A Note on Changes in Peptide Hydrolase, Esterase, and Amidase of Maturing Barley[1]

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The general pattern of protein synthesis in ripening barley has been studied by many investigators (1). Several researchers have reported on changes in carbohydrate-hydrolyzing enzymes in maturing barley (2,3,4) but there are few, if any, reports concerning enzymes which hydrolyze proteins and peptides in maturing grain.

Previous work in our laboratory has concerned the purification and characterization of several peptide hydrolases (5), esterases, and an amidase (6) in

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mature barley and malt. These include peptide hydrolase A (PHA), which hydrolyzes alpha-N-benzoyl-DL-arginine-p-nitroanilide (BAPA); peptide hydrolase B (PHB), which hydrolyzes alpha-N-benzoyl-L-arginine ethyl ester (BAEE) (5); peptide hydrolase C (PHC), which hydrolyzes alpha-naphthyl acetate (ANA) (6,7); and the amidase (LNA-ase) which hydrolyzes L-leucyl-beta-naphthylamide (LNA) (6). Changes in these enzymes during malting and in the early stages of brewing have been described (8). This paper describes the results of similar techniques used for separation and characterization of the same or similar enzymes in maturing barley.

Six-row malting barley (*Hordeum vulgare* L. var. Larker) was grown at Madison, Wis., in 1969. Samples at 7 to 37 days after heading were hand-cut from the field, frozen, lyophilized, hand-threshed, and de-awned. Moisture, Kjeldahl-N, and ash were determined according to AACC Approved Methods (9). Some general characteristics of the barley samples are given in Table I.

Extraction, dialysis, and purification of enzymes on carboxymethyl cellulose (CMC) and assays for enzyme activity and protein in column eluates have been described briefly (8) and in detail (5,6). Twenty-five grams of barley (12% moisture) was used for each extraction.

Figure 1, a and b, shows the separation of enzymes on a CMC column for barley harvested 7 days after heading. The elution pattern for barley harvested at 14 days was similar in form; patterns for the more mature barleys were similar also except that BAEE-ase was absent. Previously reported elution patterns (6) for extracts of germinated barley were comparable to those for barley harvested at 7 and 14 days. BAPA-ase (Fig. 1, a), LNA-ase, and ANA-ase (Fig. 1, b) appeared in fractions 9 to 20. In work with germinated barley (6), this ANA-ase activity has been shown to consist of at least two ANA-ases. BAEE-ase was in fractions 21 to 29 (Fig. 1, a), and was followed by two peaks of ANA-ase activity (fractions 30 to 44 and 45 to 50 in Fig. 1, b).

Identification of each enzyme studied is based on elution volume from the CMC column and on hydrolysis of synthetic substrates. The above BAPA-ase is presumed to be PHA, the BAEE-ase is presumed to be PHB, and the LNA-ase is presumed to be similar to the LNA-ase found in germinated barley. The identities of these three enzymes are better established than those of other enzymes present. The ANA-ase from germinated barley identified as PHC would appear in fractions 30 to 44. No direct comparison of the identities of these enzymes with those of germinated

TABLE I. SOME GENERAL CHARACTERISTICS OF LARKER BARLEY HARVESTED AT VARIOUS STAGES OF MATURITY

Days after Heading	Moisture at Harvest %	Kernel Weight[a] mg.	Kjeldahl-N[a] mg.	Ash[a] %
7	70.1	13.5	1.69	4.34
14	57.5	23.5	1.67	3.20
32	18.0	33.3	1.94	2.88
37	15.7	32.8	2.01	2.99

[a]Of barley dried to about 12% moisture.

