Multiple α-Amylase Components in Germinated Cereal Grains Determined by Isoelectric Focusing and Chromatofocusing[1,2]

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ABSTRACT

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Isoelectric focusing showed that germinated barley (two- and six-rowed), wheat (hard red spring and amber durum), rye, and triticale contained multiple forms of α-amylase that could be divided into low- (≤ pI 5.5) and high-pI (≥ pI 5.8) groups. Only low-pI α-amylase components were found in germinated oats, corn, millet, sorghum, and rice. Quantitative data on the proportions of high- and low-pI groups in barley, wheat, rye, and triticale were obtained by chromatofocusing. Barley contained the highest proportion (97.9%) of high-pI α-amylase and amber durum wheat the lowest (79.1%). In addition, chromatofocusing showed that germinated corn contained a group of α-amylase components having pI values between those of the high and low-pI groups. A comparison of the accurately measured α-amylase values from chromatofocusing with α-amylase component profiles obtained by isoelectric focusing revealed that determination of the relative proportions of α-amylase components on isoelectric focusing gels by visual inspection could be misleading. There was an immunochemical relationship among the low-pI α-amylase components of barley, wheat, rye, triticale, the major α-amylase components of millet, sorghum, and possibly oats, and a minor component in corn.

There have been numerous reports in the literature on the heterogeneity of α-amylases produced by germinating cereal grains (MacGregor 1983, Kruger and Lineback 1987). Many different protein separation techniques have been used, and it is often difficult to make valid comparisons between the different studies. Because isoelectric focusing on polyacrylamide gels has excellent resolving power, requires only small amounts of material, is relatively simple to carry out, and does not appear to inactivate α-amylases, it has become the method of choice for qualitative analysis of cereal α-amylases (Marchylo and Kruger 1987a). Chromatofocusing has also proved useful for analyzing these enzymes (Marchylo and Kruger 1983). This technique does not have the high resolving power of isoelectric focusing, but it does give quantitative information on the major α-amylase components present.

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The purpose of this study was to use these two techniques to compare the types of α-amylase isoenzymes produced by a number of cereals during germination. It was anticipated that this study, as well as providing useful comparative data, would identify the major α-amylase isoenzyme(s) in each cereal.

MATERIALS AND METHODS

Grain

The following grain samples were used: two-rowed barley (cultivar Klages), six-rowed barley (Bonanza), red spring wheat (Neepawa), amber durum wheat (Wakooma), triticale (Welsh), rye (Musketeer), oats (Fidler), corn (Sunnyvee), millet (Dawn), sorghum (line 954063), and rice (line Q68).

Germination Conditions

All grains were soaked for 20 min in sodium hypochlorite solution (3%) and rinsed thoroughly with deionized, sterile water. Grains of maize, rice, millet, and sorghum were steeped in water for 24 hr at 24° C before being germinated. Sterile sand (20 g) was placed in petri dishes, and sufficient water (4.5 ml) was added to saturate the sand. Twenty kernels were placed in each petri dish. Germinations were carried out in a Blue M Incubator (Blue M Electric Company, Blue Island, IL) at 18° C for barley, wheat, triticale, rye, and oats, and at 25° C for rice, sorghum, corn, and millet. Germination times were four days for barley and rye, five days for wheat and triticale, and seven days for the other grains. Germinated samples were frozen at −40° C until analyzed.

Enzyme Extraction

Ten-kernel samples of barley, wheat, rye, and triticale were extracted in a mortar with a small amount of sand and 10 ml of imidazole-HCl buffer (0.025*M*, pH 7.4) containing $10^{-4}M$ $CaCl_2$. For the other grains, samples of 20–25 kernels were extracted in a similar way with 10 ml of histidine-HCl buffer (0.025*M*, pH 6.2) containing $10^{-4}M$ $CaCl_2$. All extracts were centrifuged twice (10,000 × *g*; 10 min) and filtered through MSI 0.45-μm filters (Fisher Scientific Ltd.).

α-Amylase Activity

Grain extracts and column fractions were assayed for α-amylase using a modification of the Briggs procedure (Briggs 1961) with β-limit dextrin, prepared from waxy maize starch, as substrate.

Isoelectric Focusing

Precast, thin-layer polyacrylamide gels (Ampholine PAG plate kits, pH 3.5-9.5, LKB Producter AB, Bromma, Sweden) were used. Samples (10–80 μl) were applied to the gel on paper wicks (1 × 0.5 cm), and focusing was carried out on an Ultrophor electrofocusing unit (LKB Producter AB), cooled to 1° C. Bands of α-amylase were visualized by incubating focused gels against gels containing amylopectin β-limit dextrin as described previously (MacGregor et al 1974).

