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BRIEF COMMUNICATION

α- and β-Amylases in Seed Germination

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Abstract. During the first 24 h of germination of wheat seeds, starch is hydrolysed by free β-amylase. In the next 24 h, some amount of inactive form of β-amylase is converted into active form and this together with α-amylase synthesized *de novo* brings about the hydrolysis of starch. The amount of α-amylase is greater in seeds with embryo intact than with embryo excised after 24 h hydration. However, at later stages of seed germination α-amylase becomes predominant and the activity of β-amylase steadily diminishes.

Excellent reviews on the metabolism of germinating seed have appeared in recent years (Briggs 1973, Ching 1972 and Koller 1972), but it is rather surprising that very little information exists as regards mobilization of reserve food. In cereals, starch is hydrolysed by α- and β-amylases during seed germination. Whereas α-amylase is reported to be synthesized *de novo* through the mediation of embryo (Paleg 1960, Varner and Chandra 1964, and Chrispeels and Varner 1967), β-amylase is present in seeds in latent form (Pollock and Pool 1958). As knowledge of the mobilization of starch is likely to provide some leads to better understanding of the physiology and biochemistry of seed germination, investigations were undertaken on seeds with embryo both intact and excised for the study of activity of α-amylase, β-amylases (free and bound), the level and the rate of release of zymogen (bound form of β-amylase) and the level of maltose in germinating wheat seed.

Material and Methods

One hundred and sixty uniform healthy seeds of wheat cv. S-308 were surface sterilized and germinated on filter paper in Petri-dishes (10 cm dia) under controlled temperature condition at 28 ± 1 °C in complete darkness. These seeds were divided into two lots. Each lot was further divided into 8 groups of 10 seeds each. The seeds of lot 1 were allowed for germination while the seeds of lot 2 were deembryoed after 24 h water soaking. Five seeds from each group were sampled out for enzyme study while the remaining

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5 seeds were used for recording germination ability. Amylase activity in the germinating as well as in deembryoed seeds was determined after every 24 h interval.

Washed seeds were homogenised at 4 °C and the crude enzyme was extracted in sufficient amount of phosphate buffer (0.02 M, pH 6.9) and the final volume made up to 25 ml. Five ml of cell extract was subjected to direct estimation of β-amylase activity, while in another 5 ml, 1 ml of 0.2 M mercaptoethanol (MCE) was added and the total β-amylase activity (free + + latent) determined following the method as described by MIGIBAYASHI and SHINKE (1967). Another 5 ml of cell extract was subjected to temperature treatment (70 °C for 15 min) to denature β-amylase (KRUGER and TRACHUK 1969) and the activity of α-amylase determined from the β-amylase free cell extract following the method of BERNFELD (1955). The zymogen content was determined by subtracting the values of free β-amylase activity from the total β-amylase activity. Values are expressed as units per mg of maltose as determined by 3,5-dinitrosalicylic acid reagent. In each case the absorbance was measured with a Spectronic-20 photocolorimeter at 540 nm against blanks of starch and starch + MCE separately, and the values were calculated from a standard curve of maltose and maltose + MCE, respectively.

Results

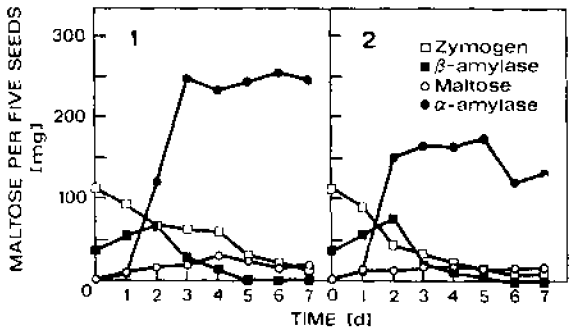
All seeds with embryo intact germinated. α-amylase was altogether absent in wheat grain, but after 1 day there was very little activity of this enzyme and it increased markedly to about 12 fold after 2 days (Figs. 1, 2). The activity of α-amylase further increased appreciably after 3 days in seeds where the embryo was left intact (Fig. 1) whereas in deembryoed seeds it did not increase much (Fig. 2). The level of α-amylase activity was much higher in embryoed seeds in comparison to deembryoed seeds at 3 days and subsequently (Figs. 1, 2). In contrast to α-amylase, β-amylase activity was observed even in seeds at zero h which increased slightly after 1 and even 2 days but decreased appreciably after 3, and 4 days, the activity being even lower than the initial values of zero h (Figs. 1, 2).

