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ENZYMATIC CHANGES AND AMYLOPLAST DEVELOPMENT IN THE MATURING BARLEY GRAIN

C. M. DUFFUS

Department of Agricultural Biochemistry, School of Agriculture, University of Edinburgh, West Mains Road, Edinburgh 9, Scotland

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Abstract—The changes in activity of various enzymes during maturation of the barley grain were recorded. Enzymes assayed were glyceraldehyde-3-phosphate:NAD and :NADP oxidoreductases, malate:NAD and :NADP oxidoreductases glyoxalate:NAD and :NADP oxidoreductases, acid phosphatase, fumarase, catalase and peroxidase. The results were correlated with structural studies of amyloplast development.

INTRODUCTION

LITTLE is known of the biochemical changes which take place in the developing cereal grain. The gross changes in the major storage materials such as protein, lipid and carbohydrates have been described by Jennings and Morton.[1] The ultrastructure of the developing wheat[2] and barley[3] endosperm has been described in detail by Buttrose. Marrè[4] has reviewed the maturation process in the castor bean seed and interpreted the course of differentiation at a molecular level by discussing the changes in enzyme activity which take place during seed development. It was found that most of the enzymes involved in basic metabolism (e.g. malate dehydrogenase, fructose-1,6-diphosphatase, aldolase, etc.) followed a similar pattern of activity throughout maturation. Thus activity increased rapidly during seed development to a maximum when maximum fresh weight was first reached, and thereafter decreased to the low levels characteristic of the mature seed. Maturation is characterized by the conversion of sugar to lipid and by the synthesis of reserve protein and phytin and these changes were correlated with the changes in enzyme activity.

The main emphasis in this paper is on the changes in enzyme activities taking place during maturation of barley seeds and correlation of the results with the development of amyloplasts.

RESULTS

Peroxidase and Catalase (Donor: H_2O_2 Oxidoreductase EC 1.11.1.7 *and H_2O_2:H_2O_2 Oxidoreductase EC* 1.11.1.6)

Figure 1 shows the variation in extractable peroxidase and catalase with age of whole grain. Activity of both enzymes remains low throughout the first 10–12 days of maturation after which it rises sharply to a maximum value around 15–20 days after anthesis. Activity then falls off rapidly with increasing maturity of the grain.

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[2] M. S. BUTTROSE, *Australian J. Biol. Sci.* **16**, 305 (1963).
[3] M. S. BUTTROSE, *J. Ultrastruct. Res.* **4**, 231 (1960).
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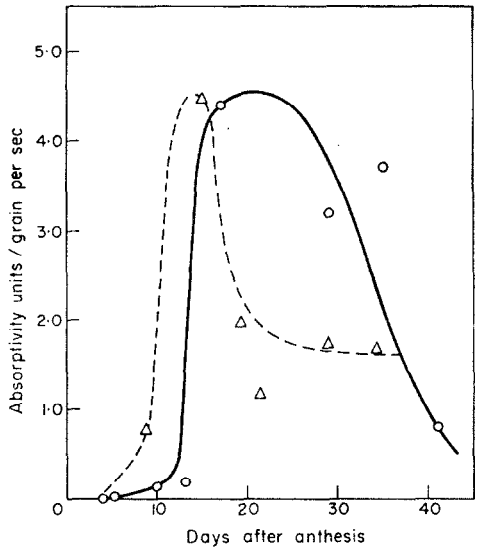

FIG. 1. CHANGES IN PEROXIDASE (○—○) AND CATALASE (△---△: VALUES $\times 10^3$) ACTIVITY DURING MATURATION OF WHOLE GRAIN.

Glyceraldehyde-3-phosphate NAD and NADP Dehydrogenases (D-*Glyceraldehyde-3 Phosphate:NAD Oxidoreductase* (*Phosphorylating*), EC 1.2.1.12 *and* D-*Glyceraldehyde-3-phosphate:NADP Oxidoreductase* (*Phosphorylating*), EC 1.2.1.13)

The activity of the NAD enzyme rises steadily throughout the early stages of maturation, increasing rapidly to a maximum about 20 days after anthesis and falling off rapidly there-