Chromatofocusing

Polybuffer exchanger 94 was equilibrated and packed as recommended by the manufacturer (Pharmacia Fine Chemicals 1980). Pharmacia K9/30 columns were packed at flow rates of 2.5 ml/min to a bed height of 24 cm. A layer (2.5 cm) of Sephadex G-25 (coarse) was put on top of the bed to ensure even sample application. A pH gradient of 7.4–4.8 was used for extracts of barley, wheat, triticale, and rye. The start buffer used was 0.025*M* imidazole-HCl, pH 7.4. Samples (2 ml) were applied by gravity, and the eluent buffer (Polybuffer 74 diluted 10 times with $10^{-4}M$ $CaCl_2$ and adjusted to pH 4.8) was pumped through the column at 20 ml/hr. A pH gradient of 6.2–4.0 was used for extracts of rice, oats, sorghum, corn, and millet. Again, 2-ml samples were applied to the column. The start buffer used was 0.025*M* histidine-HCl, pH 6.2, and the eluent buffer was Polybuffer 74 diluted 10 times with $10^{-4}M$ $CaCl_2$ and adjusted to pH 4.0. This buffer, too, was pumped through the column at 20 ml/hr. Chromatofocusing was carried out at 4° C and 2-ml fractions were collected. At least two runs were performed on each extract. The pH of the column effluent was monitored continuously with a flow-through pH-electrode assembly from LKB.

Antibody Experiments

An immune serum raised against α-amylase from developing wheat kernels was used (Daussant 1978). This serum is active against the low-pI α-amylases from germinated barley (MacGregor et al 1984), germinated wheat (Daussant 1978), and germinated triticale (Daussant and Hill 1979). Absorption of α-amylase by immune sera during isoelectric focusing was performed as described previously (Daussant and MacGregor 1979). In preliminary experiments using isoelectric focusing, the amount of serum required to complex fully with a known quantity of the low-pI α-amylase from germinated barley was determined. Using this information and knowing the proportion of low-pI α-amylase to total α-amylase activity in extracts of germinated grain (Table I), sufficient immune serum was added to the focusing gel to complex with all of the low-pI enzyme present; e.g., α-amylase in rye extracts was assumed to be composed of at least 10.6% low-pI enzyme, whereas in millet extracts, all of the α-amylase activity was assumed to consist of the low-pI component.

RESULTS AND DISCUSSION

Initially, all grains were germinated at 18° C, but synthesis of α-amylase in cereals such as sorghum and rice was very slow at that temperature. Therefore, corn, millet, sorghum, and rice were germinated at 25° C and the other cereal grains at 18° C. The temperature of germination did not affect the α-amylase banding pattern obtained after isoelectric focusing and incubation times of sufficient length were selected to permit the appearance of all major α-amylase bands. No additional bands were found after longer periods of germination.

Wheat and barley were included in this study for comparative purposes even though the various α-amylase components in these cereals have been discussed in detail elsewhere.

To facilitate comparisons, α-amylase components were divided into two groups (Fig. 1) designated as low- and high-pI (isoelectric point) groups. Because there is no evidence of a relationship among the low-pI α-amylase components of the cereals used in this study, the numerical nomenclature system suggested for α-amylases (MacGregor and MacGregor 1987), based on recommendations by the International Union of Pure and Applied Chemistry (IUPAC-IUB 1973), was not used.

The two barley cultivars used in this study gave slightly different patterns of α-amylase components after isoelectric focusing (Fig. 1). Results were similar to those reported previously (MacGregor 1978) and support conclusions from other studies that the α-amylase pattern in germinated barley is dependent on the cultivar used (Momotani and Kato 1970, Takano and Takeda 1985). α-Amylase components in the wheat and triticale samples were also similar to those in previous studies of wheat (Marchylo and Kruger 1983) and triticale (Silvanovich and Hill 1977).

Multiple forms of α-amylase have been detected in rye by both electrophoretic (Lapinski and Masojc 1983, Masojc and Lapinski 1984, Masojc 1987) and isoelectric focusing (Möttönen 1975) techniques. In the present study, several bands of α-amylase were detected after isoelectric focusing (Fig. 1) and they, too, could be divided into low- and high-pI groups. Both groups appeared less heterogeneous than those of wheat and triticale, and the high-pI group contained fewer components than barley. Because heterogeneity of α-amylase components in germinated rye varies with cultivar (Masojc and Lapinski 1984) and only one rye cultivar was used in this study, the pattern of rye α-amylase components shown in Figure 1 is not discussed in any further detail.

