IMMUNOCHEMICAL COMPARISONS OF α-AMYLASES IN DEVELOPING AND GERMINATING WHEAT SEEDS

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1. Introduction

α-Amylase activity undergoes drastic changes during the life of the wheat seeds: being high at an early stage of development, this activity drops rapidly during maturation and finally goes strikingly up again during germination [1–4]. The question arises whether the enzymes involved in these two distant life periods of the seeds are structurally identical or different.

Immunochemical methods with antibodies specific for the α-amylase of germinated seeds have been used to provide evidence that the α-amylase activity detected in maturing seeds is due to enzymes antigenically different from those synthesized during germination.

2. Materials and methods

Wheat seeds (*Triticum vulgare cultivar* Rex) were removed from the plants grown on fields at different times after anthesis and freeze-dried.

Carefully washed mature seeds were germinated at room temp on filter paper deposited on water moistened cotton. 7 days later, seeds excised of shoots and roots were freeze-dried. The length of the shoots reached 3–5 cm.

The freeze-dried developing and germinated seeds were ground and 300 mg flour were extracted with 1 ml 0.05 M veronal buffer pH 8.2 containing 0.2 M NaCl and 0.001 M $CaCl_2$. The extracts were centrifuged 15 min at 38,000 *g*, dialysed against the veronal buffer containing 0.001 M $CaCl_2$ and centrifuged again.

α-Amylase activity was measured in these extracts according to a diffusion method in agar gel containing soluble starch preincubated with β-amylase [5, 6]. Equal volumes of undiluted or diluted extracts were deposited in wells cut in the agar-starch gels (0.8% preincubated starch, 1.2% ionagar pH 5.7). After 24 hr incubation the gels were stained with an iodine solution. The diameter of the white circles occurring on a red stained background is proportional to the log concentration of α-amylase [5, 6]. The results are expressed in net diameter of the spot (diameter of the total circle–diameter of the well). The anti-α-amylase immune serum used in this study is specific for α-amylases extracted from the germinating wheat seeds [7].

In order to compare the absorption capacity of the anti-α-amylase immune serum on both types of seed extracts, different dilutions of the anti-α-amylase immune serum were deposited in wells cut in the starch-ionagar gel; after the serum was sucked in by the gel, the antigen solution i.e. extracts of germinating and maturing seeds, was deposited in these wells. After diffusion and staining, the remaining α-amylase activity detected indicates the action of enzymes which have not been precipitated by the antibodies previously deposited in the wells.

For controlling the specific action of the anti-α-amylase immune serum, parallel experiments were

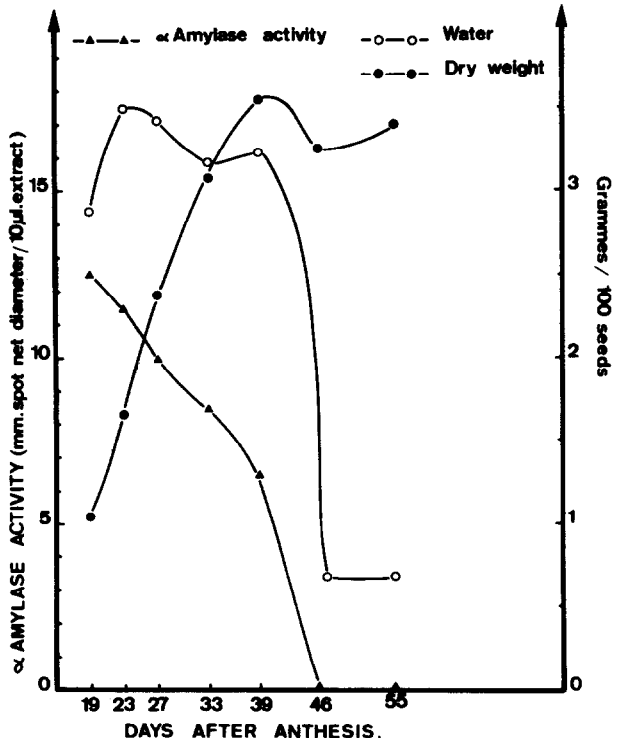


Fig. 1. Evolution of humidity and dry weight of 100 seeds picked up at different steps of maturation. Evolution of α-amylase activity in extracts of 300 mg freeze-dried seeds picked up at different steps of maturation. α-Amylase activity is expressed in mm of net diameter of reaction spot/10 µl of extracts/24 hr incubation.

conducted using another immune serum (anti plant tumor proteins) which does not contain any anti-α-amylase antibodies.

