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SHORT COMMUNICATION

ALCOHOL DEHYDROGENASE IN THE BARLEY EMBRYO

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Abstract—Alcohol dehydrogenase has been extracted from ungerminated barley embryos. During the first 8 hr of germination there is a rapid increase in the activity of this enzyme which is followed by an equally rapid decrease between 12 and 20 hr to approximately the initial level. A much slower decrease follows during the next 6 days. The enzyme preparation is specific for primary alcohols but will not oxidize methanol. Either NAD or NADP can serve as a cofactor. The optimum pH for the reaction is about 8·3.

INTRODUCTION

ALCOHOL dehydrogenase, the enzyme catalysing the oxidation of alcohols to the corresponding aldehydes, has not been demonstrated previously in barley. In fact, Cossins and Turner[1] reported that they could not detect its presence. However, it has been demonstrated in two other cereal grains, viz. oats[2] and wheat.[3]

The present studies have established that the enzyme occurs in the barley embryo and the properties of the enzyme have been investigated.

RESULTS

Alcohol dehydrogenase was readily extracted with water from isolated barley embryos. Leaving homogenates for up to 6 hr with occasional shaking led to no increase in extraction of the enzyme. No enzyme activity could be detected in similar extracts of either the aleurone or the starchy endosperm at any time throughout the first 30 hr of germination.

During the first 20 hr of germination there was an initial rapid rise in activity of the enzyme (up to 8 hr) followed by an equally rapid decrease (12–20 hr) to the initial level. Measurement of enzyme activity over the subsequent 6 days showed that the activity decreased slowly after this period to one-fifth of its original value. The maximum value of the activity was not due to any limitation in solubility of the enzyme.

In studying the kinetic properties of this enzyme, complications arise owing to the fact that, as Dalziel[4] has shown, commercial NAD contains varying amounts of potent inhibitors. Dalziel has described a method of purification but, in the present case, where a crude extract was being studied, this did not appear to be justified. Hence, results were considered comparable only where fresh solutions of a given sample of NAD were being used.

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[1] E. A. COSSINS and E. R. TURNER, *Ann. Botany* **26**, 591 (1962).
[2] J. BERGER and G. S. AVERY, *Am. J. Botany* **30**, 290 (1943).
[3] E. A. STAFFORD and B. VENNESLAND, *Arch. Biochem. Biophys.* **44**, 404 (1953).
[4] K. DALZIEL, *J. Biol. Chem.* **238**, 1538 (1963).

In a two-substrate reaction, the presence of a very large concentration of one substrate enables the kinetics of the reaction to be represented by a standard Michaelis–Menton equation with respect to the other substrate.[5] Thus Michaelis constants have been found for a number of alcohols in the presence of the non-limiting concentration of NAD used in the standard assay[6] (Table 1). It will be seen that the enzyme is specific for primary alcohols but that it is inactive with methanol.

TABLE 1. THE K_m OF BARLEY EMBRYO ALCOHOL DEHYDROGENASE WITH RESPECT TO VARIOUS SUBSTRATES

Substrate	K_m (M)
Methanol	No detectable reaction
Ethanol	0·0031
1-Propanol	0·060
2-Propanol	No detectable reaction
1-Butanol	0·029
2-Butanol	No detectable reaction
iso-Butanol	0·13
tert-Butanol	No detectable reaction
1-Pentanol	0·12
tert-Pentanol	No detectable reaction

Experimental details are given in the text. Each alcohol was studied over a range of concentrations between 0·1 and 1 M. In all cases, fresh solutions of the same sample of NAD^+ were used.

Both NAD and NADP can act as cofactors for the alcohol dehydrogenase. However, for a given concentration of ethanol the reaction is much slower with NADP than NAD. The K_m for ethanol using NADP is 0·19 M compared with 0·0031 M using NAD.

Under the conditions of the standard assay system, the pH optimum for the reaction was found to be about 8·3. It should be noted that the buffering power of the 0·032 M buffer recommended for the assay was insufficient to maintain the reaction mixture at the pH of the buffer and so the pH of each reaction mixture was determined directly at zero time. The buffer was not changed to ensure that results could be compared directly with those of other workers without having to make allowance for ion effects.