The remaining cereal grains did not contain high pI α-amylase components. Germinated oats contained a small group of closely spaced bands of α-amylase, but α-amylase components were more numerous in corn, millet, sorghum, and rice. Previous studies (Chao and Scandalios 1972, Goldstein and Jennings 1978) have shown the presence of three to four low-pI α-amylase components

in germinated corn. Results in Figure 1, however, suggest that the α-amylase system in corn is more complex than that. Several α-amylase components were detected also in millet, in agreement with previous studies that showed the presence of several α-amylase components in ungerminated grains of pearl millet (Beleia and Varriano-Marston 1981). As suggested in previous studies (Botes et al 1967, Mundy 1982, Mundy et al 1983), α-amylase from germinated sorghum was also heterogeneous. It is difficult to compare the α-amylase components described by Mundy (1982) with those detected in the present study because different pH gradients were used in the isoelectric focusing experiments. The heterogeneity of α-amylase from germinated rice has been studied in detail (Tanaka et al 1970, Daussant et al 1983). Results from these studies suggest that there are three major α-amylase components in rice, each of which may be heterogenous. Results from the present study also suggest that rice α-amylase may consist of three heterogeneous groups (Fig. 1). It is tempting to speculate that these groups correspond to the A, B, and D groups discussed by Daussant et al (1983).

It is relatively simple to compare the types of α-amylase components present in germinated kernels of different cereal grains by using isoelectric focusing. The α-amylase band pattern of a particular cereal may vary with the cultivar, but it is unlikely that high-pI components such as those present in wheat and barley will be found in oats, corn, millet, sorghum, or rice. Similarly, published evidence suggests that all germinated samples of barley, wheat, rye, and triticale contain both low- and high-pI groups of α-amylase.

Although profiles such as those shown in Figure 1 are useful, they provide only limited quantitative information about relative amounts of α-amylase components present in the extracts. Such data can be obtained by using the technique of chromatofocusing (Marchylo and Kruger 1983). Typical α-amylase profiles for the various grains studied are shown in Figures 2 and 3. The pH gradient of column effluents was monitored continuously during each run, and typical gradients are shown in Figure 2 (six-rowed barley) and Figure 3 (rice). A pH gradient of 7.4–4.8 was used for extracts of barley, wheat, rye, and triticale (Fig. 2), whereas a gradient extending from pH 6.2 to 4.0 was used for rice, oats, sorghum, corn, and millet because these cereals did not contain high-pI α-amylases (Fig. 3).

Although there was some inter-run variability in these pH gradients, resulting in small variations in the elution volume of a particular α-amylase component, there was insignificant variation in the pH at which a component was eluted. This agrees with published results from chromatofocusing studies on both α-amylase (Marchylo and Kruger 1983) and β-amylase (LaBerge and Marchylo 1983).

During chromatofocusing, proteins should be eluted at their isoelectric points, but in practice this is not so (Marchylo and Kruger 1983). Therefore, peak elution pHs shown in Figures 2 and 3 should be treated as "apparent" pI values.

It is difficult to compare α-amylase bands obtained by isoelectric focusing with the enzyme peaks obtained after chromatofocusing (e.g., compare Figs. 1 and 3). Therefore, the major α-amylase peaks from chromatofocusing were analyzed by isoelectric focusing to determine the identity of the α-amylase components in each peak. The results for corn, millet, sorghum, rice, and oats are shown in Figure 4. Similar analyses of α-amylases from germinated wheat (Marchylo and Kruger 1983) and green malt (Marchylo and MacGregor 1983) have been described in detail previously. Results obtained in the present study were similar to these and so are not shown here.

The chromatofocusing elution pattern of α-amylase components in Bonanza barley (Fig. 2) was similar to that described previously (Marchylo and MacGregor 1983). Analysis of each peak by isoelectric focusing showed that only the pI 5.4 peak contained low-pI components; the other peaks contained only high pI components. Klages barley had a less complex α-amylase profile with only two major α-amylase components. It would be premature to conclude, however, that all six-rowed and two-rowed barley cultivars would yield α-amylase profiles similar to those of Bonanza and Klages, respectively. These profiles may vary with time of germination (Marchylo et al 1985) and with the amount of α-amylase 2 inhibitor present in the barley (Weselake et al 1983) as well as with cultivar. Low-pI α-amylase components represented only a small proportion of the total α-amylase activity (Table I), as has been shown previously (MacGregor and Ballance 1980, Marchylo and MacGregor, 1983).