3. Results

α-Amylase activity is detected in extracts of seeds taken at an early stage of development. The activity decreases in the extracts as ripening develops and drops rapidly when the seeds reach their minimal water content (fig. 1).

This α-amylase activity is not high compared to the one found in the extracts of germinated seeds: the activity measured in the extract of seeds taken 19 days after anthesis lies between 3% and 5% of the activity measured in 7 days germinated seed extract.

The absorption of α-amylase enzymes with the anti-α-amylase immune serum in the extract of germinated seeds results in the inhibition of nearly all α-amylase activity (middle part of fig. 2A). The comparison of this part of the figure and the upper part of fig. 2A shows that the activity remaining in the extract after absorption with the 4-fold diluted immune serum is smaller than the activity in the 0.05 concentration of the initial extract. In other words, this immune serum concentration removes more than 95% of the α-amylase activity. Furthermore, increasing amounts of immune serum cannot absorb completely the α-amylase activity: comparison of the upper part and the middle part of fig. 2A shows that activities remaining in the extract after absorption with the twice diluted and the undiluted immune serum lie below the concentration 0.025 and slightly above the concentration of 0.01 of the initial extract; moreover, both activities are similar. Consequently, if more than 98% of the activity in the extract of germinated seeds can be removed with the anti-α-amylase immune serum, it appears nevertheless that a small part of the activity cannot be absorbed with this immune serum. This inhibition is due to the specific antibodies since the other immune serum, not specific for the α-amylase, does not modify the value of the net diameter of the reacting spot (lower part of fig. 2A).

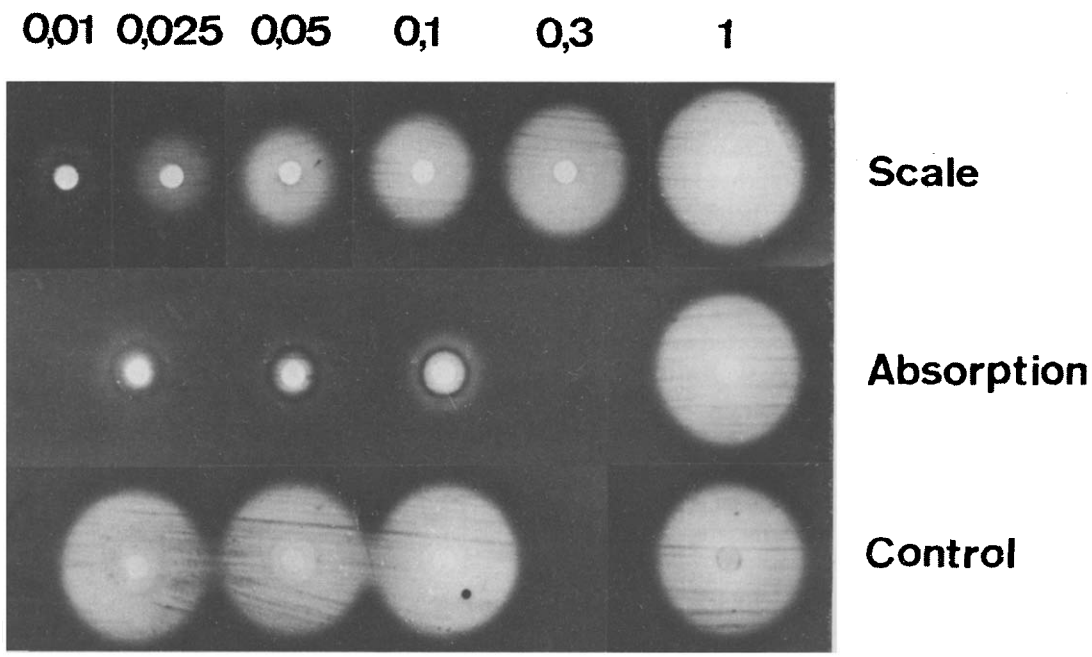
The absorption of α-amylase of developing seeds with the anti-α-amylase immune serum does not affect the amylase activity in this extract (upper part of fig. 2B).

