the IAA-treated tissues than for the control, being respectively 9.09% and 5.96% of the activity present in the pea roots. This indicates that the biosynthesis of isozyme "C" in the presence of IAA is greater than in the control and this is correlated

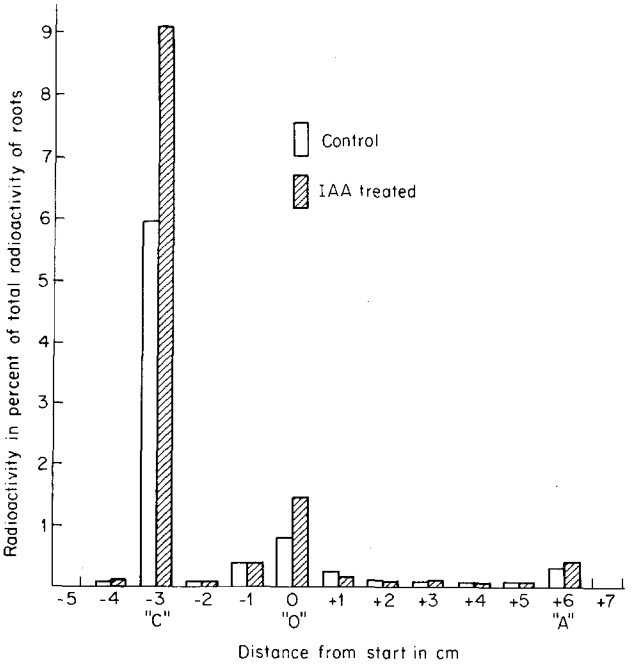

Fig. 2. Distribution of radioactivity of ^{59}Fe on electropherograms /averages of four replicates/.

with a higher peroxidase activity of this isozyme in treated tissues as compared to controls /Fig. 3/. The radioactivity of the neutral fraction "O" /Fig. 2/ was also higher in the

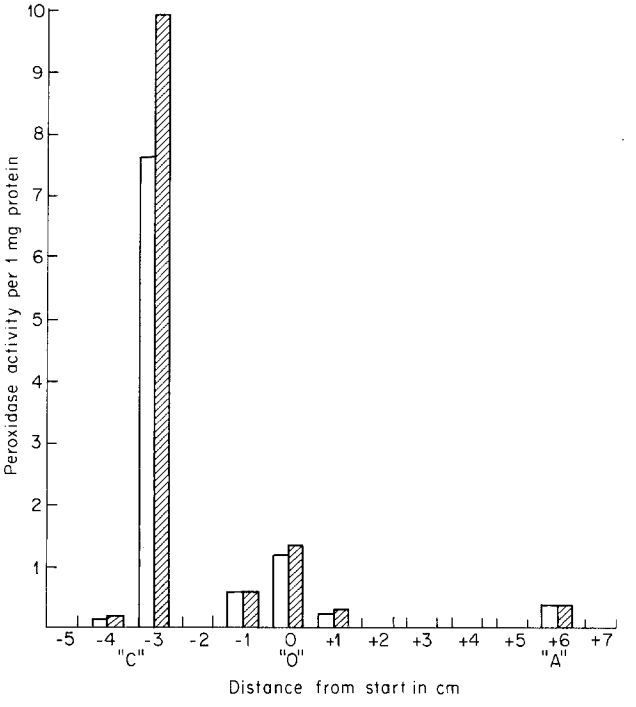

Fig. 3. Distribution of peroxidase activity on electropherograms /averages of four replicates/.

treated tissues than in controls but this cannot be considered as being real due to the possibility of contamination of this fraction with ^{59}Fe as $^{59}Fe/OH/_3$ which, in small quantities, may move into solution. The likelihood of this differences being an artifact is increased by the observation that there was no difference in peroxidase "O" activity between the treated tissues and the controls. In the case of isozyme "A", no differences either in radioactivity /Fig. 2/ nor peroxidase activity /Fig. 3/ were observed due to IAA treatment.

The peroxidase activity of extracts of IAA-treated tissues was higher than that of controls, being respectively 14.1 and 11.3 units per mg protein.