Zymogen content in wheat grain at zero h was quite high and goes on depleting with time, the decrease being more marked during the first 2 days. It is noteworthy that at that stage β-amylase activity was also at its highest peak. However, it is intriguing to note that while zymogen content continues depleting even after 2 days, the β-amylase activity goes on declining at later stages of seed germination (Figs. 1, 2).

The level of maltose in seeds at zero h was very low. It increased steadily till the 4th day but remained almost unchanged after that till the 7th day (Figs. 1, 2).

Discussion

The results presented in this paper are indicative of the fact that in the first 24 h period of seed germination, starch is hydrolysed by free β-amylase. In the next 24 h the activity of free β-amylase increases because of solubilization of a part of the zymogen. Simultaneously, a part of starch is also hydrolysed by α-amylase, which is now freshly synthesized. At later stages



Figs. 1, 2. The level of zymogen, β-amylase maltose and α-amylase in germinating seeds with embryo intact (Fig. 1) and in seeds with embryo removed after 24 h hydration with water (Fig. 2). All seeds with embryo intact germinated.

of germination the activity of α-amylase become prominent and more so in seeds with embryo than in those with excised embryo. The activity of β-amylase starts diminishing after 2 days and continues to do so still later. α-amylase is synthesized *de novo* as a function of the embryo in germinating seeds, as has already been reported by many workers (PALEG 1960, VARNER and CHANDRA 1964). It would, therefore, appear that in variant II where the embryo was removed after 24 h water soaking, the amount of α-amylase synthesis was low as compared to variant I where the embryo was left intact. The results presented in this paper are also in conformity with the work of ROWSELL and GOAD (1964a, b), who reported that β-amylase is present in starchy endosperm in free as well as in bound forms and the solubilization of zymogen is the function of hydrolytic enzymes, *e.g.* proteases. Further, it appears that at later stages of seed germination some factor is produced which inhibits the activity of β-amylase. Work is in progress in this laboratory to elucidate this point.

References

BERNFELD, O.: Amylases, α- and β. — In: COLOWICK, S. O., KAPLAN, N. O. (ed.): Methods in Enzymology. Vol. 1. P. 149. Academic Press, New York 1955.

BRIGGS, D. E.: Hormones and carbohydrate metabolism in germinating cereal grains. — In: MILBORROW, B. V. (ed.): Biosynthesis and its Control in Plants. Pp. 219—277. Academic Press, New York 1973.

CHING, T. M.: Metabolism of germinating seeds. — In: KOZLOWSKI, T. T. (ed.): Physiological Ecology. Pp. 103—205. Academic Press, New York 1972.

CHRISPEELS, M. J., VARNER, J. D.: Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. — Plant Physiol. **42** : 398—406, 1967.

KOLLER, D.: Environmental control of seed germination. — In: KOZLOWSKI, T. T. (ed.): Physiological Ecology. Pp. 2—93. Academic Press, New York 1972.

KRUGER, J. E., TRACHUK, R.: Wheat Alpha-amylases. 1. Isolation. — Cereal Chem. **46** : 219 to 226, 1969.

MIGIBAYASHI, N., SHINKE, R.: Barley and malt amylase XIII. Reconsideration on the determination method of total β-amylase in barley and types of zymogen β-amylase in barley. — Nippon Nogeikagaku Kaishi **41** : 295—303, 1967.

PALEG, L. G.: Physiological effects of gibberellic acid: 1. On carbohydrate metabolism and amylase activity of barley endosperm. — Plant Physiol. **35** : 293—296, 1960.

POLLOCK, J. R. A., POOL, A. A.: J. Inst. Brew. **64** : 151—156, 1958 cited in Briggs, D. E., 1973.

ROWSELL, E. V., GOAD, L. J.: Some effects of gibberellic acid on wheat endosperm. — Biochem. J. **90** : 11P, 1964a.

ROWSELL, E. V., GOAD, L. J.: The release of hydrolytic enzymes from isolated wheat aleurone layers activated by gibberellic acid. — Biochem. J. **90** : 129, 1964b.

VARNER, J. E., CHANDRA, G. R.: Hormonal control of enzyme synthesis in barley endosperm. — Proc. nat. Acad. Sci. U.S.A. **52** : 100—106, 1964.