The α-amylase profile from Neepawa (red spring) wheat (Fig. 2) and the α-amylase components within each peak were similar to those described in detail in a previous study (Marchylo and Kruger 1983). Only low-pI α-amylase components were found in peaks of pI 5.1, 5.3, and 5.5, and only high-pI components were present in the two major peaks. Low-pI components in wheat formed a much higher proportion of the total α-amylase activity than they did in

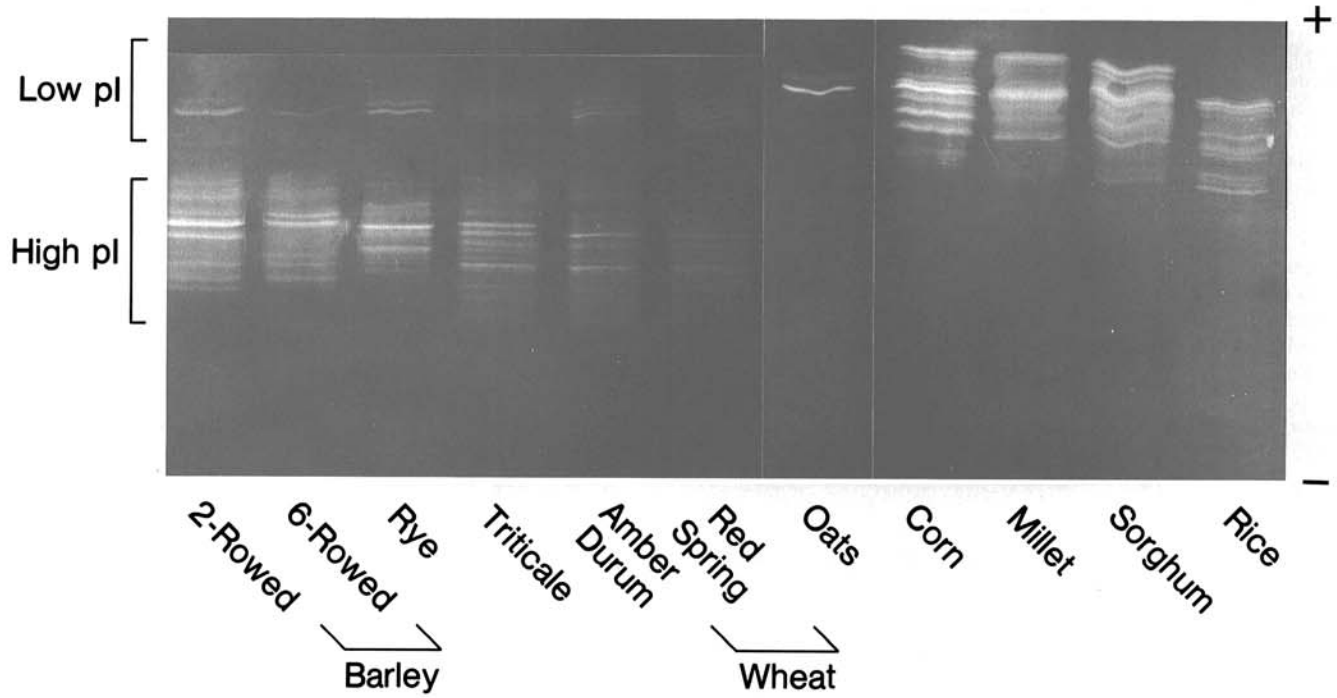

Fig. 1. Zymogram of α-amylases from germinated cereal grains. The enzymes were separated by isoelectric focusing on a pH 3.5–9.5 gradient.

barley (Table I). Similar results have been reported previously (Marchylo et al 1984). Wakooma (amber durum) wheat had a similar α-amylase profile to that of Neepawa (red spring) but a higher proportion of low-pI α-amylase components (Table I). The profile for triticale α-amylase (Fig. 2) appeared to be a combination of the profiles from rye and durum wheat, as might be expected. Again, analysis of the major peaks by isoelectric focusing showed that peaks having an apparent pI of 5.5 or less contained only low-pI components and those having a pI of 5.8 or higher contained mixtures of high-pI components.

Despite the multiplicity of α-amylase bands detected in rice extracts after isoelectric focusing (Fig. 1), only one peak of activity was found after chromatofocusing (Fig. 3). This peak consisted of the lowest pI group of α-amylase components in the rice extract (Fig. 4). The other bands of α-amylase, visualized after isoelectric focusing (Figs. 1 and 3), were not detected by chromatofocusing, suggesting that they contained only small amounts of activity. These results agree with those from previous studies that showed that the major α-amylase component in rice had a pI of 4.6 (Okamoto and Akazawa 1978) and that this was the component with the lowest pI in the rice α-amylase complex (Okamoto and Akazawa 1979).

