Production of Multiple Forms of Alpha-Amylase in Germinated, Incubated, Whole, De-embryonated Wheat Kernels[1]

B. A. MARCHYLO, J. E. KRUGER, and A. W. MacGREGOR

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ABSTRACT

α-Amylases produced in germinated wheat and incubated de-embryonated wheat kernels (*Triticum aestivum* cv. Neepawa), in the absence and presence of gibberellic acid (GA_3), were analyzed qualitatively by polyacrylamide gel isoelectric focusing (PAG-IEF) and quantitatively by chromatofocusing. α-Amylases resolved by PAG-IEF were classified into three groups, designated GIII (pI values closest to cathode), GI (pI values closest to anode), and GII (intermediate pI values). Quantitative analysis revealed that, in germinating wheat, in the absence or presence of GA_3, GIII components contributed the major proportion of activity, which decreased linearly with germination time concomitant with an increase in the proportion contributed by GI plus GII components. In the absence of GA_3, α-amylase production in incubated de-embryonated wheat was significantly lower, with the GI plus GII groups contributing the major proportion of activity. Incubation of de-embryonated wheat in the presence of GA_3 resulted in a substantial increase in α-amylase production primarily as GIII components. Relative proportions of components within the GIII and GI α-amylase groups were consistent throughout germination/incubation in the absence or presence of GA_3. These results in conjunction with results previously reported indicate that GA_3 differentially controls expression of the α-Amy-1 and α-Amy-2 genes, which code for production of the GIII and GI groups of α-amylase components in the aleurone.

Wheat α-amylase is of considerable practical importance because of its integral role in breadmaking. Although this enzyme is a necessary constituent of flour used in breadmaking, many well-documented problems are associated with the presence of excessively high levels (Bechtel et al 1964, Bloksma 1971). Elevated α-amylase levels in wheat arise primarily from preharvest sprouting (ie, germination). As a consequence, considerable research has been directed toward gaining a better understanding of α-amylase synthesis in wheat and toward characterizing wheat α-amylases.

α-Amylase synthesis in germinating wheat has been shown to be de novo (Daussant and Abbot 1969, Daussant and Corvazier 1970). The enzyme is produced in the aleurone (Rowsell and Goad 1964; Gibson and Paleg 1972, 1975), and there are indications that the scutellum also is a site of synthesis (Marchylo et al 1980a, Daussant et al 1982). Addition of gibberellic acid (GA_3) to germinating kernels (Jeffers and Rubenthaler 1974), to embryoless kernels (Khan et al 1973, Sargeant 1980, Marchylo et al 1981), and to isolated aleurone layers (Rowsell and Goad 1964, Jones 1973, Baulcombe and Buffard 1983) increases α-amylase production. α-Amylase produced during germination is heterogeneous. Early electrophoretic studies (Alexandrescu and Mihailescu 1970, Olered and Jönsson 1970) indicated that this enzyme is separable into two groups. Subsequent work revealed that these two groups are, in turn, heterogeneous (Kruger 1972; Sargeant and Walker 1978; Marchylo et al 1980b, 1981). The largest number of α-amylase components have been resolved by polyacrylamide gel isoelectric focusing (PAG-IEF) into two main groups with pI values of 6.0–6.5 and 4.5–4.8, respectively (Sargeant and Walker 1978; Marchylo et al 1980b, 1981; Sargeant 1980) and one minor group with pI values intermediate to those of the main groups (Marchylo et al 1981, Marchylo and Kruger 1983). Genetic analysis indicates that the two major groups are under independent genetic control, and the minor group appears to be controlled by the same loci as the pI 4.5–4.8 major group (Gale 1983).

Little is known about the synthesis of individual α-amylase components in germinating wheat. Qualitative PAG-IEF analysis of α-amylase produced in germinating kernels and incubating de-embryonated kernels has revealed that the relative proportion of activity contributed by the individual α-amylase groups was altered significantly in the de-embryonated kernel (Marchylo 1978, Marchylo et al 1981). Quantitative immunological analysis of wheat α-amylase produced during germination indicated that relative proportions of the two main groups changed with germination time (Sargeant 1980). The present study was undertaken to qualitatively and quantitatively follow the interrelationship between α-amylase components during germination of wheat kernels and incubation of de-embryonated wheat kernels in the absence and presence of GA_3.

