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STUDIES ON BARLEY AND MALT AMYLASES

Part XX. Comparison of Wheat Zymogen β-Amylase with Barley Zymogen β-Amylase*

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Abstract

Four types of wheat zymogen β-amylase, salt-soluble and salt-insoluble zymogen β-amylase, papain-soluble and papain-insoluble zymogen β-amylase, were confirmed in wheat by the same methods as employed in the studies on barley and malt amylases. From ground wheat salt-soluble zymogen β-amylase was isolated by gel filtration. The isolated zymogen β-amylase (Fraction I) was found to be activated into active β-amylase with 2-mercaptoethanol and papain *in vitro* and by germination *in vivo* in the same manner as barley zymogen β-amylase (Fraction B). The molecular weight of Fraction I was estimated to be 160,000 which was similar to that of Fraction B. Changes of amylase activities during wheat germination with or without the gibberellic acid treatment were also found to be similar to those of barley amylases. The gibberellic acid treatment enhanced the activation of zymogen β-amylase into active β-amylase and *de novo* synthesis of α-amylase as in the case of barley amylases.

Introduction

As have been reviewed by GEDDES,[2] REED and THORN[11] and THOMA *et al.*,[18] researches on β-amylase (EC 3. 2. 1. 2. α-1, 4-glucan maltohydrolase) in wheat have indicated that the enzyme occurs both in active and inactive forms. The inactive form, to which we have applied a term zymogen β-amylase (Z-β-A) in a broad sense including latent or bound β-amylase,[8] occupies a large part of β-amylase in ungerminated wheat. Upon germination, the active form (A-β-A) increases remarkably by activation of Z-β-A or liberation of A-β-A.

Our recent studies on barley and malt amylases have revealed that there are at least four types of Z-β-A in barley and that two different mechanisms of activation, proteolytic activation and disulfide bond cleaving activation, work successfully in full activation of Z-β-A in barley.[15]

In order to compare wheat Z-β-A with barley Z-β-A, the present investigation was undertaken to confirm types of wheatZ-β-A, isolate salt-soluble Z-β-A from wheat and pursue changes of Z-β-A during wheat germination with and without the treatment of gibberellic acid (GA) using the same methods as employed in the studies on barley Z-β-A.

Materials and Methods

Wheat samples. Seven varieties of wheat samples, two domestic and five imported samples were used in the experiments. Shirasagi wheat, one of domestic samples was harvested in Himeji district of Hyogo Prefecture in 1970.

Amylase assay. i) *α-Amylase.* One g of ground wheat or malt was extracted with 50 ml of 5 % potassium sulfate or 0.06 M phosphate buffer (pH 7.0), or 0.1 % papain. Each extract obtained was used as an enzyme solution. The enzyme assay was performed

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by the method of SANDSTEDT, KNEEN and BLISH.[14] ii) *β-Amylase.* Total β-amylase (T-β-A), A-β-A and four types of Z-β-A were measured as described previously.[16] Each activity was expressed in terms of maltose mg/one g or one grain of wheat samples. Corrections were also made for the effect of co-existent α-amylase in malt samples on β-amylase activity.

Chromatomatography of wheat and malt extracts. i) *Gel filtration on Sephadex G-75.* Sixty g of ground wheat or malt was extracted with 200 ml of 0.06 M phosphate buffer (pH 7.0) or with the same buffer containing 2 M urea. Each extract obtained was applied to Sephadex G-75 column (4x50 cm) and the elution was carried out in the same way as described previously.[16]

ii) *Ion exchange chromatography.* Twenty g of ground wheat or malt was extracted with 80 ml of 0.006 M phosphate buffer (pH 7.0) and centrifuged. Each extract was loaded on the DEAE-Sephadex A-50 column (2.8x 30 cm). The elution was performed as described previously.[16]

Wheat germination with the GA treatment. A suitable portion of Shirasagi wheat was steeped in tap water or 0.1 ppm GA intermittently for two or three days. After moisture of steeped grains was allowed to reach about 40 %, they were transferred to a germination box and germinated by timely sprinkling water or 0.1 ppm GA at 20°C for seven to eight days. A certain amount of wheat malt at various stages of germination was taken and dried at below 50°C, then ground for the enzyme extraction.