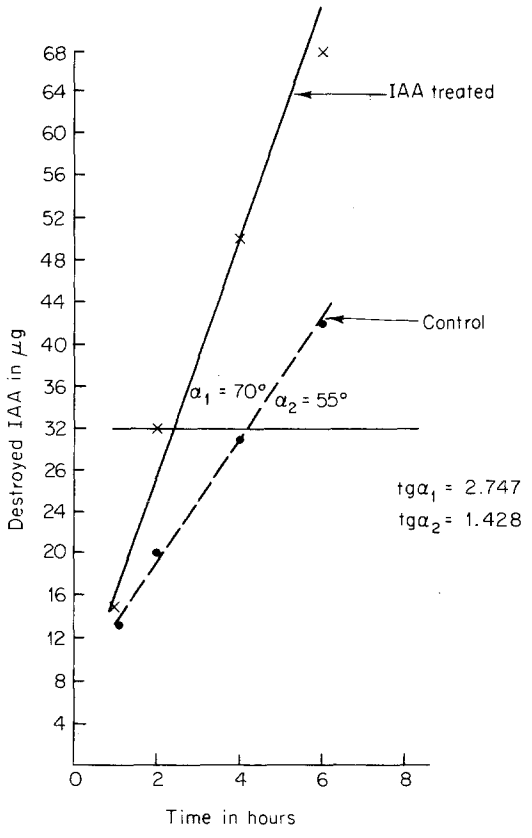

Fig. 4. Indoleacetic acid oxidase activity of extracts from pea roots /see text/.

The indoleacetic acid oxidase activities of control and IAA-treated tissues are presented on Fig. 4. Activities are expressed as the tangent of the angle of slope of the curves for the destruction of IAA in the incubation system. The activity for tissues treated with IAA was about twice that of the untreated tissues being 2.75 and 1.43 respectively.

D i s c u s s i o n.

There are many reports in the literature of the separation of proteins and peroxidase on starch or polyacrylamide gels using electrophoresis /Hamil and Brewbaker 1969, Sargent 1969, Alvarez 1968/. However it is difficult to determine quantitatively the activity of particular isozymes

by this method due to inaccuracies in the application of the sample to the gel and difficulties associated with the elution of the isozymes from the gel. The method employed in the current investigation has numerous advantages over these previous methods.

1/ Inorganic ^{59}Fe present in the tissue following infiltration, is - under the conditions used /buffer pH 8.5/ - in the form of $^{59}Fe/OH/_3$, which remains on the origin during electrophoresis and is not appreciably eluted from the starch with water, thus enabling separation of inorganic ^{59}Fe from ^{59}Fe incorporated into peroxidase isozymes.

2/ Peroxidase isozymes are readily extractable quantitatively from the starch.

3/ The high water-holding capacity of starch prepared in this way, enables the use of large sample volumes /about 0.2 ml/.

According to many authors, the enzymatic system termed auxinoxidase includes peroxidases /Siegel and Galston 1967, review by Pilet and Gaspar 1968, Alvarez and King 1969/ It is likely that it is one particular peroxidase isozyme which has a high ability to destroy IAA /Macnicol 1966/. In several publications it has been indicated that peroxidase activity is increased by the treatment of plants with IAA /Siegel and Galston 1967, Polevoy and Stroganova 1968, Lavee and Galston 1968, Alvarez and King 1969/.

Macnicol /1966/ investigated the distribution of peroxidase isozymes in pea plants. The auxinoxidase activity of one particular isozyme /isozyme C_3/, isolated from the roots of peas, was much higher than that of other isozymes, isolated from aerial parts of the plant.

In the present study, it was found that the intensity of biosynthesis and peroxidase activity of isozyme "C" was higher than for other isozymes and, moreover, that under the influence of IAA the intensity of biosynthesis and peroxidase activity increased in relation to controls. The indoleacetic acid oxidase activity of extract from tissues treated with IAA was also higher than in controls.

Hence, under the influence of IAA treatment, the biosynthesis of some peroxidase isozymes is intensified and this is in agreement with the suggestion made by Galston

and Dalberg /1954/ and Pilet /1964/ that this may be an adaptive enzyme.

Under the influence of IAA treatment, the physiological equilibrium between IAA and auxinoxidase, which is characteristic for the particular tissue and the particular conditions, is probably disturbed by the exogenous IAA. This results in a higher turnover of certain peroxidase fractions connected with the indoleacetic acid oxidase system.

A c k n o w l e d g m e n t s .

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