MATERIALS AND METHODS

Preparation and Incubation/Germination of De-embryonated and Whole Wheat Kernels

Intact embryos were excised from wheat kernels (hard red spring wheat cv. Neepawa) after being soaked at room temperature (2 hr) in $10^{-3} M$ $CaCl_2$ with or without $10^{-4} M$ gibberellic acid (GA_3) to facilitate dissection as described previously (Marchylo et al 1981). Care was taken to ensure that all embryonic tissue (including the scutellum) was detached from the endosperm. Groups of 10 de-embryonated kernels were placed crease down into sterilized sand (2 g, contained in small plastic weighing boats) saturated with $10^{-3} M$ $CaCl_2$ with or without $10^{-4} M$ GA_3. The weighing boats were placed in petri dishes and incubated from one to six days in a germination cabinet maintained at 18°C and 96% relative humidity. The moisture level in the sand was maintained throughout the incubation period by periodic addition of water. Whole wheat kernels were soaked and germinated under identical conditions. Following incubation/germination, the petri dishes were sealed with tape and stored at −19°C for subsequent analysis. All incubation/germinations were duplicated.

Extraction

Ten kernel lots, including the sand on which they were incubated or germinated, were extracted as described previously (Marchylo and MacGregor 1983).

Column Preparation

Polybuffer exchanger™ 94[2] was equilibrated and packed as described previously (Marchylo and MacGregor 1983), with one modification. In this study, Pharmacia K 9/30 columns were used with bed heights of about 18 cm.

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[2]Polybuffer 94 is the exclusive trademark of Pharmacia Fine Chemicals AB, Uppsala, Sweden.

Sample Application and Elution

Extracts were applied and eluted as previously indicated (Marchylo and Kruger 1983, Marchylo and MacGregor 1983). Extract volume applied was dependent on the α-amylase activity of the sample and ranged between 1 and 6 ml.

α-Amylase Activity Analysis

Total α-amylase activity in extracts and eluent fractions were determined as described by Kruger and Tipples (1981) with a Perkin-Elmer model 191 Grain Amylase Analyzer. If required, extracts or fractions were diluted with 0.2M sodium acetate buffer (pH 5.5) containing $10^{-3}M$ $CaCl_2$ before activity analysis.

Isoelectric Focusing and Detection of α-Amylases

Extracts and eluent fractions were analyzed using premade thin-layer polyacrylamide gel plates (Ampholine PAG plate kits, pH 3.5–9.5, LKB Producter AB, Bromma, Sweden) as described previously (Marchylo and Kruger 1983, Marchylo and MacGregor 1983). Following isoelectric focusing, α-amylases were detected by a β-limit dextrin-plate technique (MacGregor et al 1974, Marchylo et al 1980b). Incubation times for the β-limit dextrin-polyacrylamide gel sandwich were varied according to the activity present in extracts and eluent fractions.

RESULTS

Production of α-Amylase in Germinating Wheat Kernels

Ten kernel lots of wheat (cv. Neepawa), germinated at 18°C from one to six days on moist sand in the absence and presence of gibberellic acid (GA_3), were extracted, assayed for α-amylase activity, and the time course increase in total activity plotted (Fig. 1A,C). Ungerminated kernels were assayed and found to contain negligible activity. In both the absence and presence of GA_3, α-amylase activity increased rapidly up to five days of germination, after which the rate of synthesis decreased. However, GA_3 induced production of larger quantities of α-amylase activity. Similar time course production of α-amylase in germinated wheat was reported previously (Khan et al 1973, Sargeant 1978, Marchylo et al 1981).