Estimation of molecular weight. Molecular weights of isolated enzyme fractions were estimated from the Andrews' plot in the same way as described previously.[17]

Results

Multiple forms of Z-β-A in wheat.

Types of Z-β-A in two domestic and five imported wheat samples were investigated by the same methods as employed in barley Z-β-A. The results obtained, as shown in Table 1, indicated that there were also four types of Z-β-A in wheat and that Manitoba No. 2 was rich in both T-β-A and salt-soluble Z-β-A while Shirasagi was poor in A-β-A, salt-soluble and papain-insoluble Z-β-A.

Chromatography of wheat extract on Sephadex G-75.

Ground wheat (Shirasagi and Manitoba No. 2) was extracted with 0.06 M phosphate buffer containing 2 M urea and the extract was subjected to gel filtration. As an elution pattern of Shirasagi wheat is shown in Fig. 1, two peaks of β-amylase (Fraction I and II) were detected. In the case of Manitoba No. 2, two peaks of β-amylase were also obtained.

Activation of Fraction I into Fraction II.

The Shirasagi extract was incubated with

Table I. Distribution of Zymogen β-Amylases in Wheat Samples

Wheat Samples	T-β-A	A-β-A	Salt		Papain	
			Soluble Z-β-A	Insoluble Z-β-A	Soluble Z-β-A	Insoluble Z-β-A
Shirasagi	20.35	8.70	2.10	9.60	3.90	0.30
Norin No. 61	20.95	11.85	2.50	5.95	2.50	1.00
Manitoba No. 2	38.35	19.20	4.00	16.80	3.05	2.15
America Hard Winter	27.15	12.50	4.35	11.15	3.00	1.90
America Western White	24.10	12.60	2.85	6.30	4.00	1.45
Australia Victoria	26.75	14.35	4.55	7.85	4.90	1.30
Western Australia	26.90	13.40	4.35	9.35	4.05	1.55

Note : each activity was expressed in terms of maltose mg/g.

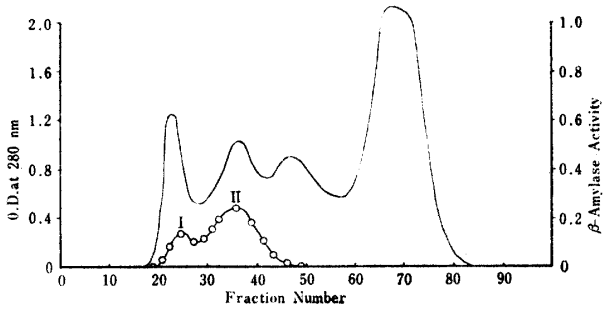

Fig. 1. Gel Filtration of 2 M Urea Extract from Shirasagi Wheat on Sephadex G-75.

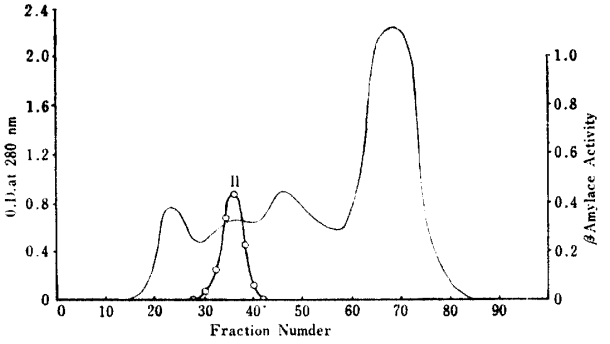

Fig. 2. Gel Filtration of Wheat Extract Reduction on Sephadex G-75.