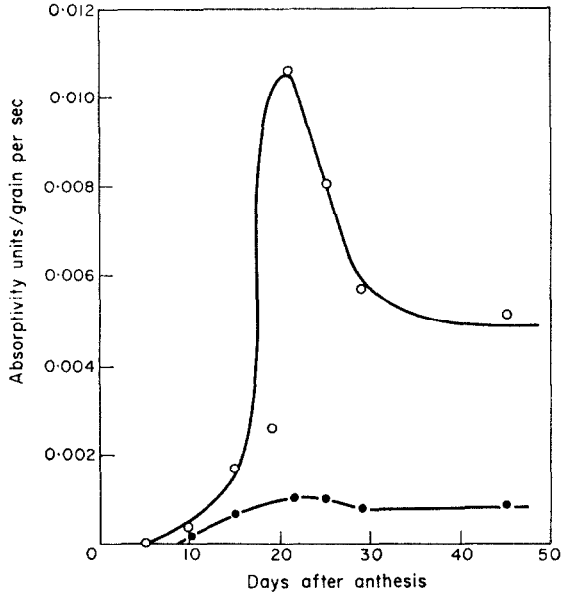

FIG. 2. CHANGES IN GLYCERALDEHYDE-3-PHOSPHATE NAD (○—○) AND NADP (●—●) DEHYDROGENASES ACTIVITY DURING MATURATION OF WHOLE GRAIN.

after (Fig. 2). The NADP enzyme has a much lower activity and shows little variation throughout the maturation period.

Malate NAD and NADP Dehydrogenases (L-*Malate*:*NAD Oxidoreductase*, EC 1.1.1.37 *and* L-*Malate*:*NADP Oxidoreductase*)

Results are similar to those for the glyceraldehyde-3-phosphate dehydrogenases except that the peaks of activity are rather later in the time scale (Fig. 3). Again the NADP enzyme is present but the activity is very low.

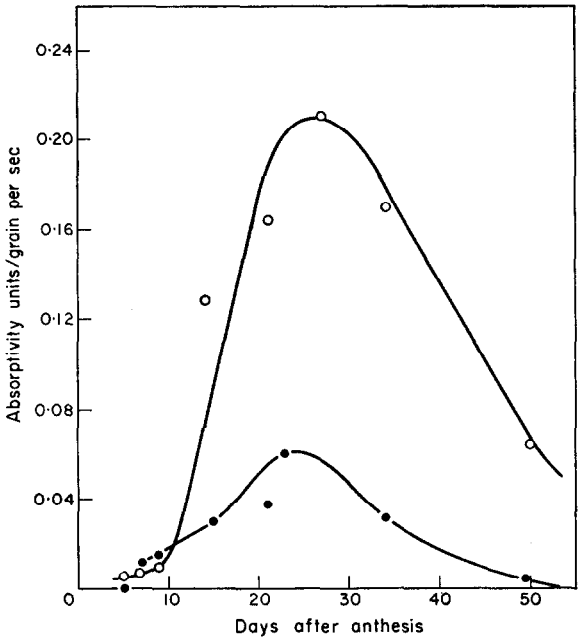

FIG. 3. CHANGES IN MALATE NAD (○—○) AND NADP (●—●: VALUES ×10²) DEHYDROGENASES ACTIVITY DURING MATURATION OF WHOLE GRAIN.

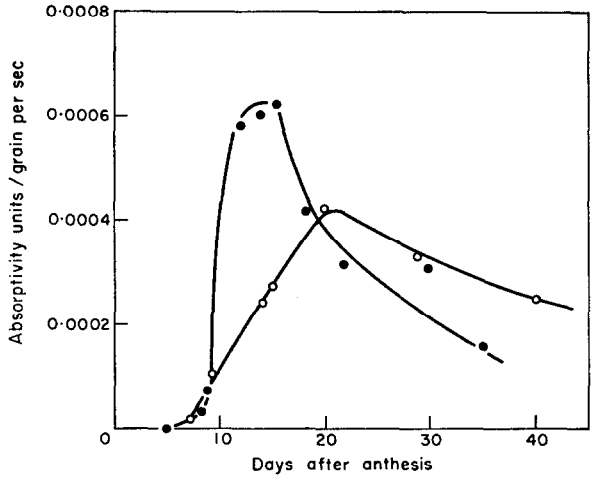

FIG. 4. CHANGES IN GLYOXALATE NAD (○—○) AND NADP (●—●) DEHYDROGENASES ACTIVITY.

Glyoxalate NAD and NADP Reductases (*Glycollate:NAD Oxidoreductase*, EC 1.1.1.26 *and Glycollate:NADP Oxidoreductase*)

Again, both NAD and NADP enzymes are present (Fig. 4), in this case with similar (and low) activities. The maximum for the NADP enzyme occurs slightly earlier in the maturation period and falls off more rapidly than that for the NAD enzyme.

Acid Phosphatase (*Orthophosphoric Monoester Phosphohydrolase* EC 3.1.3.2)

Activity rises steadily throughout maturation with a maximum value (Fig. 5) around 25–30 days after anthesis.

