The Effect of Deuterium Oxide on the Gibberellicacid-induced α-Amylase in Germinating Barley Seeds *

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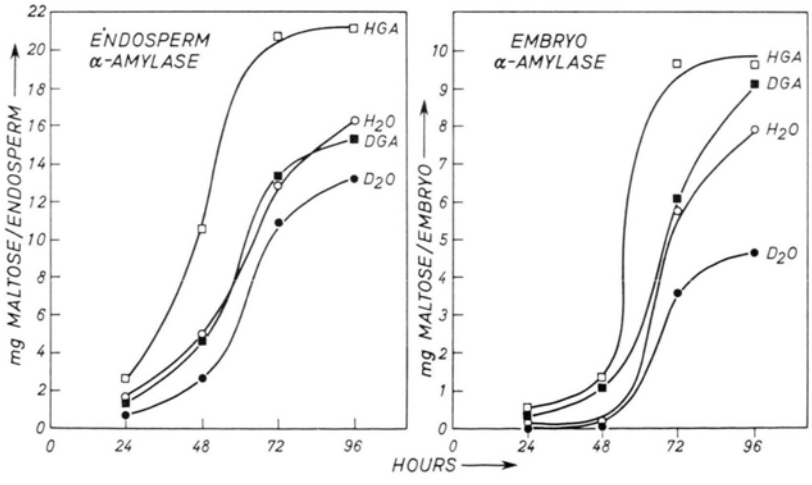
During the course of our investigations on the effect of D_2O on the germination of barley, we have observed that the formation of sugars from starch is highly retarded at all the concentrations of D_2O [6]. Although, earlier reports [1-3] do not indicate any significant retardation of amylase activity by D_2O, subsequent studies [4, 5] on isotope rate ratio for porcine pancreatic α-amylase suggest that D_2O influence the rate of action of this enzyme. It was therefore thought worthwhile to investigate the effect of D_2O on amylase activity in germinating barley seeds variety Himalaya. The modifying influence of Gibberellic acid (GA) on α-amylase of normal seeds as well as embryoless seed-halves, incubated in various concentrations of D_2O and GA, was also studied.

Results and Discussion

Figs. 1 and 2 respectively show the β- and α-amylase activity in endosperm and embryo at various stages of germination in H_2O and 50% D_2O. β-amylase (Fig. 1) is not considerably affected as compared to α-amylase (Fig. 2) and the slight differences in β-amylase activity between treatments unlike that of α-amylase activity, remain more or less constant throughout the experimental period. It is now well established tha β-amylase is already present in the dormant barley seeds while amylase is synthesized *de novo* [8, 9]. It is therefore, possible that the marginal differences in β-amylase (Fig. 1) activity between the H_2O and D_2O treatments noted here, could be explained on the basis of isotope effect on the rate of reaction. However, in the case of α-amylase (Fig. 2) it appears that D_2O interferes with its synthesis. This could be due to either insufficient supply of GA or inhibition of response to GA. GA in 1.45×10^{-4} M concentration could nullify the inhibitory influence of 50% D_2O completely both in the embryo and the endosperm. Thus, the compensating effect of GA tends to support the former possibility viz., insufficient GA supply.

In another experiment, the effect of different concentrations of GA and D_2O on embryoless seed-halves was examined. The data in table 1 show that (i) even without GA the seed-halves show α-amylase activity which is markedly inhibited by 100% D_2O but only slightly by 50% of D_2O and (ii) GA at 10^{-6} M proved optimal in H_2O as well as in 50% D_2O and at 10^{-4} M in 100% D_2O.

Thus it is apparent (table 1) that there exist in the embryoless seed-halves precursors which are not de-



Fg. 1. Change in the β-amylase activity of endosperm and embryo during germination of barley in H_2O and 50% D_2O with or without 1.45×10^{-4} M GA; HGA and DGA respectively representing H_2O and D_2O in the presence of GA.