Two major peaks of α-amylase were obtained from oat extracts after chromatofocusing (Fig. 3). Both peaks were heterogeneous (Fig. 4) with one peak (pI 4.6) containing two and the other (pI 4.8) three α-amylase components. Because the various α-amylase components were not separated well by isoelectric focusing, it is difficult to match individual components in the chromatofocusing peaks with those in the total extract. Such identification could be accomplished more easily by using a shallow pH gradient for isoelectric focusing. α-Amylase components in the pI 4.8 peak (Fig. 3) might be expected to have higher pIs than components in the pI 4.6 peak on analysis by isoelectric focusing. This was not observed, however (Fig. 4). Possible reasons for this type of discrepancy between results obtained by isoelectric focusing and those obtained by chromatofocusing have been discussed previously (Marchylo and Kruger 1983, Pharmacia Fine Chemicals 1980-81).

Only one major peak of α-amylase activity was eluted during chromatofocusing of germinated sorghum extracts (Fig. 3). This

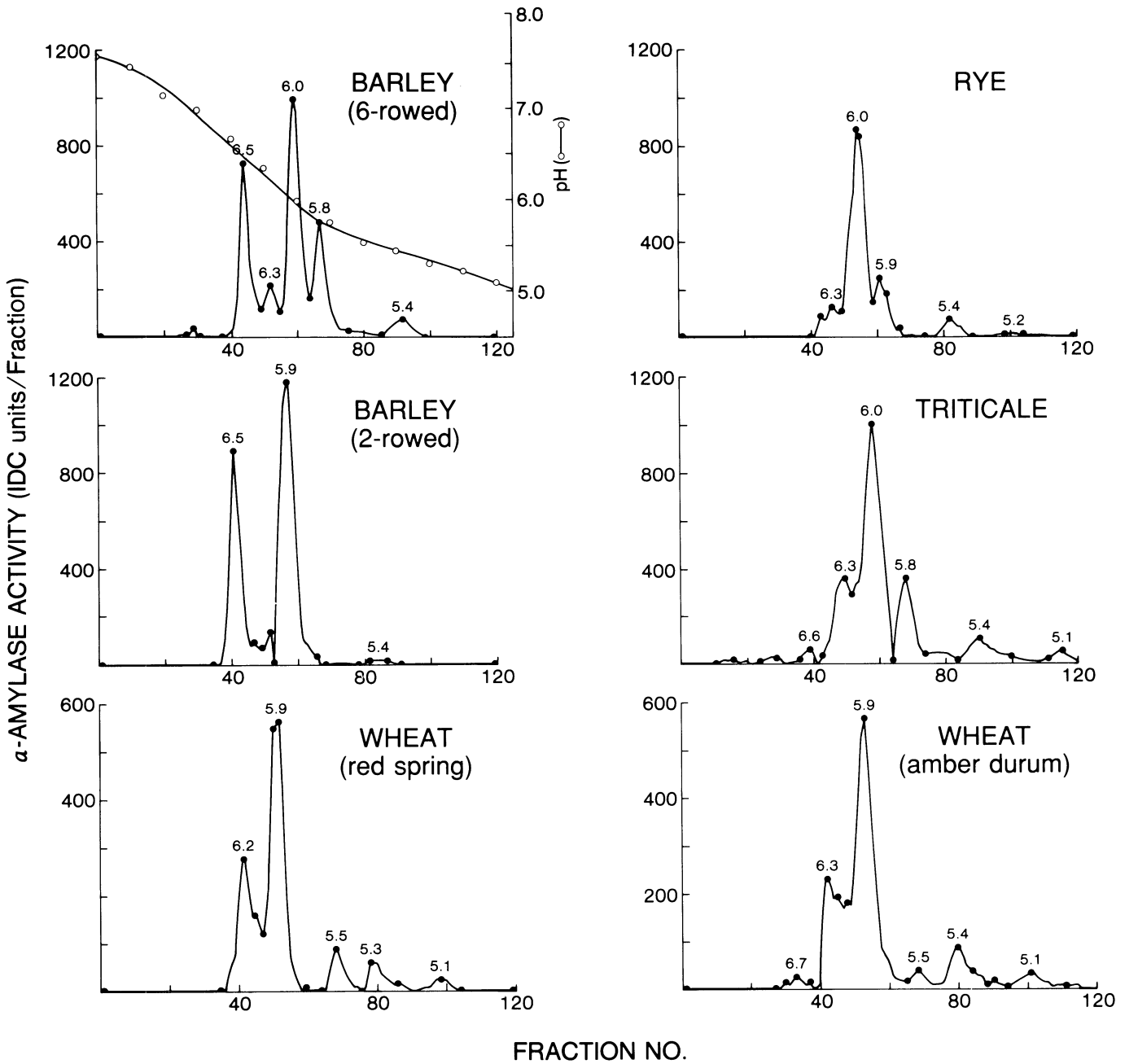

Fig. 2. Separation of α-amylases by chromatofocusing on a pH 7.4–4.8 gradient (-o-o-o- pH gradient).