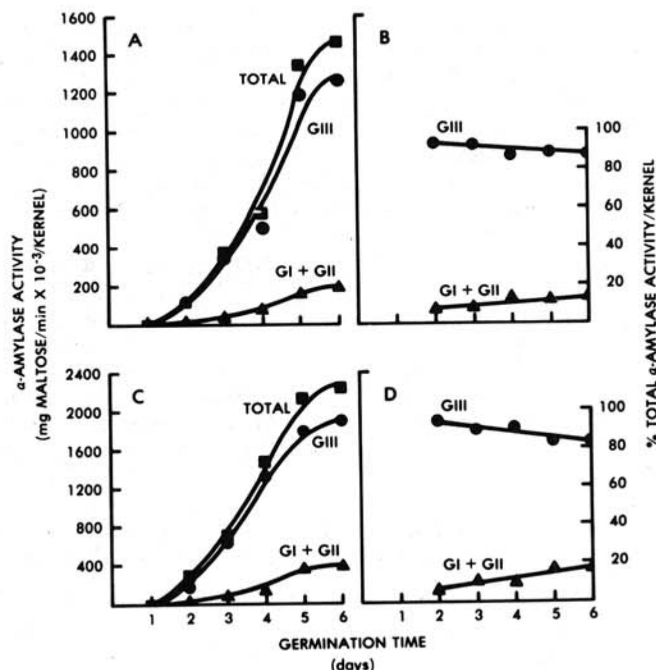

Fig. 1. A, C, Formation of α-amylase activity in germinated wheat. Activities of GIII and GI + GII groups of α-amylases were determined following chromatofocusing and were normalized to 100% recovery. **A** = in the absence of GA_3, **C** = in the presence of GA_3. **B, D,** Proportions of GIII and GI + GII groups of α-amylases relative to total GIII + GII + GI activity. GI, GII, and GIII α-amylases are as denoted in Fig. 2. **B** = in the absence of GA_3, **D** = in the presence of GA_3.

The difference in rates of production and degree of GA_3 induction observed were probably cultivar dependent.

The pattern of α-amylase components at each stage of germination was determined by polyacrylamide gel isoelectric focusing (PAG-IEF). Identical patterns of α-amylase components were obtained at each germination stage in the absence or presence of GA_3. Longer incubation time of the β-limit dextrin-polyacrylamide gel sandwich was required at early germination stages in order to detect all components. The lack of effect of GA_3 on α-amylase patterns has been reported also for germinating barley (MacGregor 1978). A zymogram of five-day germinated wheat α-amylase is shown in Fig. 2A as an example of the α-amylase patterns obtained.

For discussion purposes, α-amylase components were classified into three groups (GI, GII, and GIII) according to a previously described nomenclature (Marchylo et al 1980a, 1980b, 1981). Visual analysis of zymograms indicated that the major proportion of total α-amylase activity was contributed by GIII components with substantially lower levels present in the form of GI components. Only trace levels of GII components were visible. This technique, however, only represents a qualitative estimate of the activity associated with resolved α-amylase components (MacGregor and Daussant 1981). The recently developed chromatofocusing technique was used to fractionate the α-amylase and quantitatively determine the proportion of activity contributed by each α-amylase group. This is a column chromatographic technique that elutes proteins from an ion-exchange column in order of isoelectric pH (Pharmacia Fine Chemicals 1980; Sluyterman and Elgersma 1978; Sluyterman and Wijdenes 1978, 1981a, 1981b). Extracts from all germination stages were analyzed, with the exception of one-day germinated samples, which exhibited activity levels too low for meaningful analysis. Elution profiles were essentially the same for all samples with respect to the number of peaks resolved and elution pH. Slight differences in elution volumes were observed as a result of minor variations in pH gradient between analyses. However, all peaks focused at reproducible elution pHs as previously reported (Marchylo and Kruger 1983). Elution profiles obtained for five-day-germinated wheat, in the absence or presence of GA_3, are illustrated in Fig. 3A and B, respectively, as examples of typical profiles. Eight peaks of activity were resolved, with peaks 2 and 3 contributing the major proportion of activity.

Peak fractions were selected and subjected to PAG-IEF to determine the identity of α-amylase components resolved by chromatofocusing. All components present in original extracts were recovered following chromatofocusing as shown in Fig. 4 for five-day germinated wheat in the presence of GA_3. Peaks 1–3 were composed primarily of GIII α-amylase components; peak 4 represented GII components; peaks 5–8 were composed of GI components. The GIII and GI components were separated completely from each other, although some overlap of GIII and GII components was evident. Partial resolution of components

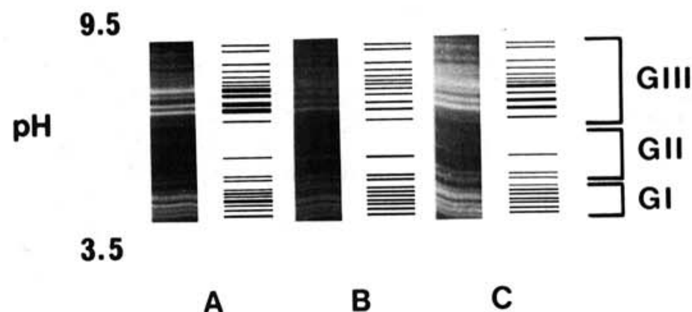

Fig. 2. Polyacrylamide gel isoelectric focusing, in a pH 3.5–9.5 gradient, of α-amylases extracted from: **A,** five-day-germinated wheat in the presence of GA_3; **B,** five-day-incubated de-embryonated wheat in the absence of GA_3; **C,** five-day-incubated de-embryonated wheat in the presence of GA_3. GI, GII, and GIII groups of α-amylase are as described previously (Marchylo et al 1980b, 1981).

within GIII and GI groups was obtained. For example, the three major GI components present in the initial extract were resolved into peaks 5, 6, and 8, and one of the four major GIII components was found in peak 2, while the remaining three focused in peak 3. Identical results were obtained in the absence of GA_3. A more complete discussion of the elution behavior during chromatofocusing of major and minor germinated wheat α-amylase components was described previously (Marchylo and Kruger 1983).

