INTERACTION OF METALLIC IONS AND CHELATING AGENTS IN INDOLEACETIC ACID DESTROYING ENZYMES

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SUMMARY

A number of metallic ions abolished the inhibition of IAA-oxidase activity caused by DIECA. Contrary to WEBER (1970), in our opinion, these results do not give any information on the metal naturally occurring in the IAA-oxidase molecules. The restoration of the IAA-oxidase activity is caused by the removal of the chelating substance by the metallic ions.

1. INTRODUCTION

It is generally accepted that indoleacetid acid (IAA) destroying enzymes are metallo-enzymes. Mostly iron is supposed to be present in the IAA-oxidase molecules.

Recently WEBER (1970) demonstrated that the destruction of IAA by extracts of pea and barley seedlings was inhibited by ethylenediaminotetraacetic acid (EDTA). The enzyme activity was restored after addition of Zn^{++} or Mg^{++} ions. No restoration was found with Cu^{++}, Fe^{++}, Ca^{++}, Co^{++}, Ni^{++}, Cd^{++}, or Mn^{++} ions. Based on these findings Weber suggested that the metal occurring in the IAA-oxidase molecules is probably zinc or magnesium.

In a previous paper (JANSSEN 1970) it was demonstrated that solidiumdiethyldithiocarbamate (DIECA) inhibits the destruction of IAA by IAA-oxidase of pea roots. Unpublished experiments showed that this inhibition could be abolished by both Fe^{+++} and Cu^{++} ions. These experiments have now been repeated and extended with some other metallic ions. From the results it is clear that with these reactivation experiments no information can be obtained on the kind of metal present in the IAA-oxidase molecules.

2. MATERIALS AND METHODS

From root-tips of 65 hours old seedlings of *Pisum sativum* L. cv. 'Vlijmsche Gele Krombek' an extract was prepared in phosphate-citrate buffer solution of pH 5 as discribed before (Jansen 1969). The crude enzyme preparation was partically purified by means of Sephadex G 25 columns.

The IAA-oxidase activity was measured in a reaction mixture consisting of 0.2 ml IAA 10^{-3} g/ml, 05 ml p-coumaric acid 10^{-5} g/ml, 0.5 ml DIECA 10^{-4} g/ml, 1.0 ml of the purified enzyme preparation, 2.3 ml phosphate-citrate buffer solution of pH 5.0 and 0.5 ml of a 0.1 or a 0.01 % solution of the metal-com-

pound to be tested. The remaining IAA was determined with the Salkowski reagent according to TANG & BONNER (1947).

The experiments were performed in daylight at about 20°C.

3. RESULTS AND DISCUSSION

The IAA-oxidase activity of extracts from pea roots was inhibited by DIECA. After addition of Cu^{++}, Fe^{++}, Fe^{+++}, Zn^{++}, Cd^{++}, Pb^{++}, Co^{++}, or Ni^{++} ions the enzyme activity was at least partially restored. No restoration was found after addition of Mn^{++}, Mg^{++}, or Ca^{++} ions. WEBER (1970) found only reactivation after adding Zn^{++} or Mg^{++} ions.

The fact that so many metals are able to abolish the inhibition by DIECA of IAA-oxidase activity makes it unlikely that these experiments give information on the metal present in the IAA-oxidase molecules. The metals restoring the IAA-oxidase activity can be chelated by DIECA (see ALBERT & GLEDHILL 1947). If a metal forms a complex with a chelating substance not only the metal but also the chelating substance is removed. The restoration of the IAA-oxidase activity after addition of metallic ions, therefore, is probably caused by the removal of the chelating substance.

The results presented in this paper differ from those of WEBER (1970) who worked with extracts from pea and barley seedlings. He reported that manganese at a concentration of 0.05 M did not inhibit the IAA-oxidase activity of extracts from barley. This also holds true for the extracts of pea seedlings (personal communication). In our experiments with extracts from pea roots manganese inhibited the IAA-oxidase activity even at a concentration of 0.0005 M. The reason for the different sensitivity to metallic ions of the extracts used is not clear.

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