peak had a low apparent pI value of 4.3 and contained the two lowest pI components detected by isoelectric focusing (Fig. 4). This is in agreement with results of Mundy (1982), who showed that the major α-amylase component in germinated sorghum had the lowest pI. Again, it is not obvious from isoelectric focusing studies (Figs. 1 and 4) that most of the α-amylase activity in germinated sorghum is associated with these particular components. The components having slightly higher pI values (visible as a doublet in Figures 1 and 4) were associated with the small α-amylase peak of pI 4.6 shown in Figure 3.

Chromatofocusing separated germinated corn α-amylase into a number of fractions (Fig. 3), but the resolution was not as high as that achieved by isoelectric focusing (Fig. 1). The major peak, representing about 74% of the total enzyme activity, had an apparent pI value of 4.9 and contained a number of α-amylase components (Fig. 4). Unlike sorghum, these were not the lowest pI components; these were found in the small peak of pI 4.3. The higher pI peaks shown in Figure 3 were associated with the higher pI bands of activity detected after isoelectric focusing (Fig. 1). These represented a small proportion of the total activity and were designated as α-amylase components of intermediate pI (Table I) because they did not fit easily into either the high or low-pI groups. This group of α-amylases was a distinctive feature of corn α-amylase profiles.

The chromatofocusing elution pattern of α-amylase from germinated millet also showed a number of peaks of activity (Fig. 3). The major peak, representing 61% of the total activity, had a low-pI value of 4.2 and consisted of α-amylase components having the lowest pI values as determined by isoelectric focusing (Fig. 4). Previous studies have shown that mature millet contains a number of α-amylase components, but the component with the lowest pI appeared to have the highest activity (Beleia and Varriano-Marston 1981). The source of α-amylase in mature millet has not been determined, but if it were associated with premature germination, then the results of the present study would be in good agreement with those reported by Beleia and Varriano-Marston (1981). Several smaller peaks of α-amylase were resolved by chromatofocusing (Fig. 3) and were found to be associated with the α-amylase components of higher pI detected by isoelectric focusing (Figs. 1 and 4). Examination of the results from isoelectric focusing studies (Figs. 1 and 4) would not suggest that the α-amylase components of lowest pI contribute most to the total α-amylase activity, emphasizing again that care must be exercised when making visual evaluations of relative amounts of enzyme activity