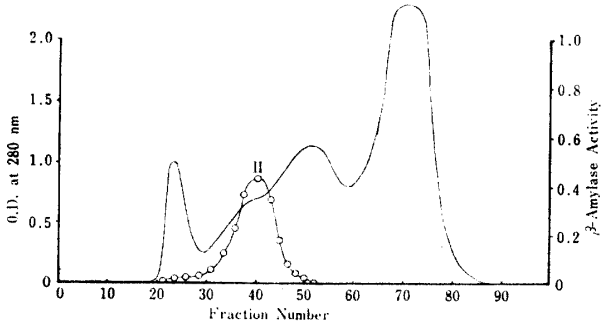

Fig. 3. Gel Filtration of Wheat Malt Extract on Sephadex G-75. Sample : Shirasagi wheat malt germinated for 8 days.
—— Protein concentration,
○——○ β-Amylase activity

0.2 M 2-mercaptoethanol (2-ME) containing 0.1% papain at room temperature for 24 hr. Then, it was loaded on the Sephadex column in the same way. As shown in Fig. 2, Fraction I was found to disappear to be activated into Fraction II. The similar results were obtained in the case of Manitoba No. 2. Furthermore, Fig. 3 shows an elution pattern of wheat malt extract (Shirasagi), indicating that Fraction I was activated into Fraction II during germination. In relation to the reversible transformation *in vitro* between Fraction B (a homopolymer type of Z-β-A) and Fraction C (a basic unit of A-β-A) of barley β-amylase,[17] Fraction II of wheat β-amylase was incubated with 0.3 M potassium ferricyanide or 0.01 M hydrogen peroxide at room temperature for 24 hr in the presence of 0.01 M EDTA and 2 M urea and subjected to gel filtration. The elution pattern showed that no clear transformation occurred from Fraction II to Fraction I.

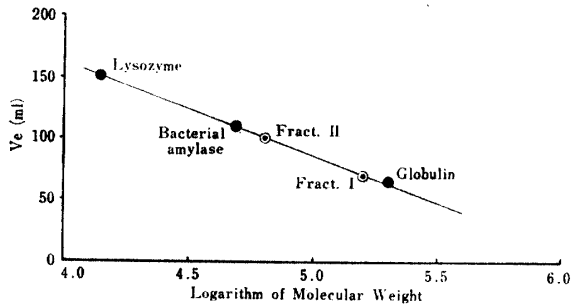

Fig. 4. Estimation of Molecular Weight of Wheat Amylases on Sephadex G-100.

Molecular weights of Fractions I and II.

Fractions I and II were isolated by repeating gel filtration on Sephadex G-75 and ion exchange chromatography on DEAE-Sephadex A-50 and concentrated by a collodion bag. Each fraction purified was loaded on the Sephadex G-100 column for estimation of the molecular weight. Fig. 4 shows an Andrews' plot of Fractions I and II, from which the molecular weight of Fraction I was estimated to be about 160,000 and that of Fraction II, to be about 64,000

Changes of wheat amylases during germination.

Fig. 5 shows changes of salt-soluble A-β-A and Z-β-A, α-amylase activities during germination with and without the GA treatment. α-Amylase which was not detected in ungerminated wheat, as seen in Fig. 5, increased remarkably with the advancement of germination days. Salt-soluble A-β-A also increased while salt-soluble Z-β-A decreased and disappeared in the 8-day or 10-day malt sample. The GA treatment clearly accelerated these changes of wheat amylases during germination.

Chromatographic behavior of salt-soluble

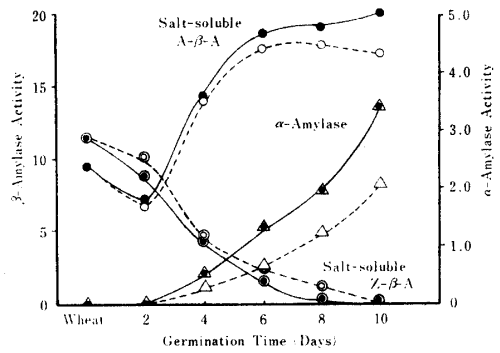

Fig. 5. Changes of Wheat Amylases during Germination with and without GA Treatment.

——— GA-treated ····· Untreated

wheat β-amylases.

