BIOSYNTHESIS AND MODIFICATIONS OF α- AND β-AMYLASES IN GERMINATING WHEAT SEEDS

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1. Introduction

Considerable changes occur in the enzyme composition of wheat seeds during the developmental processes of germination [1a, b]. The increases in α- and β-amylase activities are particulary striking [2]. The increase in β-amylase activity seems to be due primarly to activation of the bound form of the enzyme existing in ungerminated seeds, but the possibility remains that part of the enzyme could be concurrently synthesized *de novo.*

Evidence for induced enzyme synthesis can be demonstrated by combining the technique of labelled amino acid incorporation into proteins with those of enzyme purification and equilibrium sedimentation [3–4]. By combining the techniques of labelled amino acid incorporation into proteins and immunoelectrophoresis, it was our aim to investigate further the occurrence of α-amylase and the increase in free β-amylase in wheat during germination and to determine to what extent the increases were due to *de novo* synthesis or precursor activation.

In this paper, we show that the increase in α-amylase activity is attributed to *in vivo* synthesis of its two isoenzymes. In contrast, the modifications of β-amylase, antigenically identified, in ungerminated seeds are not due to synthesis of new enzyme molecules but are assigned to the activation of a preexisting enzyme precursor.

2. Materials and methods

Thirty seeds of *Triticum vulgare* var. *Capelle* were washed in ether, dried and soaked in 5 ml water containing 10 μCi each of ^{14}C-labelled lysine, glycine, leucine and phenylalanine. The seeds were placed on filter paper soaked in water containing the labelled amino acids. Seven days later, 10 seeds excised of shoots and roots were ground in a mortar in one ml veronal buffer 0.05 M pH 8.2 as used in the subsequent electrophoresis. At the same time, 10 ungerminated wheat seeds were extracted in the same manner with 1.3 ml of the buffer. The extracts were centrifuged ½ hr at 18,000 *g* and dialysed against the veronal buffer to remove free amino acids and small peptides.

Immunoelectrophoresis according to Grabar and Williams [5] and amylase characterization after immunoelectrophoresis were performed according to techniques previously described [6]. The autoradiograms were obtained by exposing the films for one month directly against the dried gel. The following immune sera were used: 1) An anti-ungerminated wheat seed proteins previously prepared [6]; 2) An anti-barleymalt α-amylase. (This latter serum can be used for the wheat enzyme since identical reactions are obtained between α-amylases of germinated barley and wheat seeds using such immune sera [7]); and an anti β-amylase prepared with the purified wheat en-

zyme. (This purification involved ammonium sulfate precipitation, DEAE cellulose chromatography and G-100 Sephadex gel filtration).

3. Results

The changes occurring in wheat amylases during germination are reported in fig. 1. The immune sera were selected to detect proteins corresponding to the enzymes under investigation from the other proteins of the extracts. The enzyme-antibody precipitin bands corresponding to the amylases studied were confirmed by enzymatic characterization reactions made directly on the immunoelectrophoretic patterns.

These immunoelectropherograms demonstrate the presence of isoenyzme systems for β-amylase in resting seeds, and α-amylase in germinating seeds. Further studies on the nature of these two isoenzymic systems are still underway. The changes occurring in the electrophoretic mobility of the β-amylase during germination are obvious. The α-amylase patterns are characterized by a lack of reaction between the anti α-amylase immune serum with the proteins extracted from ungerminated seeds. Similar experiments for β-amylase show that the enzyme fraction extracted from resting seeds with only reducing agents (mercaptoethanol 0.3%) is antigenically identical to the enzyme extracted with saline solutions. No detectable protein, antigenically related to the α-amylase characterized in the germinated seeds, could be extracted from ungerminated seeds by reducing agents.

The immunoelectropherograms of proteins extracted from both ungerminated seeds and from seeds grown on labelled amino acid solutions and their corresponding autoradiograms are reported in fig. 2.