The activity contributed by each group of α-amylases was determined at each germination stage by totaling the activity present in fractions comprising each group. Fractions within peaks 1–3 were totaled to give GIII activity, whereas fractions in peaks 4–8 were totaled to give GI plus GII activity. Peak 4 (GII components) was combined with GI peaks because of the low activity levels associated with this group. At all germination stages, the GIII components contributed the major proportion of activity produced in the germinating wheat kernel (Fig. 1A, B). However, the proportion of activity contributed by GIII components decreased linearly (from about 93 to 87%, $r^2 = 0.771$) with germination time, concomitant with a proportional increase in GI + GII activity. Similar changes in proportions of GIII and GI + GII activity were observed for wheat germinated in the presence of GA_3 (Fig. 1C, D), with GIII activity decreasing linearly (from about 94 to 84%, $r^2 = 0.755$) concomitant with a proportional increase in GI + GII activity.

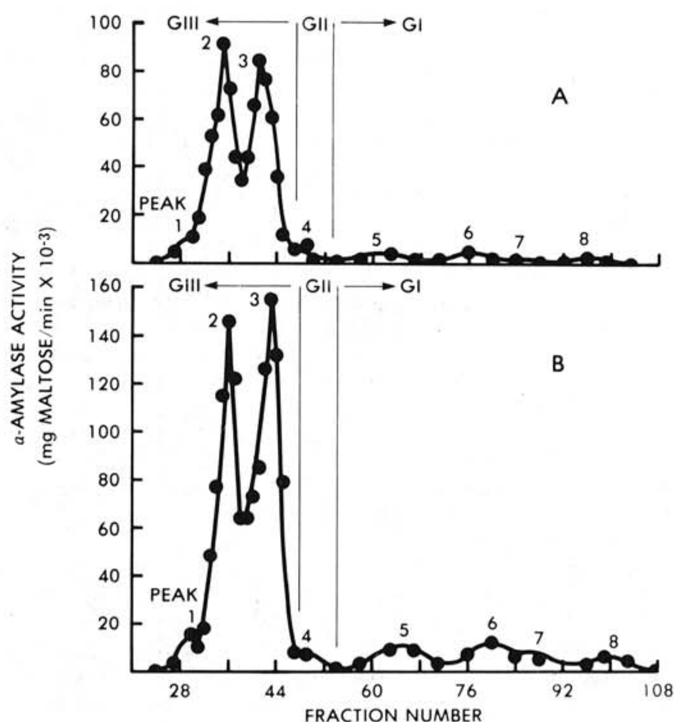

Fig. 3. Separation by chromatofocusing of α-amylases from wheat germinated for five days. **A** = in the absence of GA_3; **B** = in the presence of GA_3. GI, GII, and GIII α-amylases are as denoted in Fig. 2.

Production of α-Amylases in De-embryonated Wheat Kernels

The time course production of α-amylase in de-embryonated wheat kernels (Fig. 5A) differed significantly from that in germinated wheat (Fig. 1A). In both cases, enzyme was produced rapidly during the first five days of incubation/germination. However, the activity in de-embryonated kernels decreased dramatically between five and six days of incubation, whereas in the germinating wheat kernel, production of α-amylase continued increasing but at a somewhat slower rate. The maximum activity attained in the de-embryonated wheat kernel was about 7% of the maximum produced in the germinating wheat kernel.

Addition of GA_3 to the incubation medium altered the time course production of α-amylase in the de-embryonated kernel (Fig. 5C) such that it resembled the time course production in the germinating whole seed (Fig. 1C). α-Amylase production in incubating de-embryonated kernels increased about 10-fold in the presence of GA_3, but the maximum level attained was appreciably lower (about 60%) than the maximum activity attained in the germinating whole grain in the presence of GA_3.

De-embryonated wheat at each stage of incubation, in the absence or presence of GA_3, contained the same complement of α-amylase components found in germinating wheat, as determined by PAG-IEF analysis (Fig. 2). However, the relative intensity of α-amylase bands was significantly different in incubated de-embryonated grains. In the absence of GA_3, the intensity of GIII components appeared to be reduced, relative to the GI and GII components, as compared to the whole grains. In addition, the intensity of GII components relative to GI components appeared to have increased. Thus, in incubated de-embryonated wheat, on a visual qualitative basis, the GI + GII components contribute a significantly higher proportion of activity to the total, as compared to the germinating whole seed. In the presence of GA_3, the intensity of GIII bands in incubating de-embryonated wheat appeared to be restored to the levels present in the germinating whole seed. However, the proportion of visual activity in the de-embryonated grain contributed by GI and GII components appeared to remain elevated even in the presence of GA_3 when compared to proportions observed in the germinating whole grain.

