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NOTE

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Types of Amylases in Rice Grains

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Amylases in cereal grains have recently been elucidated not to concern *de novo* synthesis of starch during ripening, but to play an important role in decomposing starch and supplying energy necessary for germination of cereal grains.[1~2] Compared with numerous investigators of amylases in barley, wheat and other cereal grains,[3] very few have been concerned with those in rice grains. It was reported during 1930's that there were 3 kinds of amylases in rice grains, the starch-liquefying, the dextrin-forming and the sugar-forming enzymes.[4] These different enzymes were considered to correspond to 3 stages of starch decomposition; liquefaction, dextrinization and saccharification. However, extensive investigations thereafter have shown that both liquefaction and dextrinization are catalyzed by one enzyme (α-amylase) and saccharification, by another (β-Amylase).[5~7] Since then rice grains have generally been conceded to contain α- and β-amylase.

Our recent work on cereal amylases have been focused on those in rice grains and revealed that there was no measurable α-amylase activity in ungerminated rice grains. This is clearly inconsistent with the above conception of amylases in rice grains. The present paper deals with types of amylases in ungerminated and germinated rice grains.

Enzymic assays essentially followed the methods described by Kneen and Sandstedt for α-amylase,[8] and by Bernfeld for β-amylase.[9] Various types of inactive β-amylases (zymogen β-amylases) were assayed after the treatment of activation with 0.1 M mercaptoethanol and 0.1% papain as detailed in the previous paper.[10]

Table I shows the amounts of amylases in various brown rice samples. As clearly seen, α-amylase activity could not be detected in all the samples tested. This fact may add an experimental support to the view that ungerminated cereal grains generally contain no α-amylase activity.[11] At the same time it may not be neglected that there are some reports on α-amylase of cereal grains which appears at earlier stages of ripening and disappears rapidly.[12~14] The fate of the enzyme is, however, still an open question.

As also shown in Table I, the brown rice samples were found to contain both active and zymogen β-amylase activities, which were different with the samples tested. Total β-amylase, salt-soluble and salt-insoluble zymogen β-amylases, papain-soluble and papain-insoluble zymogen β-amylases were measured by the same methods that were used for those in barley and

TABLE I. DISTRIBUTION OF VARIOUS TYPES OF AMYLASES IN RICE GRAINS

Variety of rice	α-Amylase	Total β-amylase	Active β-amylase	Salt-soluble Z-β-A	Salt-insoluble Z-β-A	Papain-soluble Z-β-A	Papain-insoluble Z-β-A
Takanenishiki	—	12.2	1.7	4.8	5.9	2.6	3.3
Yamadanishiki	—	13.8	3.0	1.9	8.9	4.0	4.4
Gohyakumangoku	—	11.4	3.3	3.2	4.9	4.6	1.2
Sasanishiki	—	12.6	2.1	1.9	8.9	4.3	4.4
Fujiminori	—	12.2	1.5	5.0	5.7	3.0	2.6
Kinmaze	—	14.8	2.3	5.1	7.4	3.3	5.0
Nihonbare	—	12.4	1.9	5.5	5.0	2.7	2.5
Honenwase	—	12.8	2.0	5.7	5.1	4.2	1.8
Fukunohana	—	13.2	2.6	6.6	4.0	2.0	2.2
Yamahoshi	—	13.2	3.6	1.6	7.7	5.1	2.7
Akebono	—	13.4	2.5	4.1	6.8	4.0	2.9
Kinki-33	—	16.4	1.9	3.3	11.2	5.0	6.9
Yoneshiro	—	13.4	2.2	2.0	9.2	4.3	4.9
Shiokari	—	11.0	1.4	2.5	7.1	3.0	4.2
Shinsenbon	—	11.2	2.6	3.4	5.2	3.0	2.0

β-Amylase activity was expressed in terms of maltose mg liberated in 3 min at 30°C by 1 g of gound rice samples. Total β-amylase activity consists of active β-amylase, salt-soluble zymogen β-amylase, papain-soluble and papain-insoluble zymogen β-amylase activities. Salt-insoluble zymogen β-amylase activity consists of papain-soluble and papain-insoluble zymogen β-amylase activities.

wheat grains. The results obtained were found to be very similar to those in the cases of barley and wheat grains.[2]

Figure 1 A shows a gel filtration pattern of protein extract from ungerminated rice seeds. β-Amylase activity was found in three peaks (I, II and III). No α-amylase activity was detected in any of the peaks. This is quite in agreement with the results in Table I. Figure 1 B also shows a gel filtration pattern of protein extract from the germinated seeds. It should be noted from Figs. 1 A and 1 B that germination resulted in

