The Inhibitory Effect of some Antibiotics on Increase in o-Diphenol Oxidase Activity during Incubation of Sliced Sweet Potato Tissue

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The marked increase in o-diphenol oxidase activity which developed in incubating slices of sweet potato roots was suppressed by administration of actinomycin D, puromycin and blastcidin S. This suggests that the rise in enzyme activity resulted from de novo synthesis of enzyme protein during incubation. The formation of component III of o-diphenol oxidase which occurred in response to cutting, was strongly inhibited by supplying the above chemicals.

It has been found that o-diphenol oxidase [E.C. 1.10.3.1 o-quinol: O2 oxidoreductase] activity in sweet potato roots was enhanced in response to either cutting or infection.1-3) The rise of o-diphenol oxidase activity in injured plant tissue has been observed by many researchers, as reviewed by Farkas and Király.4) In slices of sweet potato roots, o-diphenol oxidase activity increased markedly in a sigmoidal fashion during incubation for 4 days. The activity after 4 days was nearly 5-fold over that of untreated healthy roots.5) Furthermore, component III, a form of o-diphenol oxidase not present in healthy roots was found in both cut and infected tissues.6) Antibiotics such as actinomycin D, puromycin and blastcidin S have been utilized in studies on protein biosynthesis since the mode of action of these chemicals has been clarified.5-10) This paper deals with the inhibitory effects of the above mentioned chemicals on the pattern of increase in o-diphenol oxidase during incubation of slices of sweet potato roots.

MATERIALS AND METHODS

Experimental Materials and Infiltration Methods Sweet potato roots (variety, Norin 1) harvested at the Kariya farm, Aichi, were stored at 10~11°C until used. The roots were scrubbed in water, immersed for 30 minutes in 0.1% sodium hypochlorite solution to sterilize the outer parts, and rinsed in running tap water. Cross sectional cuts, 2 mm in thickness, were made from the roots by means of a bacon slicer. Disks 19 mm in diameter, were cut from the slices with a cork borer. These disks were placed in a petri dish and incubated at 28~30°C under high humidity, for about 25 hours. After this preincubation period the disks were immersed in

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0.002 M potassium phosphate buffer, pH 6.8, with or without antibiotics, and continuously stirred with a magnetic stirrer for 1 hr. at room temperature. Then they were taken out from the solutions blotted with filter paper, and further incubated in petridishes at 28\(\sim\)30\(^\circ\)C until processed for enzyme extraction. The antibiotics, actinomycin D, puromycin and blastcidin S, were added to the aforementioned buffer to a final concentration of \(1.6\times10^{-4}\)M, \(1.0\times10^{-3}\)M and \(2.4\times10^{-6}\)M, respectively. When added to the reaction mixture described below, these compounds had no effect on the activity of \(\alpha\)-diphenol oxidase.

**Enzyme Extraction from the Tissues**

After a specified period of incubation, acetone powders were prepared from the disks. Two slices, about 1.6 g, were homogenized for 2.5 min. with 30 ml of cold acetone (\(-30\)\(^\circ\)C) containing 0.1% ascorbic acid in a Homoblender (Nihon Seiki Co. Ltd.). The homogenate was filtered on a Büchner funnel. The residue was washed with 60 ml of cold acetone and 20 ml of cold ether, and then dried to a powder in a desiccator over P\(_2\)O\(_5\) in vacuo. The dried powder was suspended in 6 ml of 0.05 M potassium phosphate buffer, pH 6.8, ground in a mortar and the suspension centrifuged at 8,000\(\times\)g for 10 min. The supernatant solution was used for assay of \(\alpha\)-diphenol oxidase activity.

**Assay of \(\alpha\)-Diphenol Oxidase Activity**

\(\alpha\)-Diphenol oxidase activity was determined using an oxygen electrode assembly as previously reported. Sodium caffeate was used for the substrate.

**Polyacrylamide-Gel Electrophoresis**

Polyacrylamide-gel electrophoresis was carried out following the method of Ogita et al., with a slight modification. The zones of \(\alpha\)-diphenol oxidases in polyacrylamide-gel after electrophoresis were detected as follows: the gel was immersed for about 10 min. in a solution made up of equal volumes of 0.9% sodium caffeate (pH 6.0) and 0.1% \(p\)-phenylenediamine. The position of the enzyme on the gel was revealed by the formation of a darkviolet colored reaction product. Fresh solutions of \(p\)-phenylenediamine were prepared and mixed with the substrate solution immediately before use in the above assay. The apparatus and the materials for gel electrophoresis were obtained from Futaba Shoji Co., Osaka, Japan.