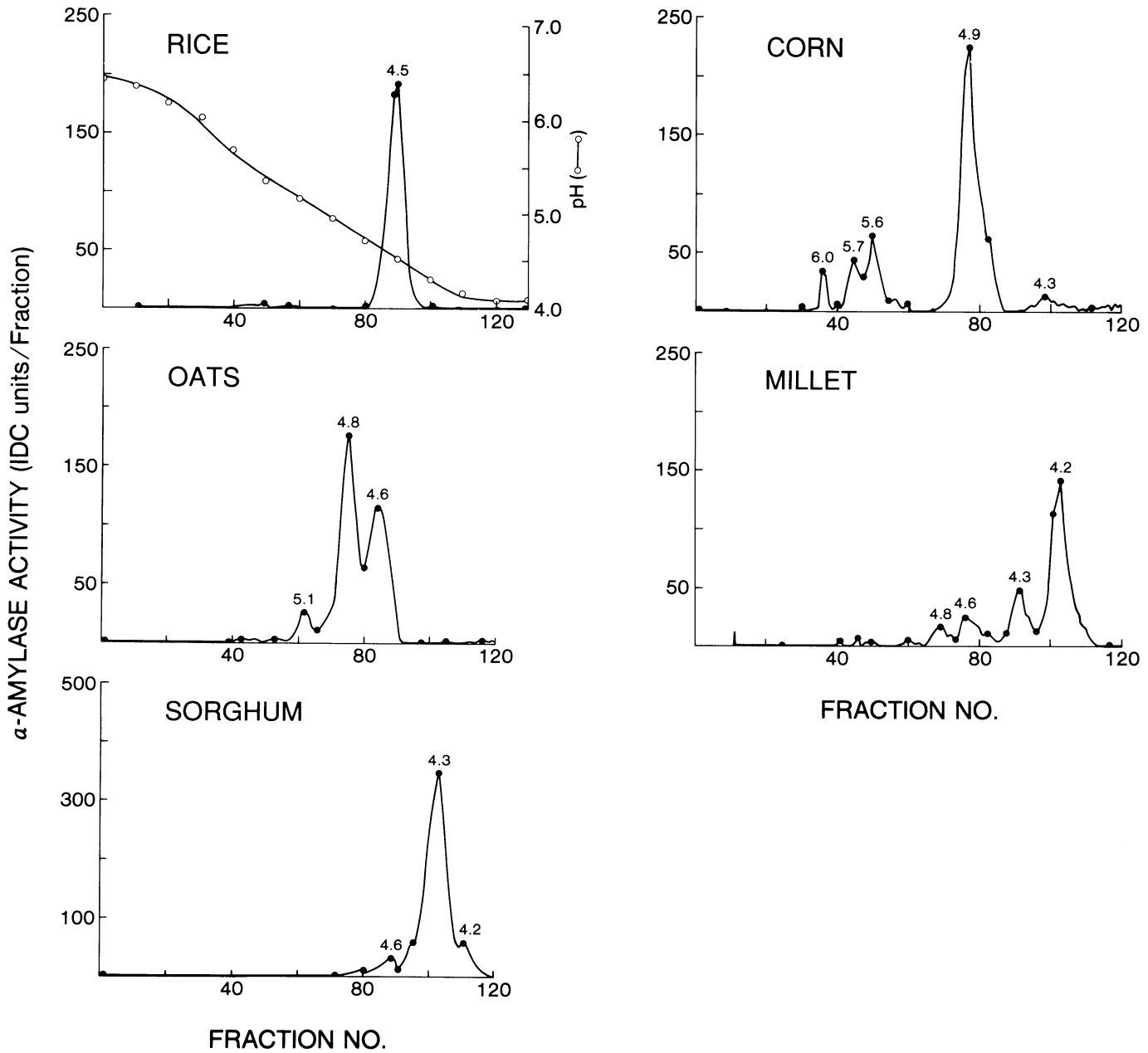

Fig. 3. Separation of α-amylases by chromatofocusing on a pH 4.0–6.2 gradient (-o-o-o- pH gradient).

on gels after isoelectric focusing.

In general, recovery of enzyme activity after chromatofocusing was acceptable (Table I). Low recoveries were obtained always, however, with extracts of oats and sorghum. The reason for this has not been determined, but it is possible that α-amylases from these particular grains are unstable under the conditions used for chromatofocusing. If some components are less stable than others, then the activity profiles shown in Figure 3 may not be representative of the total α-amylase in either oats or sorghum.

Previous studies have shown that α-amylases from different cereal grains have some common antigenic sites (Daussant and Grabar 1966, Alexandrescu et al 1975). For example, an immune serum prepared against α-amylase from immature wheat kernels (Daussant 1978) reacted with the low-pI α-amylase components in germinated wheat (Daussant and Renard 1976, Daussant 1978), germinated triticale (Daussant and Hill 1979), and germinated barley kernels (MacGregor et al 1984). These results indicate that some immunochemical similarity exists between the low-pI α-amylase components of barley, wheat, and triticale.

Extracts of germinated samples of all of the cereal grains used in this study were treated with immune serum raised against immature wheat α-amylase to see if an immunochemical relationship existed among all the low-pI α-amylase components. The serum, not unexpectedly, reacted with the low-pI components of barley, wheat, triticale, and also rye, as shown by the absence of these components after isoelectric focusing of extracts treated with immune serum (Fig. 5). High-pI components were not affected. Results with the other grains were not as clear-cut (Fig. 6). Only the minor, lowest pI component of corn α-amylase appeared to react, whereas the major component of both millet and sorghum was removed by the immune serum. The major components of rice were not affected, but the lowest pI components of oats were removed from the sample by the immune serum. These results suggest that there may be a relationship between the low-pI α-amylases of barley, wheat, triticale, and rye, the major low-pI α-amylase components of millet, sorghum, and possibly oats, and a minor component in corn. Such a conclusion should be tested by a much more rigorous immunochemical study of α-amylases from these various cereal grains.