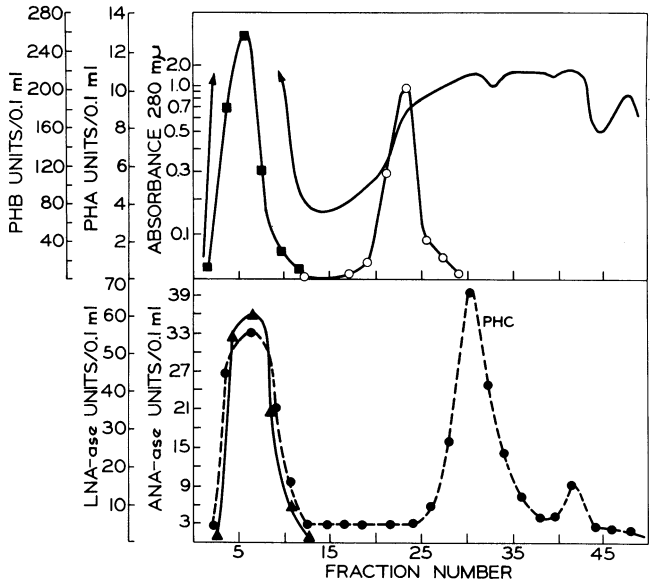

Fig. 1. Elution pattern of enzymes from CMC column. Line, O.D. 280 nm; squares, BAPA-ase; triangles, LNA-ase; open circles, BAEE-ase; solid circles, ANA-ase.

TABLE II. ENZYME ACTIVITIES IN BARLEY SAMPLES HARVESTED AT VARIOUS STAGES OF MATURITY AND DRIED TO APPROXIMATELY 12% MOISTURE

	Days after Heading							
	7		14		32		37	
Enzyme	d	e	d	e	d	e	d	e
BAPA-ase[a]	3.0	250	2.7	130	0.74	25	0.80	28
LNA-ase	6.6	550	6.8	325	6.5	220	7.8	270
ANA-ase (fractions 10–20)	12.4	1140	10.0	480	6.5	225	6.1	215
BAEE-ase[b]	16.0	1340	7.1	340	0	0	0	0
ANA-ase (fractions 30–44)[c]	22.9	1920	3.6	170	12.2	415	11.5	405
ANA-ase (fractions 45–50)	2.7	230	7.0	335	1.2	42	2.6	90

[a]Presumably PHA.

[b]Presumably PHB.

[c]Presumably PHC.

[d]Units per kernel, dry basis.

[e]Units per g., dry basis.

barley has been made, however, and this identification is only tentative. The enzymes are therefore referred to in this paper by names derived from the substrates hydrolyzed.

Table II shows the total activity in each enzyme peak from CMC for barley samples harvested at various stages of maturity. Although there was a decrease in LNA-ase and ANA-ase of fractions 45 to 50 during maturation on a weight basis,

there was practically no overall change on a kernel basis. On the other hand, the drop in capacity to hydrolyze BAPA and ANA (fractions 10 to 20 and 30 to 44) during maturation is larger by far than the increase in kernel weight, as indicated by a decrease in enzymatic activities both on a weight and a kernel basis. BAEE-ase activity is absent in mature barley, but is high both in immature and malted grain. These results indicate that, except for LNA-ase, the amounts of the enzymes studied decrease during maturation to a low level in mature barley. Upon germination, the levels of all these enzymes increase (8).

Literature Cited

1. HARRIS, G. The structural chemistry of barley and malt, chapter 10. In: Barley and malt; Biology, chemistry, technology, ed. by A. H. Cook. Academic Press: New York (1962).
2. PIENDL, A. Brautechnologie und Molekularbiologie. Brauwissenschaft 22: 175 (1969).
3. DUFFUS, C. M. Alpha-amylase activity in the developing barley grain and its dependence on gibberellic acid. Phytochemistry 8: 1205 (1969).
4. WICKBERG, H., and ENEBO, L. Ripening process and enzyme activity of barley. Svensk Bryggeritidskr. 77: 16 (1962) (from J. Inst. Brewing 68: 439 [1962]).
5. BURGER, W. C., PRENTICE, N., KASTENSCHMIDT, J., and MOELLER, MARY. Partial purification and characterization of barley peptide hydrolases. Phytochemistry 7: 1261 (1968).
6. BURGER, W. C., PRENTICE, N., MOELLER, MARY, and KASTENSCHMIDT, J. Hydrolysis of alpha naphthyl acetate and L-leucyl-beta-naphthylamide by barley enzymes. Phytochemistry 9: 33 (1970).
7. BURGER, W. C., PRENTICE, N., and MOELLER, MARY. Peptide hydrolase C in germinating barley. Plant Physiol. 46: 860 (1970).
8. PRENTICE, N., BURGER, W. C., and MOELLER, MARY. Activity patterns of three peptide hydrolases and an amidase during malting and brewing. Cereal Chem. 48: 587 (1971).
9. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. AACC Approved methods (formerly Cereal laboratory methods, 7th ed.). The Association: St. Paul, Minn. (1962).

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