Attempts to purify the enzyme were unsuccessful. Precipitation with ammonium sulphate or organic solvents in the cold resulted in virtually complete loss of activity.

DISCUSSION

The changes in enzyme activity of the barley embryo during germination are in agreement with those observed in other seeds.[1,7,8] Presumably this reflects a change in the metabolism of the embryo from a relatively inert state, in which glycolysis provides sufficient energy, to one of rapid growth for which aerobic metabolism is essential.

The specificity of the enzyme appears to be similar to that of the wheat-germ alcohol dehydrogenase.[3] However, the wheat-germ enzyme, unlike that of barley, will oxidize

[5] M. DIXON and E. C. WEBB, *Enzymes*, 2nd edition, p. 70. Longmans, London (1964).
[6] B. L. VALLEE and F. L. HOCH, *Proc. Nat. Acad. Sci. U.S.* **41**, 327 (1955).
[7] H. BARTELS, *Planta* **55**, 573 (1960).
[8] A. MAFFEI-FACCIOLI, *Boll. Soc. Ital. Biol. Sper.* **35**, 2166 (1959).

methanol. The only other plant alcohol dehydrogenase studied in detail, that obtained from the pea,[1,9] is much less specific and will oxidize primary, secondary and tertiary alcohols.

The pH dependence of the enzyme compares with that of preparations from other sources, all of which show a pH optimum between 8 and 9.[10]

EXPERIMENTAL

The barley used throughout was a sample of Maris Badger from the 1965 harvest. The grain was dehusked by treatment for 2 hr with 50 per cent (v/v) H_2SO_4 which apparently has no adverse effect on the embryo.[11]

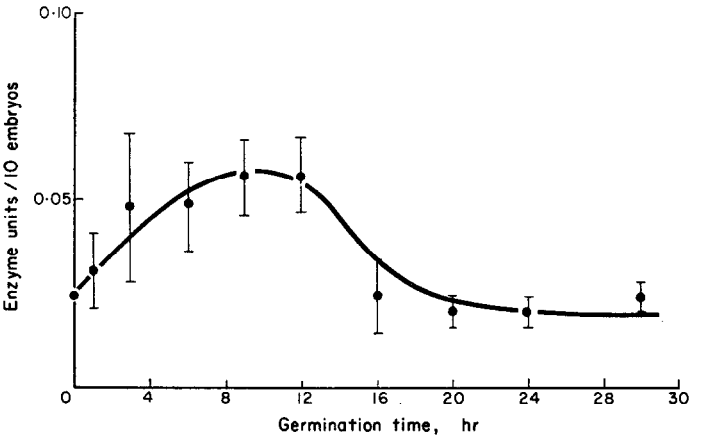

FIG. 1. CHANGE IN ACTIVITY OF BARLEY EMBRYO ALCOHOL DEHYDROGENASE DURING THE EARLY STAGES OF GERMINATION.
One enzyme unit reduces 1 μmole of NAD per min at 25° under the conditions described in the text. The bars represent the standard deviations of the points.

All alcohols used were redistilled before use. Commercial samples of NAD and NADP were used while other chemicals were of AR grade. Double-glass-distilled water was used throughout.

The embryos were removed from the barley grains and the residual endosperm divided into aleurone and starchy endosperm by the method previously described.[12] The aleurone and starchy endosperm were each extracted with 5 ml water for each 10-grain sample, while the embryos were extracted with 1 ml water. The samples were ground in a glass homogenizer. In the case of the aleurone layers, powdered glass was added. Once the cells were completely disrupted, the insoluble residues were removed by centrifuging. The supernatant was used as the enzyme preparation.

Dehusked barley was germinated on water-saturated filter paper in 9-cm Petri dishes. It was left to grow at 25° and samples were taken at appropriate times.

The enzyme activity was determined by the method of Vallee and Hoch.[6]

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[12] J. H. DUFFUS, *J. Inst. Brewing* **72**, 569 (1966).