HISTOCHEMICAL LOCALIZATION OF ALCOHOL DEHYDROGENASE IN THE COTYLEDONS OF PISUM SATIVUM L. DURING GERMINATION

C. KOLLÖFFEL

Botanisch Laboratorium, Utrecht

SUMMARY

A high level of alcohol dehydrogenase activity could be demonstrated in fresh sections of cotyledons of peas germinated for one up to three days. This activity is localized mainly in the epidermis, the hypodermis, the procambium and certain cells of the storage parenchyma.

Several papers on alcohol dehydrogenase (alcohol: NAD oxidoreductase, EC 1.1.1.1, ADH) in peas have been published, but none of them contained data on the histochemical localization of this enzyme. In relation with earlier work on in vitro ADH activity (KOLLÖFFEL 1968) it was attempted now to determine the localization and the activity of ADH at different times during germination.

The reduction of nitro-blue tetrazolium by electrons transferred from ethanol via NAD and NADH: nitro-blue tetrazolium reductase (NADH: NBT reductase) was taken as evidence for the activity of ADH. In fact, therefore, the activity of the alcohol: NBT reductase complex is measured and the NADH: NBT reductase is localized.

Freshly cut freehand sections of pea cotyledons were washed in phosphate buffer (0.05 M, pH 6.4) and incubated in a medium containing: ethanol (0.4 M), NAD (0.0013 M), nitro-blue tetrazolium chloride (0.25 mg/ml) and phosphate buffer (0.05 M, pH 6.4). After an incubation period of 30 min, the sites of enzyme activity stain bluish purple. Control sections incubated in the absence of substrate or NAD developed a very weak colour only. Further evidence of ADH activity was sought by incubating sections in a medium containing instead of ethanol several other alcohols. A weak reaction was obtained with methanol, but with propanol or butanol as substrates the reactions were about equal to that with ethanol. The addition of iodo-acetic acid (10^{-3} M) or phenyl-mercury acetate (10^{-4} M) to the incubating medium completely inhibited the reaction whereas the addition of potassium thiocyanate (10^{-4} M) or sodium azide (10^{-3} M) inhibited the reaction only partially.

The diformazan deposits were uniformly distributed in the cytoplasm of the cells, but not throughout the cotyledons. Initially, there is a high level of activity in the epidermis, the hypodermis, the procambium and in certain cells of the storage parenchyma. They all are still active four days from the onset of germination. But after seven days they show only a very weak reaction. The majority of the cells of the storage parenchyma, however, showed initially a weak reaction and no visible reaction after about four days.

Macroscopically, there is only a slight difference between the colour intensity of the diformazan deposits of sections of cotyledons of peas germinated for one day or for three days respectively. However, the ADH activity of extracts prepared from cotyledons three days after the onset of germination was considerably lower than that of extracts from cotyledons germinated for one day (KOLLÖFFEL 1968). Preliminary experiments, suggest that this discrepancy is the result of the presence of some inhibitor of ADH which could be extracted from cotyledons of peas germinated for several days, but not from cotyledons of peas germinated for one day. Further experiments are now in progress to confirm this suggestion.

REFERENCE

KOLLÖFFEL, C. (1968): Activity of alcohol dehydrogenase in the cotyledons of peas germinated under different environmental conditions. *Acta Bot. Neerl.* **17**:70–77.