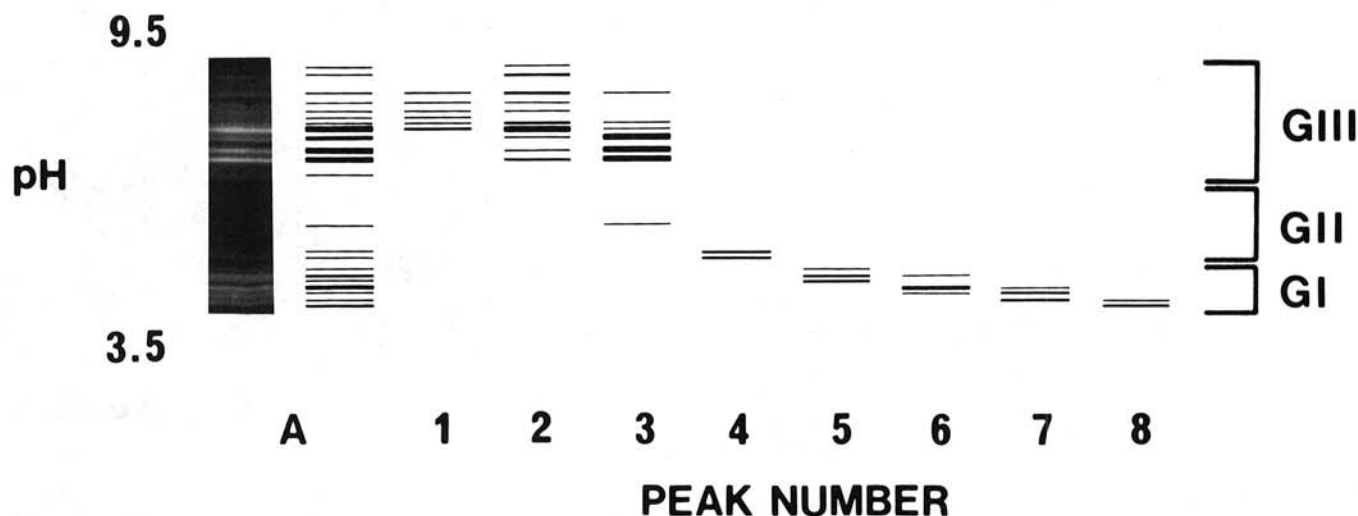

Fig. 4. Polyacrylamide gel isoelectric focusing, in a pH 3.5–9.5 gradient, of α-amylases in an extract of five-day-germinated wheat in the presence of GA_3, A, and in the peak fractions of peaks 1–8 separated by chromatofocusing (Fig. 3).

Extracts of de-embryonated kernels, incubated in the absence or presence of GA_3, then were subjected to chromatofocusing to quantitatively determine the proportions of activity contributed by each group of α-amylase components. De-embryonated kernels incubated for one to three days in the absence of GA_3 and one day in the presence of GA_3 produced very low levels of α-amylase and were not analyzed. Elution profiles, at each incubation stage in the absence or presence of GA_3, were identical with respect to the number of peaks eluted and the elution pH of each peak. Elution profiles for five-day incubated de-embryonated kernels in the absence and presence of GA_3 are illustrated in Fig. 6A and B, respectively, as an example of typical profiles. Polyacrylamide gel isoelectric focusing analysis of peak fractions indicated that, for all incubation stages, each peak basically represented the same components as described for the germinating whole kernel (Fig. 7). All components were eluted during chromatofocusing with the exception of one GII component. Further PAG-IEF analysis showed that this component eluted between peaks 3 and 4 (results not shown).

Quantitative analysis of elution profiles showed that, at each incubation stage in the absence of GA_3, the GI + GII components contributed a major proportion of α-amylase activity, increasing from 55% after four days of incubation to 63% after six days (Fig. 5A, B). In contrast, throughout incubation, GIII components contributed the major proportion of activity (75.6% ± 0.9%, $\bar{x} \pm S\bar{x}$) in de-embryonated kernels incubated in the presence of GA_3 (Fig. 5C, D).