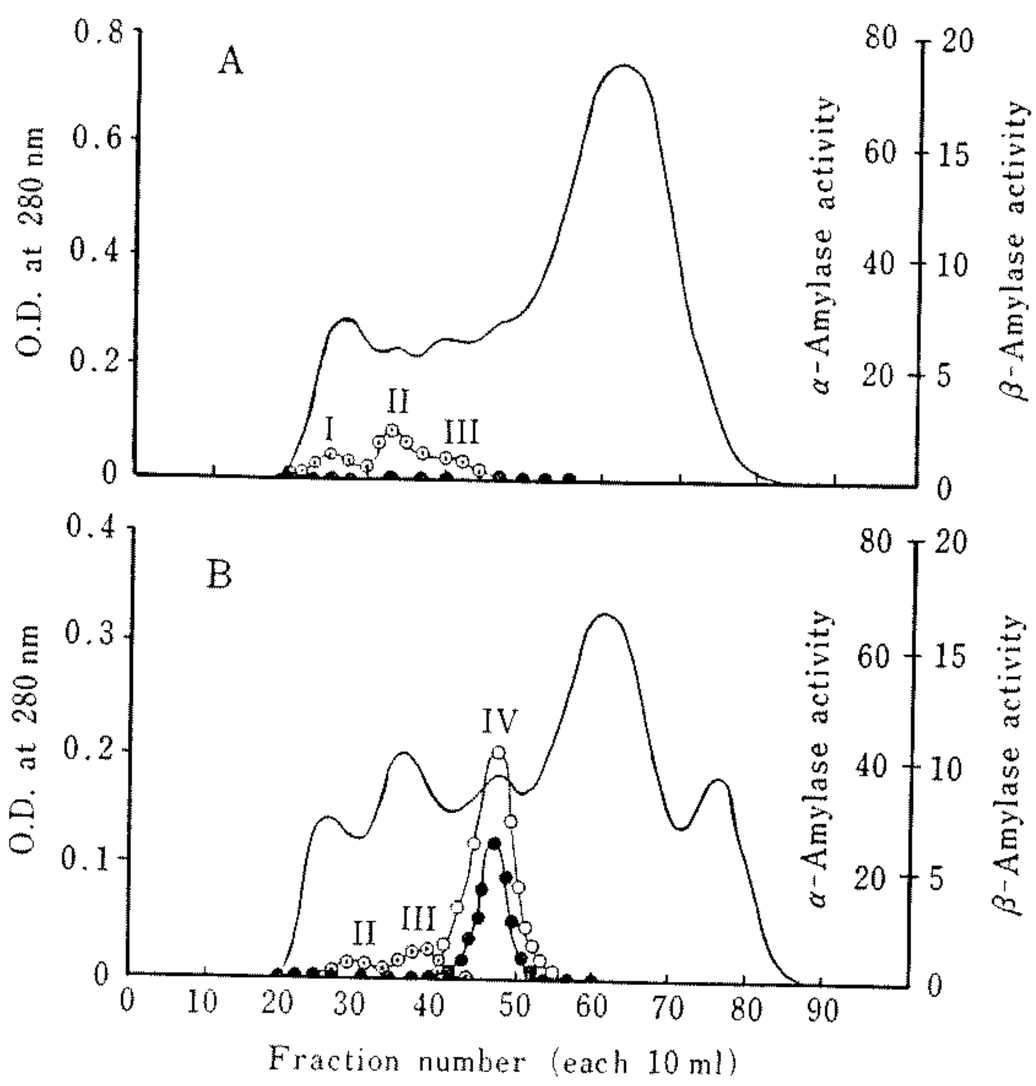

FIG. 1. Gel Filtration of Protein Extract from Ungerminated and Germinated Rice Seeds.

A: Ungerminated rice seeds. B: Germinated rice seeds.
A hundred g of ungerminated rice seeds (Takanenishiki, non-glutinous) was extracted with 500 ml of 5% potassium sulfate. The extract was saturated with ammonium sulfate up to 70%. The precipitate obtained was dialyzed against 1/15 M phosphate buffer (pH 7.0) and 30 ml of the dialyzate was loaded on the Sephadex G–75 column (4×45 cm) and eluted with the same buffer. The same procedure was carried out with the germinated sample.
——, Protein concentration; ●—●, α-amylase; ○—○, active β-amylase; ⊙—⊙, salt-soluble zymogen β-amylase.

disappearance of peak I and in appearance of a new peak (IV). Enzymic assay showed that peak IV contained α-amylase, active β-amylase and no zymogen β-amylase. Peaks I, II and III were also found to consist of salt-soluble zymogen β-amylases of different molecular weight and to be activated into active β-amylase by mercaptoethanol and papain *in vitro* and by germination *in vivo*. These fractions of zymogen β-amylases, as seen in the cases of salt-soluble zymogen β-amylases in barley,[10] had a small amount of saccharogenic activities without the treatment of activation. Accordingly, each activity of active β-amylase in Table I may correspond to the total saccharogenic activity of salt-soluble zymogen β-amylases without the treatment of activation.[15]

The results reported in this paper are of interest in two ways. In the first place, we find it difficult to avoid the conclusion that ungerminated rice grains contain only β-amylase like other cereal grains and that α-amylase appears remarkably in germination. Secondly, types of β-amylases in ungerminated and germinated rice grains are similar to those in other cereal grains. Further investigation of amylases in rice grains is now in progress and comparison of their enzymic properties with those of other cereal grains will appear elsewhere.

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