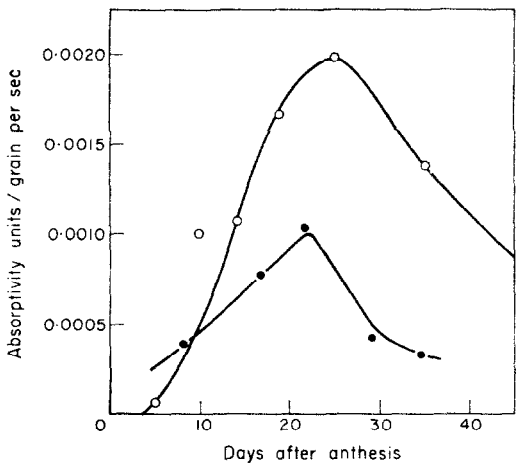

FIG. 5. CHANGES IN ACID PHOSPHATASE (○—○) AND FUMARASE ACTIVITY (●—●) DURING MATURATION OF WHOLE GRAIN.

Fumarase (L-*Malate Hydro-lyase*, EC 4.2.1.2)

Activity rises slowly to a maximum around 21 days after anthesis and decreases steadily throughout the later stages of maturation (Fig. 5).

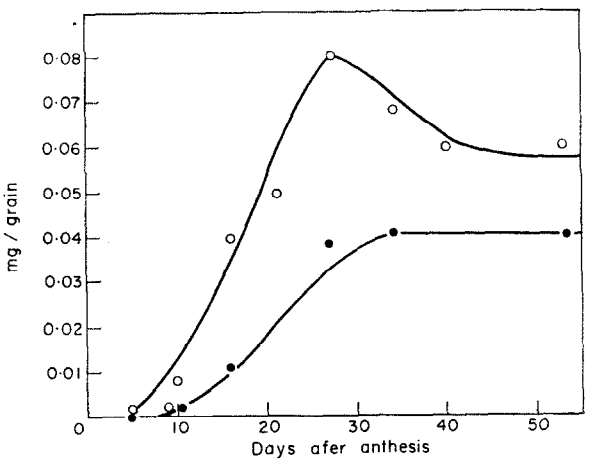

FIG. 6. CHANGE IN FRESH (○—○) AND DRY (●—●) WEIGHT DURING MATURATION OF WHOLE GRAIN.

Fresh and Dry Weights

The results (Fig. 6) are very similar to those for developing whole wheat grain.[1] Dry weight reaches a plateau around 30 days after anthesis and fresh weight a maximum value around 25 days after anthesis.

Light Microscopy

Starch-containing plastids could be seen in the barley grain from 5 days after anthesis. During the period 5–21 days after anthesis the starch granules grow rapidly to fill the plastid and any internal structures previously visible become obscured. The size of the starch granule reached a maximum around 21 days after anthesis. In addition to the large amyloplasts, a considerable number of smaller starch granules could be seen.

DISCUSSION

The ultrastructure of the developing barley grain has been described by Buttrose.[3] The proplastids of the young endosperm, from which amyloplasts subsequently develop, were seen as early as 5 days after anthesis. Invaginations into the plastid stroma parallel to the inner membrane illustrate their similarity to the normal chloroplast. Initiation of starch granules in proplastids was observed to continue for about 14 days after anthesis.

Light microscopy was used in the present case to follow the gross detail of amyloplast development throughout grain maturation and the results agreed substantially with those described above. The smaller starch granules were also described by Buttrose[3] and are derived apparently from the amyloplast itself.

The period of decay of proplastid internal structure coincides with the rapid increase in activity of the two enzymes catalase and peroxidase. Perhaps both enzymes are products of this plastid disintegration. One consequence of increased peroxidase activity may be a marked decrease in indole acetic acid concentration.[5] That this may have an important effect on enzyme levels in the endosperm has been shown by MacLeod and Palmer.[6]

The development of plastids into starch-accumulating particles or amyloplasts is accompanied by a rapid increase in the enzymes of carbohydrate metabolism, glyceraldehyde-3-phosphate NAD and NADP dehydrogenases. Again, the maximum in activity coincides with the maximum size of the amyloplast. While both enzymes may be concerned with hexose synthesis the NADP enzyme has been assigned to a primarily photosynthetic role.[7] Smillie and Fuller[8] found it only in green plants and in oxygen evolving photosynthetic organisms. Although it does have a broadly similar pattern of activity over the period of maturation, this activity is much less than that for the NAD enzyme. The latter enzyme is thought to participate in the dark reactions of photosynthesis,[8] possibly by means of an NADPH transhydrogenase, and certainly about 30 per cent of the whole cell activity of spinach leaves has been localized in the chloroplast.[9]

Alternatively the NAD enzyme may be involved in glycolysis. The results agree well with those of Hageman and Arnon[10] for pea seeds and leaves who found that the developing seed had much less NADP glyceraldehyde-3-phosphate dehydrogenase than NAD enzyme. On the other hand the same enzymes in green leaves had almost equal activities.