Radioactivity is detected in the precipitin bands corresponding to the two α-amylase isoenzymes but not for the band corresponding to the β-amylase. On the α-amylase pattern there is also a third constituent nearest the anode, which is only detected on the autoradiogram. Until direct enzymatic characterization can prove that this weak constituent contains amylase activity, it cannot be ascertained whether or not this represents a third isoenzyme of the α-amylase. The comparative immunoelectropherograms using immune serum to the proteins of ungerminated seeds reflect the changes, persistance and disappearance of proteins already present in the resting seeds [6]. The radioactivity is detected in only a few of the precipitin bands.

4. Discussion

The increasing α-amylase activity in wheat seems to be controlled by gibberellin [8]. In barley it is now established that the gibberellin evoked α-amylase is produced by *de novo* synthesis [4]. This may suggest that the increase in α-amylase activity in cereals during germination could be generally ascribed to synthesis of the enzyme. The radioactivity detected in the precipitin bands corresponding to the two α-amylase isoenzymes demonstrates that at least part of the molecules of both isoenzymes have been synthesized *de novo*. Moreover, if an inactive precursor of the enzyme

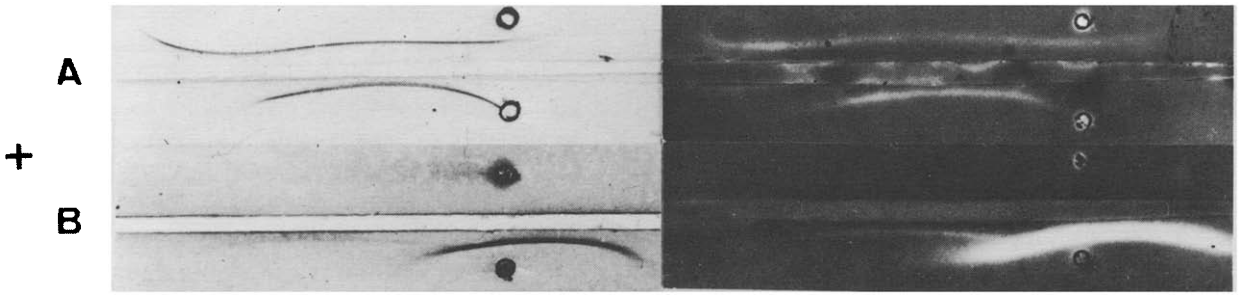

Fig. 1. Immunoelectropherograms of wheat proteins extracted from ungerminated seeds (wells above the canals) and from germinated seeds (wells below the canals). Immune sera used in the canals were: A anti β-amylase, B anti α-amylase, Left: Protein stained pattern. Right: amylase characterized pattern.