Fig. 6 shows chromatography of wheat and malt extracts on the DEAE-Sephadex column. Two peaks of β-amylase were found in both wheat and malt samples. The first peak was considered to correspond to Fraction II and the second peak, to Fraction I of gel filtration (Fig. 1). However, all the activity of the second peak did not disappear during germination as in the case of Fraction I (Fig. 3), and A-β-A remained even in the 8-day malt both with and without the GA treatment. Therefore, the second peak was thought to be composed of both salt-soluble Z-β-A and A-β-A. The A-β-A of the second peak seemed to be chromatographically different from that of the first peak. Fig. 6 also shows that the GA treatment accelerated the activation of salt-soluble Z-β-A of the second peak into A-β-A of the first peak.

Discussion

Types of Z-β-A confirmed in wheat were essentially similar to those of barley Z-β-A.[15] However, the result obtained in the fractionation of wheat β-amylases were considerably different from those in barley β-amylases. Firstly, although three fractions of β-amylase (Fractions A, B and C) were obtained from the barley extract,[16] only two fractions (Fractions I and II) were done from the wheat extract (Fig. 1). A simple comparison among β-amylase fractions in barley and wheat suggests that Fraction I in wheat corresponds to Fraction B in barley and Fraction II in wheat, to Fraction C in barley. Figs. 2 and 3 also suggest that Fraction I is composed of salt-soluble Z-β-A which can be activated into A-β-A (Fraction II) with 2-ME and papain *in vitro* and by germination *in vivo*. Secondly, the treatment of Fraction II with oxidizing reagents did not form Fraction I although Fraction B in barley was polymerized from Fraction C *in vitro*.[17] Therefore, it seems that no reversible transformation between Fractions I and II occurs *in vitro* and that Fraction I is not such a homopolymer type of Z-β-A as Fraction B in barley. There are some papers to declare that wheat Z-β-A is a heteropolymer composed of A-β-A and wheat proteins like glutenin.[5,12,13] Thirdly, Fraction II in wheat was found to have the different molecular weight (64,000) from that of Fraction C (54,000) in barley, while Fraction I and Fraction B were found to have the same molecular weight (160,000). As reported previously,[16] Fraction B was recognized to be a trimer of Fraction C, but Fraction I may not be a trimer of Fraction II on the basis of calculation of the molecular weight. TKACHUK and TIPPLES[19] isolated three components of salt-soluble β-amylase from wheat flour and estimated their molecular weight all to be 64,200. This value is in good agreement with that of Fraction II isolated in our laboratory. Fourthly, there was no fraction in wheat corresponding to Fraction A (a heteropolymer type of Z-β-A) in barley whose molecular weight was 280,000.[16]

The results obtained in the pursuit of changes in wheat amylases were essentially similar to those in barley amylases. Although the presence of α-amylase has been reported during growth and maturation of wheat,[3,6,7] α-amylase was not detected in ungerminated wheat. The fate of α-amylase during maturation and dormancy of wheat is one of the interesting subjects to study in future. As seen in Figs. 5 and 6, the GA treatment enhanced α-amylase and A-β-A activities. The effect of GA on the cereal seed germination has been recognized as mainly to enhance hydrolytic enzyme activities.[10] FLEMING and JOHNSON[1] reported the presence of two GA-like substances in malted wheat and