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[6] A. M. MacLeod and G. H. Palmer, *New Phytol.* **68**, 295 (1969).
[7] G. A. Hudock and R. C. Fuller, *Plant Physiol.* **40**, 1205 (1965).
[8] R. M. Smillie and R. C. Fuller, *Biochem. Biophys. Res. Commun.* **3**, 368 (1960).
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[10] R. H. Hageman and D. I. Arnon, *Arch. Biochem. Biophys.* **57**, 421 (1955).

Lipid is present in most of the tissues of the barley grain and it has been shown[1] that endosperm lipid content increases during the first 4 weeks of wheat grain maturation. It is probable that the malic dehydrogenase and glyoxalate reductase are concerned in pathways of lipid metabolism. Glyoxalate NADP reductase has been shown to be localized in leaf chloroplasts[11] and malate NADP dehydrogenase is probably present there also.[12] A scheme for acetyl CoA synthesis in plastids involving both these enzymes has been proposed by Stumpf *et al.*[13] Part, at least, of the NADH malic dehydrogenase and of the NADH glyoxalate reductase is present in plastids and therefore may be also associated with the amyloplast. The increase in the amount of lipid formed probably correlates with the increase in the number of intracellular membranes abundant in endosperm cells during maturation.[3]

That a reducing environment, conducive to lipid and, indeed, carbohydrate synthesis, may prevail in the maturing grain is borne out by the decrease in activity of the mitochondrial enzyme fumarase throughout the later stage of maturation. Certainly, in developing castor bean endosperm—a lipid synthesizing tissue—mitochondrial enzyme activity decreases from the onset of the constant fresh weight phase.[4] A supply of reduced nucleotides should then be available for both lipid and carbohydrate synthesis.

It will be noted that the peak of activity for NADPH glyoxalate reductase is somewhat earlier in the time scale than that for the NADH enzyme and corresponds roughly in time to the period of rapid increase in catalase and peroxidase activity. This may be correlated with a switch from purely plastid lipid synthesis to intracellular membrane synthesis.

The presence of various hydrolytic enzymes other than phosphatase, of which α-amylase is one, has been demonstrated in the developing grain.[14,15] The activity of such enzymes increases rapidly during germination and contributes to the mobilization of storage material. Their significance to the developing grain is obscure. It may be that, like α-amylase, phosphatase activity is controlled by gibberellic acid during the maturation process.

EXPERIMENTAL

Plant Material

The two-row barley, *Hordeum distichum* (L.) Lam. cv. Maris Baldric, was used. The conditions of growth and the method used to determine the date of anthesis were as described by Merritt and Walker.[16] Grain could be stored for periods of up to 2 months at −12° without loss of enzymatic activity.

Extraction of Enzymes

Enzymes were extracted using a glass homogenizer followed by brief centrifugation as previously described.[15] The extraction medium was the buffer used in the subsequent assay systems.

Enzyme Assays

Enzymes were assayed using Unicam SP500 (single beam) and SP800 (double beam) spectrophotometers. Catalase,[17] peroxidase[18] (using *O*-dianisidine as hydrogen donor), acid phosphatase,[19] glyceraldehyde-3-

[11] C. M. THOMPSON and C. P. WHITTINGHAM, *Biochim. Biophys. Acta* **143**, 642 (1967).
[12] M. D. HATCH and C. R. SLACK, *Biochem. Biophys. Res. Commun.* **34**, 589 (1969).
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phosphate NAD and NADP dehydrogenases,[10] malate NAD and NADP dehydrogenases,[20] glyoxalate NAD and NADP reductases,[21] and fumarase[22] were assayed by standard techniques. Activities were expressed in absorptivity units per sec per grain.

Light Microscopy

Samples for examination were homogenized gently in M NaCl and filtered through two layers of muslin. The solutions were examined using a light microscope equipped with phase contrast (magnification × 1000). The preparations were stained for starch when necessary with I_2/KI.

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I also wish to thank Dr. Trevor Walker of the Scottish Plant Breeding Station, Pentlandfield, East Lothian, for his kindness in supplying the plant material.

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