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**RESULTS AND DISCUSSION**

In Fig. 1 is shown the inhibitory effect of actinomycin D and puromycin on the increase in \(\alpha\)-diphenol oxidase activity. In control disks not infiltrated with antibiotics, \(\alpha\)-diphenol oxidase activity increased slowly during the first 25 hrs., and then increased rapidly thereafter. Such an increase in activity was prevented by addition of either actinomycin D or puromycin. Of the 3 compounds tested, blastcidin S suppressed the increase in \(\alpha\)-diphenol oxidase most effectively (Fig. 2). Here as in the previous experiment, \(\alpha\)-diphenol oxidase activity in the untreated disks increased markedly after the initial 25 hr. lag.

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Effect of Antibiotics on Increase in o-Diphenol Oxidase

FIG. 2. The Inhibitory Effect of Blastcidin S on the Development of o-Diphenol Oxidase Activity during Incubation of Tissue Disks from Sweet Potato Roots.

After preincubation for 25 hrs. in petri dishes, disks of sweet potato root tissue were placed in a solution of $2.4 \times 10^{-6}$M blastcidin S in 0.002M potassium phosphate buffer under condition described in Materials and Methods. The curves show o-diphenol oxidase activity in extracts of C, control disks and B, disks infiltrated with blastcidin S. The abscissa shows hours of incubation. The pointer, 1, indicates the time when disks were transferred from petri dishes to the solutions described above. The ordinate shows o-diphenol oxidase activity in units per disk.

Phase. However, addition of blastcidin S after preincubation in petri dishes for 25 hrs. almost completely prevented the increase in o-diphenol oxidase activity. After an incubation period of 67 hrs., enzyme samples were prepared from healthy roots, untreated disks and disks treated with blastcidin S, and subjected to polyacrylamide-gel electrophoresis. The electrophoretic patterns thus obtained are depicted in Fig. 3. Component III present in extracts of untreated disks was absent in extracts of disks treated with blastcidin S and freshly prepared root extracts. The amount of component IIb, the synthesis of which appears to increase in response to cutting was greatly decreased in extracts of the treated disks. A diminution in the amount of component IIa was also detected in the blastcidin S treated material (Fig. 3).

The fact that enhancement of o-diphenol oxidase activity in slices of sweet potato roots was inhibited by addition of actinomycin D, puromycin and blastcidin S, known inhibitors of nucleic acid and protein biosynthesis, strongly suggests that the rise in enzyme activity results from de novo synthesis of the enzyme protein and not from activation of a proenzyme. In other studies, it has been shown that slicing and infection of sweet potato roots is followed by marked activation of various enzymes, leading to intense alteration of metabolism. Kanazawa, Shichi and Uritani found that the increase in peroxidase [EC 1.11.1.7 Donor: H$_2$O$_2$ oxidoreductase] ac-

FIG. 3. Diagrams of Polyacrylamide-gel Electrophorograms.

The diagrams represent electrophoresis patterns of enzyme preparations from H, untreated healthy root; C, control disks; B, disks supplied with blastcidin S. Disks with or without inhibitors were incubated for a total of 67 hrs. including the 25 hr. preincubation period. Conditions for electrophoresis were 60 volt/cm, 2.5 m amp for 3 hrs. Bands labeled IIa, IIb and III represent areas on the gel which were positive for o-diphenol oxidase activity and are therefore labeled o-diphenol oxidase components IIa, IIb and III.
tivity in slices of sweet potato roots was inhibited by treatment with actinomycin D, puromycin, etc. These authors suggested that the observed increase was due to net synthesis of peroxidase.\textsuperscript{12)} Similarly, Minamikawa and Uritani found that increase in phenylalanine deaminase [EC 4.3.1.5 L-phenylalanine ammonia-lyase] activity in sweet potato root in response to wounding or fungal infection was suppressed by administration of such inhibitors.\textsuperscript{14)} These findings are in accordance with incipient active changes of protein metabolism in the injured tissues as previously shown.\textsuperscript{15)} In the experiments on peroxidase and phenylalanine deaminase, the inhibitors were supplied by vacuum infiltration. The technique of vacuum infiltration, however, could not be used in the case of \textit{o}-diphenol oxidase, as the vacuum infiltration itself suppressed further induction of \textit{o}-diphenol oxidase.\textsuperscript{3)}

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\textsuperscript{13)} Y. Kanazawa, H. Shichi and I. Uritani, \textit{This Journal}, 29, 840 (1965).
\textsuperscript{14)} T. Minamikawa and I. Uritani, \textit{This Journal}, 29, 1021 (1965).