An attempt also was made to determine whether the relative proportions of components within a group changed in whole grains or de-embryonated grains during germination/incubation. Only an estimate could be obtained because of overlap between peaks and lack of resolution of all components. For this analysis, peak 4 (GII components) was analyzed separately from GI components so that a clearer picture of changes within the GI group would be obtained. An estimate of any changes in activity of GII components relative to the activity of all GI components also was included. Peaks 1 and 2, and 6 and 7 were combined because of overlap between these peaks. Only three germination/incubation stages (days 4,5,6) were analyzed because, in earlier stages, it was difficult to obtain an accurate estimate of the activity associated with the smaller peaks. Within the limitations of this analysis, the relative proportions of components resolved with the GIII and GI groups remained basically constant in whole and de-embryonated wheat kernels through germination/incubation in the absence or presence of GA_3. Average relative proportions within each group are shown in Table I. The proportion of GII activity relative to GI activity was consistent within germinated wheat and within incubated de-embryonated wheat. However, the proportion of GII relative to GI activity in the de-embryonated kernels was almost double that in the whole kernels. Gibberellic acid did not affect the GII to GI proportions.

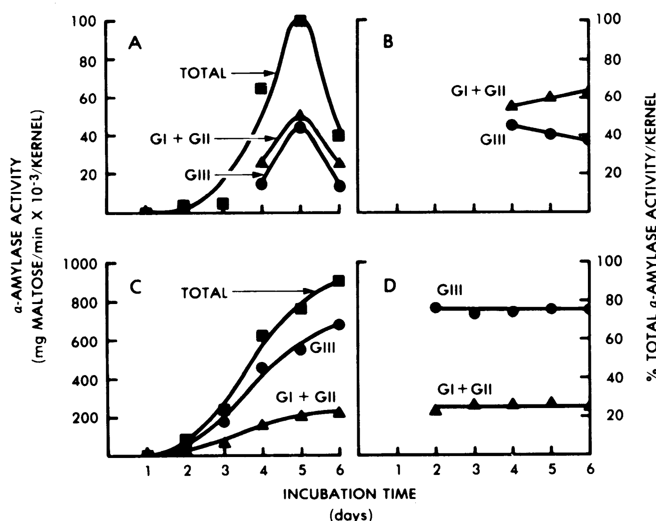

Fig. 5. A, C, Formation of α-amylase activity in incubated de-embryonated wheat. Activities of GIII and GI + GII groups of α-amylases were determined following chromatofocusing and they were normalized to 100% recovery. **A** = in the absence of GA_3, **C** = in the presence of GA_3. **B, D,** Proportions of GIII and GI + GII groups relative to total GIII + GII + GI activity. GI, GII, and GIII α-amylases are as shown in Fig. 2. **B** = in the absence of GA_3, **D** = in the presence of GA_3.

DISCUSSION

Qualitative and quantitative results reported in this study show that, in germinating wheat the GIII components (Fig. 2A) contribute the major proportion of α-amylase activity. However, the proportion decreased linearly with germination time concomitant with a linear increase in the proportion of GI + GII components. This result is consistent with immunoelectrophoretic work (Sargeant 1980), which indicated that the major proportion of α-amylase protein in germinating wheat was present as GIII components and that this proportion decreased during germination. The presence of GA_3 induced synthesis of higher α-amylase levels during germination, but proportions contributed by GIII and GI + GII α-amylases remained basically the same as those observed for wheat germinated in the absence of GA_3. Linear changes in proportions of these groups could reflect differences in turnover rate or rates of synthesis between groups.

Recently, Daussant et al (1982) reported that GI α-amylase components are synthesized much later in germination than GIII components. This was not found in the present study since significant levels of GI activity were detected quantitatively after two days of germination and qualitatively after one day of germination. Extrapolation of the linear curves illustrated in Fig. 1B and D shows that only about 5–6% of the total activity present after one day of germination would be contributed by GI + GII components. Thus, very little activity would be associated with these components because of the low levels of activity present in one-day-germinated wheat, and it is correspondingly difficult to detect these components. This study, then, presents evidence that all α-amylase components are synthesized in unison.