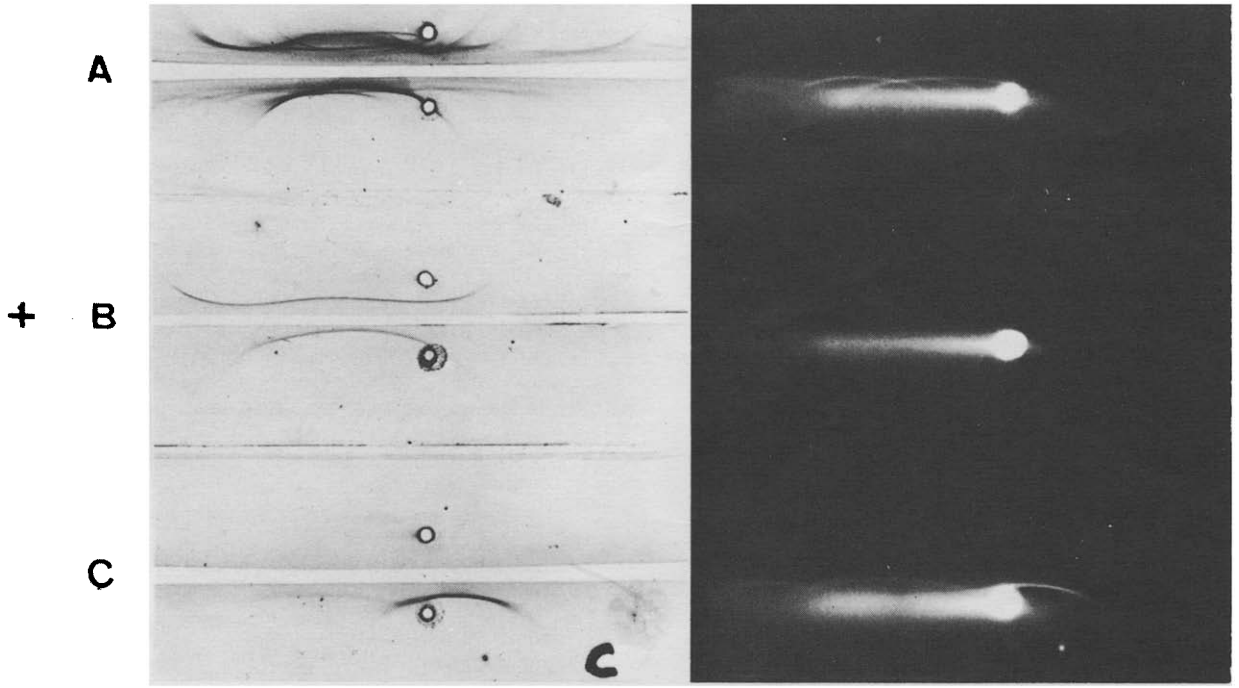

Fig. 2. Immunoelectropherograms of proteins extracted from ungerminated seeds (wells above the canals) and from germinated seeds (wells under the canals).
Immune sera used: A: anti proteins extracted from ungerminated wheat seeds
B: anti β-amylase
C: anti α-amylase
Left : protein stained pattern.
Right: autoradiogram.

was present among the proteins extracted from ungerminated seeds, its structural analogy with the enzyme would have caused it to react with the anti α-amylase immune serum, as found for several other precursors and their corresponding anti-enzyme immune sera [9a,b]. The lack of reactivity between the immune serum and the proteins extracted from ungerminated seeds, therefore indicates that there is no precursor of the enzyme among these proteins. This plus the fact that no α-amylase-like proteins have been detected in a bound form in ungerminated seeds, as for β-amylase, indicates that most if not all of the two α-amylase isoenzymes have been synthesized and do not result from activation of a precursor. The localization of the site of synthesis of the two isoenzymes remains to be determined. It is worth noting that α-amylase is not the only protein to be synthesized during germination. This is shown by radioactivity detected in other precipitin bands corresponding to some of the other proteins already present in the resting seeds.

β-Amylase seems to be subjected to another kind of modification during germination. In resting wheat seeds, part of the enzyme which is not extractable with saline solutions is bound by disulfide bridges to the glutenin, an insoluble wheat protein [10a, 10b]. The increase in β-amylase activity during germination is generally ascribed to a progressive activation of the bound enzyme, but the question still remains whether part of the increase could be due to a concurrent synthesis of the enzyme. Although labelled leucine and glycine represent the most important amino acids in wheat β-amylase composition [11], no incorporation could be detected in the enzyme characterized by the

precipitin reaction with the antibodies (fig. 2B). It seems therefore that the modifications of this enzyme occurring during germination do not include new synthesis of a fraction of the enzyme.

The immunochemical protein characterization proved useful for the identification of the eventual precursor of an enzyme. This method, in combination with the *in vivo* incorporation of labelled amino acids into proteins, has demonstrated the capacity of these techniques to detect synthesis of definite constituents in protein mixtures. They may provide information concerning eventual simultaneous activation of a precursor and the synthesis of an enzyme; they therefore represent a useful tool in the study of protein turnover and enzyme induction in plants.

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