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SHORT COMMUNICATION

PURIFICATION AND SOME PROPERTIES OF ALCOHOL DEHYDROGENASE FROM MAIZE

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INTRODUCTION

ALCOHOL dehydrogenase is an enzyme which is widely distributed in nature. Detailed biochemical studies have been done with animal ADH,[1-5] and yeast ADH,[1,6,7] while the properties of the plant enzyme has still not been fully clarified. Plant alcohol dehydrogenase has been studied in pea,[8-14] maize,[15-16] peanut,[17] rice,[18-19] cereals[20-23] and fir seeds.[24] A number of authors[8,9,17,20] tried to purify the enzyme capable of catalysing the oxidation of ethanol; most successful of these was Pattee *et al.*[17] who isolated it from peanut.

RESULTS AND DISCUSSION

We succeeded in purifying alcohol dehydrogenase (ADH) (E.C. 1.1.1.1) from 2-day-old seedlings of maize 41-fold. The enzyme preparation was purified by thermal denaturation, acetone precipitation and fractionation with $(NH_4)_2SO_4$ to 35, 50, 70% saturation. The fraction obtained by salting-out with $(NH_4)_2SO_4$ in the 35–50% range of saturation contained the major part of alcohol dehydrogenase activity and was applied to the chromato-

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graphic column. Neither heating the extract nor additional precipitation with acetone brought any substantial purification of the enzyme. Only salting out with $(NH_4)_2SO_4$ led to an increase of specific activity and roughly 2·5-fold purification. The same degree of purification was also achieved when, instead of acetone fractionation, basic proteins were precipitated with a 2% protamine sulphate solution. Chromatography on a column of DEAE-cellulose resulted in another 15-fold purification of maize ADH as compared to the sulphate fraction. Chromatography on G-200 Sephadex gave no further substantial purification.

The Michaelis constant for ethanol was $1{\cdot}8 \times 10^{-4}$ M, the pH optimum was 8·7. Reagents reacting with —SH groups were used to detect a requirement for free SH groups for activity in the case of animal[1] and yeast[1] ADH, and experiments with the pea enzyme[8–10] show that these play a role similar to that of plant ADH. Experiments with reagents which form metal chelates have confirmed, that the animal, yeast and pea enzymes are metalloproteins.[1,8,10] We have found in the course of studying the influence of substances with SH groups on maize alcohol dehydrogenase, that L-cysteine (10^{-4} M) and mercaptoethanol (10^{-3} M) enhance the activity of the preparation to a slight degree. The activity of the enzyme was 10–42% inhibited by addition of iodoacetamide, EDTA, $B_4O_7^{2-}$, Zn^{2+} and Mg^{2+}, but was unaffected by *p*-chloromercuribenzoate, Cu^{2+} and Hg^{2+} (all at 10^{-4} M except EDTA at 10^{-3} M).

The maize enzyme did not oxidize lower primary saturated aliphatic alcohols such as propanol, butanol and hexanol. The reaction rate with the unsaturated analogue of the C_3 aliphatic alcohol was greater than that of ethanol. The plant enzyme did not oxidize methanol, colamine, methoxyethanol, ethoxyethanol, 1,3- and 1,4-butanediol, isoamyl-, isooctyl- and isopropyl alcohol, cyclohexanol, diethylene glycol and cinnamyl alcohol.

The activity of alcohol dehydrogenase was studied during ontogenesis in plants germinating under natural as well as artificial anaerobiosis, in light and in dark. The activity of ADH depends on the ethanol concentration in plant tissues: with ethanol amounts rising in so-called natural anaerobiosis or, when plants are kept in an atmosphere of nitrogen, the activity of alcohol dehydrogenase also increases. The ADH preparation obtained from green plants is more active than ADH from etiolated plants. In this case again there is a relationship between the enzyme activity and ethanol content.

Key Word Index—Zea mays; Gramineae; maize; alcohol dehydrogenase; enzyme purification.