Embryo excision not only altered the proportions of α-amylase components synthesized but also dramatically decreased the potential of the aleurone to produce α-amylase such that, at best, only 7% of the activity produced in the germinated whole grain was synthesized. This result is consistent with the generally accepted idea that the embryo serves as a source of gibberellins required for induction of α-amylase synthesis in the aleurone. α-Amylase synthesis in the de-embryonated grain in the absence of GA_3 may be indicative of endogenous levels of GA_3 or other activating substances in the endosperm or it may reflect limited aleurone

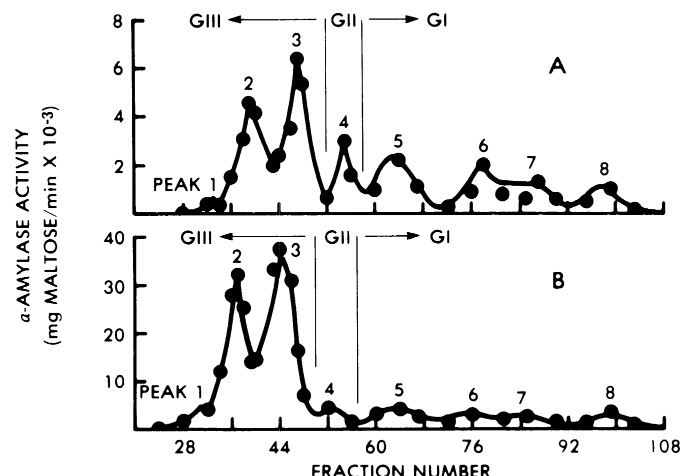

Fig. 6. Separation by chromatofocusing of α-amylases from de-embryonated wheat incubated for five days. **A** = in the absence of GA_3, **B** = in the presence of GA_3. GI, GII, GIII denotes groups of α-amylase components as shown in Fig. 2.

potential to synthesize enzyme in the absence of GA_3.

Quantitative analysis of incubated de-embryonated wheat kernels showed that up to 63% of the total activity was contributed by GI + GII components. As in germinating wheat, the proportion of activity contributed by GIII components decreased during incubation concomitant with an increase in GI + GII components.

Addition of GA_3 to the incubation medium induced synthesis of much higher α-amylase levels in the de-embryonated grain, but the maximum level achieved was only about 40% of the maximum activity produced in the germinating whole seed in the presence of GA_3. In addition, in the presence of GA_3 the time course production of enzyme in the de-embryonated grain was basically the same as that exhibited by germinated wheat. Induction by GA_3 of α-amylase synthesis by the aleurone has been well documented, but it is unclear why comparable levels of α-amylase are not produced by the de-embryonated grain incubated in the presence of GA_3. Recent reports have shown that the embryo can play an important role in α-amylase synthesis in germinating grain (Gibbons 1980a, 1980b; Daussant et al 1982). Possibly the shortfall in α-amylase production results from the loss of α-amylase production by the embryo. Alternately, as suggested by Khan et al (1973) and MacGregor (1983), optimal α-amylase synthesis by the aleurone may depend on factors, other than GA_3, that are supplied by the embryo.

Quantitative analysis showed that, throughout incubation, GIII components contributed about 76% of the total activity produced by the aleurone, and this contribution did not decrease during incubation, as was the case in germinating wheat. The reason for this is unclear. The proportion contributed by the GI + GII components (about 24%) remained higher than the GI + GII contribution in germinating wheat. Thus, production of α-amylase in the de-embryonated kernel, even in the presence of exogenously applied GA_3, does not correspond to production in the germinating whole seed. These results suggest that care must be taken before using results obtained from studies of α-amylase synthesis in de-embryonated grains or isolated aleurone layers, to explain synthesis in the germinating whole grain.

In a previous report (Marchylo et al 1981), qualitative PAG-IEF analysis suggested that higher levels of GI + GII α-amylase activity were produced in incubated de-embryonated wheat as compared to germinated wheat (cv. Cypress). From this observation it was concluded that the embryo repressed synthesis of these components in the germinating whole grain. Quantitative analyses done in the present study showed this conclusion to be false. In fact, production of GIII activity is reduced by about 96% in the incubated de-embryonated grain, as compared to GIII production in the germinating whole grain. In contrast, synthesis of GI + GII activity is reduced by about 75%. This results in the observed increase in the relative proportion of activity contributed by GI + GII components. Thus, it would appear that the embryo or factors produced by the embryo are required for optimal production of GIII activity and to a lesser extent for production of GI + GII activity. This supposition was confirmed by incubation of the de-embryonated grain in the presence of GA_3, which induced about a 20-fold increase in GIII activity as compared to a fourfold increase in GI + GII activity. Thus, synthesis in the aleurone of GIII components as compared to GI + GII components is controlled differentially by GA_3. Recently, Gale (1983) reported that the GIII and GI groups of components are under independent genetic control. It then would appear that expression of these genes is controlled differentially by GA_3. Reports (Jacobsen and Higgins 1982, Callis and Ho 1983) have shown that a similar system of α-amylase genetic control exists in Himalayan barley aleurone cells.