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[1] M. L. CALDWELL and S. E. DOEBBELING, J. biol. Chemistry **123**, 479 [1938].
[2] M. L. CALDWELL, S. E. DOEBBELING, and F. C. VON WIEKELIN, J. Amer. chem. Soc. **61**, 125 [1939].
[3] D. L. FOX, J. cellular comparat. Physiol. **6**, 405 [1935].
[4] M. FLASHNER and A. LUKTON, Biochim. biophysica Acta [Amsterdam] **146**, 596 [1967].
[5] D. E. KOSHLAND, JR., J. A. YANKEELOV, JR., and J. A. THOMA, Federat. Proc. **21**, 1031 [1962].
[6] M. K. BHANDARKAR, S. BHATTACHARYA (nee BOSE), and B. K. GAUR, Communicated to Physiol. Plantarum [Copenhagen] [1970].
[7] B. I. S. SRIVASTAVA and W. O. S. MEREDITH, Can. J. Bot. **40**, 1257 [1962].
[8] L. PALEG, Plant Physiol. **36**, 829 [1961].
[9] J. E. VARNER and G. RAMACHANDRA, Proc. nat. Acad. Sci. USA **52**, 100 [1964].

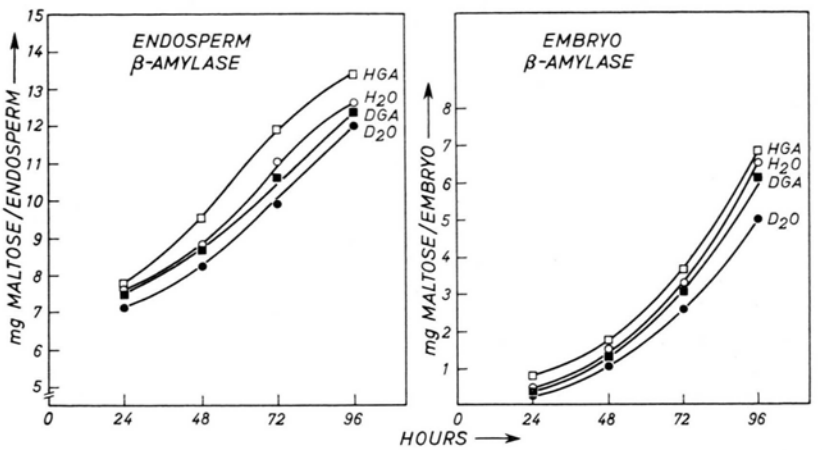

Fig. 2. Change in the α-amylase activity of endosperm and embryo during germination of barley in H_2O and 50% D_2O with or without 1.45×10^{-4} M GA; HGA and DGA same as in Fig. 1.

Treatment	O.D. at 510 mμ			
	— GA	10^{-1} M GA	10^{-1} M GA	10^{-1} M GA
H_2O	0.26	0.41	0.27	0.18
50% D_2O	0,21	0.35	0.33	0.26
100% D_2O	0.11	0.25	0.29	0.35

Table 1. Effect of various concentrations of GA on α-amylase induction in H_2O and D_2O treated seeds. Embryoless seed-halves were sterilized with 1% Na-hypochlorite and washed with sterile water; 5 of them were transferred into each vial containing 1 ml of incubating media (H_2O, 50% D_2O, 100% D_2O with or without GA, 100 μg streptomycin, 10 mM $CaCl_2$). α-amylase was measured [7] after incubation for 72 hours at 30 °C. The value are an average of 2 different experiments.

pendent on GA for their conversion to active enzymic form. It has been proposed that this α-amylase synthesis and/or release is dependent on the imbibition level of seeds [10]. Further it has already been shown that seeds soaked in D_2O do not reach a complete state of hydration even after 24 hours [11, 12].

In the light of these findings it is likely that D_2O has interfered in the hydration, the effect being concentration dependent thus impairing GA-independent α-amylase synthesis. Coincidentally, the GA concentration inducing maximum synthesis of α-amylase in D_2O (100%) happens to be same as earlier found to bring about maximum D_2O absorption by seeds [12]. This would suggest that GA might in part be overcoming the D_2O effect by enhancing the imbibition. On the other hand, the finding that α-amylase synthesis, inhibited by D_2O, could be overcome by increasing concentrations of exogenous GA further supports our assumption that there is a lowered availability of endogenous GA in presence of D_2O.

It is inferred that D_2O-induced inhibition of α-amylase may be due to reduction in the imbibition ability of seeds as well as in the supply of GA necessary for *de novo* synthesis of this enzyme.

[10] K. H. YUNG and J. D. MANN, Plant Physiol. **42**, 195 [1967].

[11] S. BHATTACHARYA (nee BOSE), M. K. BHANDARKAR, and B. K. GAUR, Physiol. Plantarum [Copenhagen] **22**, 1025 [1969].

[12] M. K. BHANDARKAR, M. Sc., thesis, University of Bombay, India, 1967.