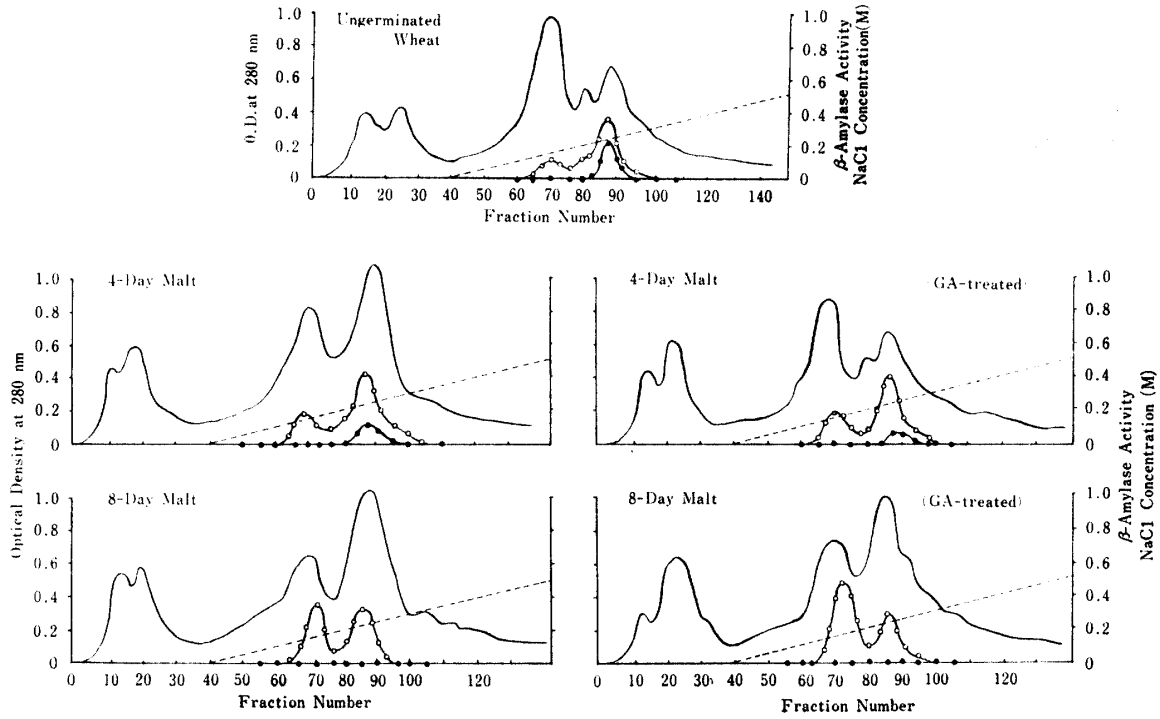

Fig. 6. Chromatography of Wheat and Malt Extracts on DEAE-Sephadex A-50.

Sample: Shirasagi wheat, ——— Protein concentration, ····· NaCl concentration O—O A-β-A, ●—● Z-β-A

their increase in quantity during the first three or four days of germination. This period of germination, as seen in Fig. 5, corresponds to that of rapid increase in α-amylase and A-β-A. The results in chromatography of wheat and malt extracts on DEAE-Sephadex A-50 indicated that there are multiple forms of A-β-A in wheat. In relation to some papers on multiple forms of cereal amylases,[4,9] further detailed studies on types of amylases in wheat should be necessary in future.

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麦芽アミラーゼに関する研究（第20報）

小麦 Zymogen β-amylase と大麦 Zymogen β-amylase の比較

新家　龍，西羅　寛

要　約

大麦 Zymogen β-amylase（Z-β-A）の研究に用いたと同様の実験方法により，小麦中のZ-β-Aについて研究した。まず，小麦中のZ-β-Aの存在形態について調べた結果，大麦中の場合と同様4つの型のZ-β-A，すなわち塩類可溶性および不溶性Z-β-A，パパイン可溶性および不溶性Z-β-A の存在が認められた。次に，粉砕小麦試料を2M尿素で抽出し，Sephadex G-75 カラムにてゲル濾過した結果，2つのアミラーゼ画分（Fraction I およびII）が得られた。Fraction I は2-メルカプトエタノールやパパインによつて，あるいは発芽現象によつて Fraction II に活性化されることなどから，Fraction I はZ-β-A 画分であり，Fraction II は活性 β-アミラーゼ画分であることを認めた。また，Fraction I はゲル濾過法による分子量が16万であり，大麦中の塩類可溶性 Z-β-A，Fraction Bに対応することがわかつた。さらに，小麦発芽中のアミラーゼの消長について調べた結果，大麦発芽中のそれとほぼ同じ結果が得られ，とくにジベレリン酸処理発芽において，Z-β-A の活性化および α-アミラーゼ合成の顕著な促進が認められた。