These experiments demonstrate that antibodies which react with enzymes bearing more than 98% of the α-amylase activity in extracts of 7 days germinated seeds do not react with α-amylases present in extracts of seeds taken 19 days after anthesis.

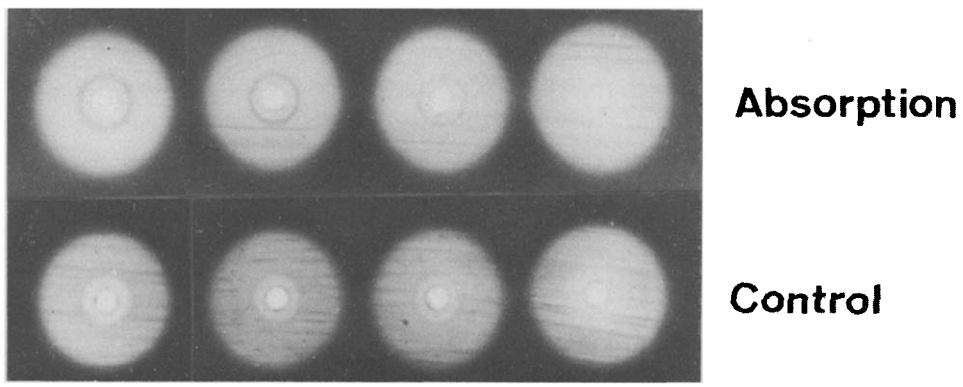
Further experiments conducted with extracts of seeds taken 23 days and 27 days after anthesis provide similar results.

4. Discussion

α-Amylases in crude water extracts of developing and germinating seeds display differences in electrophoretic mobilities [8]. This result does not necessarily imply that distinct molecular species are involved in these two extracts: in fact, such differences occur-



A



B

Fig. 2A. α-Amylase activity in extracts of 7 days germinating seeds before and after immunoabsorption. The initial extract was first diluted 15-fold for these experiments. Scale: α-Amylase activity of different dilutions of this extract. Aliquots of this extract were diluted in order to get a scale ranging from this concentration (1), on the right, to a 100-fold smaller concentration (0.01) on the left. Absorption: α-Amylase activity after absorption with the anti-α-amylase immune serum. From the left to the right the 4 wells were first filled with, respectively, undiluted, twice diluted, four-fold diluted immune serum, and veronal buffer. After the solution had been sucked in by the gel, the wells were filled again with the extract (concentration 1 of the scale). Control: α-Amylase activity after absorption with an immune serum not specific for α-amylase. The dispositions of the serum and the extract are identical to the disposition depicted for the preceeding part of the figure. Fig. 2B. α-Amylase activity in extracts of developing seeds picked up 19 days after anthesis before and after immunoabsorption. The initial extract was not diluted for these experiments. Absorption: α-Amylase activity after absorption with the anti-α-amylase immune serum. From the left to the right the 4 wells were first filled with, respectively, undiluted, twice diluted, four-fold diluted immune serum, and veronal buffer. After the solutions had been sucked in by the gel, the wells were filled again with the extract. Control: α-Amylase activity after absorption with an immune serum not specific for α-amylase. The dispositions of the immune serum and the extract are identical to the disposition depicted for the preceeding part of the figure.

ring during germination and detected on wheat β-amylases have been ascribed to modifications concerning already existing proteins rather than to disoccurrence of an enzyme population and synthesis of a different new one [7]. Nevertheless, α-amylase occurring in germinating wheat seeds has been proved to be synthesized during germination [7]. Therefore it appears that at least part of the constituents of α-amylase occurring in developing and germinating seeds concerns different molecular species.

The absorption experiments reported in this study provide evidence that the enzymes synthesized during germination structurally differ from the α-amylase detected in developing seeds and precise that more than 98% of the activity detected in germinating seeds is borne by the new protein types. The nearly 2% remaining activity in extracts of germinated seeds after immunoabsorption may be ascribed to enzymes of the developing period still existing at this germination step. Nevertheless, it cannot be excluded that this residual activity is due to the limit of the absorption technique used. This question calls for further studies.

Studies on barley α-amylase indicated that the mechanism governing α-amylase synthesis in the maturing seeds is similar to that in germinating seeds and is controlled by gibberellic acid [9]. If this is also true for the wheat enzymes one could consider that the hormonal regulation of the α-amylase remains the same during these life periods of the seeds but concerns different loci of the genome.

Acknowledgement

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