The relative proportions of components within the GIII and GI α-amylase groups remained consistent during germination/incuba-

TABLE I
Relative Proportions Within a Group of α-Amylase Peaks Separated by Chromatofocusing in Whole and De-embryonated Wheat Kernels During Germination/Incubation

Group	Relative Proportion of Peak Activity Within a Group (%)					
	GIII		GII	GI		
Peak Number	1 + 2[a]	3[a]	4[b]	5[b]	6 + 7[b]	8[b]
Germinating wheat[c]	49.1	50.9	11.5	37.7	47.9	15.2
Germinating wheat[c] + GA_3	46.7	53.4	12.4	35.8	47.9	16.4
Incubating de-embryonated wheat[c]	44.1	55.9	21.1	39.5	46.9	13.6
Incubating de-embryonated wheat + GA_3[c]	46.3	53.7	21.7	34.9	47.2	17.3
Overall average[d] ($\bar{x} \pm S\bar{x}$)	46.0 ± 0.8	53.5 ± 0.8	···	36.8 ± 0.6	47.7 ± 0.6	15.6 ± 0.7

[a] As percent of total GIII activity (peak 1 + 2 + 3).
[b] As percent of total GI activity (peak 5 + 6 + 7 + 8).
[c] Average of six, five, and four days of germination/incubation.
[d] n = 12. Averaged values within a group do not necessarily add up to 100% due to rounding.

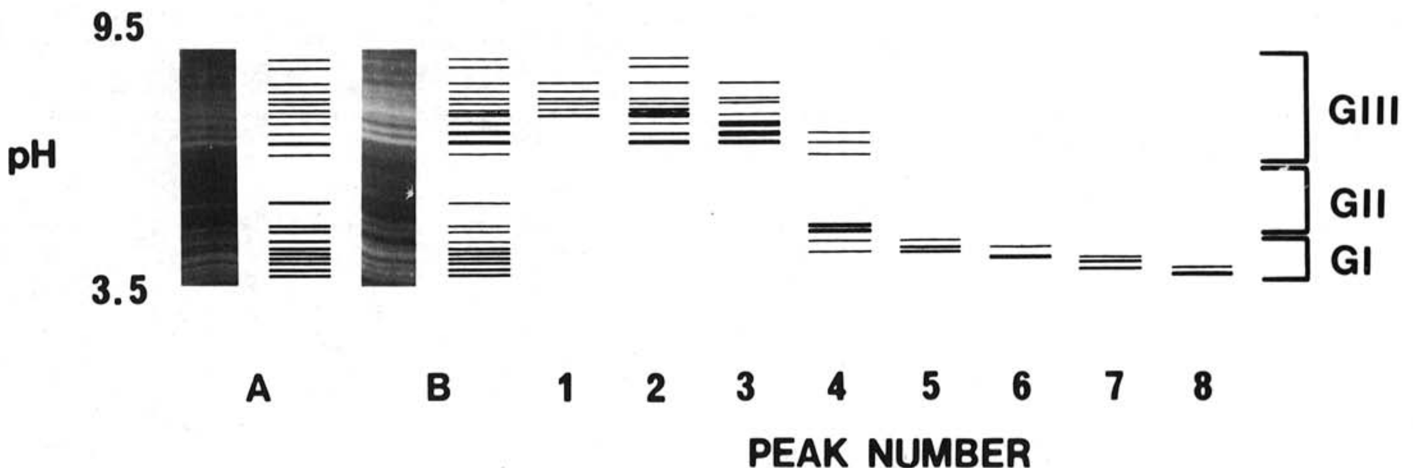

Fig. 7. Polyacrylamide gel isoelectric focusing, in a pH 3.5–9.5 gradient, of α-amylases in: **A**, an extract of five-day incubated de-embryonated wheat in the absence of GA_3; **B**, the presence of GA_3; and in the peak fractions of peaks 1–8 separated by chromatofocusing (Fig. 6A,B).

tion of the whole and de-embryonated wheat in the presence and absence of GA_3. This is compatible with Gale's (1983) proposal that the GIII and GI α-amylases are genetically controlled by the α-Amy-1 and α-Amy-2 genes, respectively. Gale (1983) also suggested that the GII components are encoded by the same genes as the GI components. However, as shown in Table I, the proportion of the GII components relative to GI components almost doubled in the incubated de-embryonated grain, in the absence or presence of GA_3. If the GI and GII components were encoded by the same genes, then it would be expected that the proportion of GII to GI activity would have remained constant. Further work will be required to clarify the relationship between the GI and GII groups of components.

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