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SHORT COMMUNICATION

AN ESTERASE FROM BARLEY ENDOSPERM*

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Abstract-An esterase isolated from barley endosperm which hydrolyzes a-naphthyl acetate has a mol. wt. of 29,500 and an isoelectric pH of 6.2. Other esterases and peptide hydrolase C were also present. The mol. wt. of the esterase was lower than those of the barley enzymes peptide hydrolases A, B, and C (56,000, 80,000, and 39,500 respectively), and L-leucyl-β-naphthylamidase (56,000). The esterase showed no phosphatase activity.

INTRODUCTION

GERMINATED barley contains several enzymes which hydrolyze a-naphtbyl acetate (ANA-ases).[1] One of these (ANA-ase IV), a peptide hydrolase,[2] has been designated as peptide hydrolase C (PHC). Extraction of distal halves and of the starchy endosperm tissue of ungerminated barley kernels revealed the presence of PHC or a similar enzyme and the properties of this enzyme were investigated.

RESULTS AND DISCUSSION

Figure 1 shows the distribution of esterases separated by electrofocusing. The enzyme in fractions 50-60 (isoelectric pH 5·8) is believed to be PHC, and only a small amount is present. The bulk of the ANA-ase activity in starchy endosperm resides in fractions 70-74 and in fractions 88-98. Since the latter consisted of at least two components, the ANA-ase of fractions 70-73 (isoelectric pH 6·2) was chosen for further purification.

Analytical disc gel electrophoresis[3] of fractions 70-73 separated the proteins into three main and four minor components, as illustrated in Fig. 2. The esterase was the slowest-moving component of the mixture. Unlike PHC this esterase appeared to be very unstable in purified form under these conditions, since about 70 per cent of the activity was lost during gel filtration. Tests with 0·02% NaN_3 in the assay procedure did not show inhibition. No phosphatase activity was detected.

The mol. wt. of the esterase is 29,500, whereas that of PHC from Trophy malt, prepared as described previously,[2] is 39,500; mol. wts. for peptide hydrolases A and B[4] and L-leucyl-naphthylamidase[1] are 56,000, 80,000 and 56,000 respectively.

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[1] W. C. BURGER, N. PRENTICE, M. MOELLER and J. KASTENSCHMIDT, *Phytochem.* **9, 33** (1970).
[2] W. C. BURGER, N. PRENTICE and M. MOELLER, *Plant Physiol. 46,860* (1970).
[3] M. MOELLER, W. C. BURGER and N. PRENTICE, *Phytochem.* **8, 2153** (1969).
[4] W. C. BURGER, N. PRENTICE, J. KASTENSCHMIDT and M. MOELLER, *Phytochem. 7, 1261* (1968).

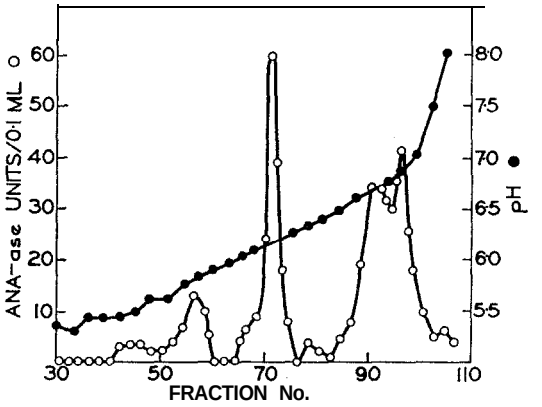

FIG. 1. SEPARATION OF ENZYMES BY ELECTROFOCUSING.

The function of the esterase described here, as well as that of the other esterases in the starchy endosperm is not known. The starchy endosperm has been regarded as a storage tissue for protein and carbohydrate. The β-amylase is located mainly around the periphery of the kernel and in the scutellum,[5] and lipase occurs mainly in the embryo and aleurone tissue,[6] while the tissue used in our work consisted of the inner portion of the endosperm.

EXPERIMENTAL

Aleurone and embryo tissue were abraded from a commercial sample of pearled Larker barley. The starchy endosperm (50 g) was ground finely, extracted for 1 hr at 4° with 150 ml water. The suspension was

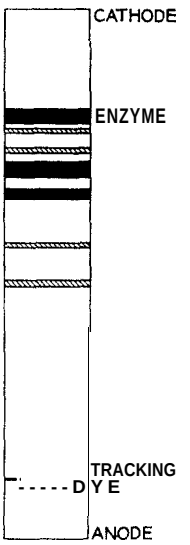

FIG. 2. PURIFICATION OF ESTERASE BY DISC GEL ELECTROPHORESIS.
Major component. Minor component.

[5] C. ENGEL, *Biochim. Biophys. Acta* 1, *42 (1947).*
[6] A. M. MACLEOD and H. B. WHITE, *J. Inst. Brew. 68, 487 (1962).*

stirred occasionally. After centrifuging at 30,000 g, the sediment was washed with water (50 ml) and re-centrifuged. The combined extracts were dialyzed against 3 x 1300 ml 0·005 M succinate buffer pH 5·5.

The preparation was purified by carboxymethyl cellulose (CMC) chromatography as described previously' except that succinate instead of acetate buffers were used.

The esterase fraction (containing PHC) from CMC chromatography was electrofocused[2] with ampholyte (pH range 5-7). A second extract of starchy endosperm (50 g) was treated similarly, the two preparations from electrofocusing combined, and further purified by disc gel electrophoresis.[3] For the purifications achieved by the three treatments (Table 1) the Lowry method[4] was used to obtain the protein values. The protein for the product from disc electrophoresis is an estimate.

TABLE 1. PURIFICATION DATA

Purification step	Preparation No.	Vol. (ml)	ANA-ase (U/0·1 ml)	Protein (mg/ml)	Specific activity (U/mg protein)
Dialyzed extract	1	175	8	2.6	31
	2	180	7	2·3	31
Carboxymethyl cellulose	1	22	24	1.9	126
	2	23	30	1.7	177
Electrofocus*		8	29	0.19	1520
Electrophoresis*		4	10.4	< 0.05	> 2080

* Pooled eluates from the two preparations.

The mol. wt. was determined with Sephadex G-100 equilibrated with 0·05 M succinate, 0·1 M NaCl, 0·02% NaN_3, pH 6·0. The standard proteins were aldolase, ovalbumin, chymotrypsinogen A, and ribonuclease A (Pharmacia Fine Chemicals, Inc.).

Phosphatase activity was determined with p-nitrophenylphosphate as substrate.