Barley, wheat, rye, and triticale are closely related and belong to the subfamily Pooideae and the tribe Triticeae (Campbell 1985). α-Amylases from germinated samples of these cereals have certain similarities, including an obvious division into two groups. There is abundant evidence that most of the enzyme is synthesized in the aleurone layer (see Hill and MacGregor 1988 for review) and consists largely of high-pI components (Table I). Small amounts of α-amylase appear to be produced in embryos (including scutella) of barley (Briggs 1964, MacGregor and Marchylo 1986, Ranki and Sopanen 1984), wheat (Marchylo and Kruger 1987b), and rye (Wagenaar and Lugtenborg 1973). It is probable that triticale embryos would also synthesize some α-amylase. Low-pI enzymes from barley, wheat, rye, and triticale reacted strongly with antibodies raised against α-amylase from developing wheat kernels (Fig. 5), indicating that structural relationships exist among these enzymes. No high-pI components were detected in α-amylases from the other cereal grains included in this study. This may explain the absence in these grains of the inhibitor to the high-pI α-amylase group that has been detected in barley, wheat, rye, and triticale (Weselake et al 1985).

There is good evidence that the scutella of rice (Okamoto and Akazawa 1979) and of sorghum (Koehler 1981, Aisien 1982, Aisien and Palmer 1983, Aisien et al 1983) are major sources of α-amylase during germination. These results, along with those showing that embryos of barley (MacGregor and Marchylo 1986) and of wheat (Marchylo and Kruger 1987b) appear to produce predominantly

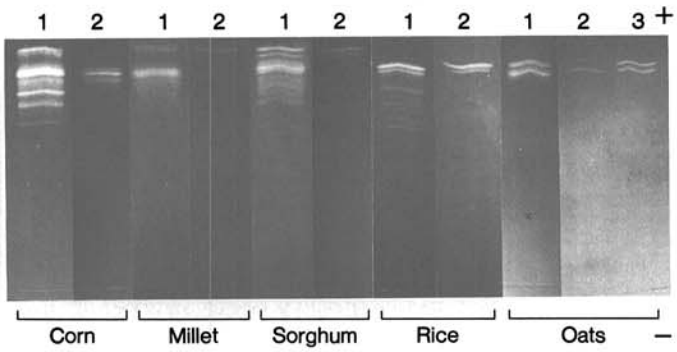

Fig. 4. Isoelectric focusing analysis (pH 3.5–9.5 gradient) of major α-amylase peaks obtained by chromatofocusing (Fig. 3). **1,** original extracts; **2,** pI 4.9 peak from corn, pI 4.2 peak from millet, pI 4.3 peak from sorghum, pI 4.5 peak from rice, and pI 4.6 peak from oats; **3,** pI 4.8 peak from oats.

TABLE I
Relative Proportions of High- and Low-pI α-Amylase Groups in Germinated Cereal Extracts Determined by Chromatofocusing[a]

Cereal	Cultivar	Average Recovery (%)	Relative Proportions (%)[b] High pI	Low pI
Red spring wheat	Neepawa	87.0	83.8	16.2
Amber durum wheat	Wakooma	80.2	79.1	20.9
Triticale	Welsh	78.1	83.5	16.6
Rye	Musketeer	78.6	89.4	10.6
6-Rowed barley	Bonanza	82.2	97.9	2.1
2-Rowed barley	Klages	99.3	97.9	2.1
Rice	Q68	83.8	0	100.0
Oats	Fidler	46.0	0	100.0
Sorghum	954063	42.1	0	100.0
Corn	Sunnyvee	77.2	0 (26.0)[c]	74.0
Millet	Dawn	75.4	0	100.0

[a] The pI cut-off points were based on results of isoelectric focusing patterns; peaks having an apparent pI of 5.5 or less contained only low-pI components, whereas peaks having an apparent pI of 5.8 or greater contained high-pI components.
[b] As percentage of activity recovered after chromatofocusing.
[c] α-Amylase components of intermediate apparent pI.

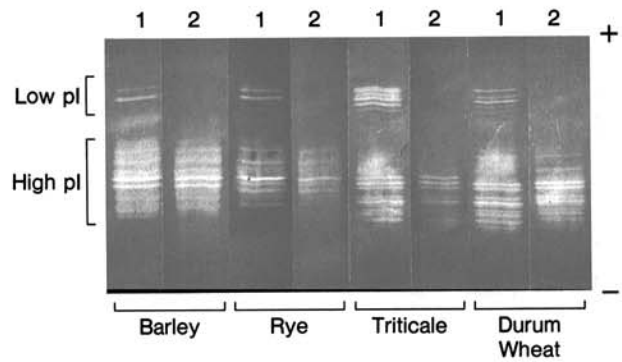

Fig. 5. Zymogram of α-amylases in **1,** untreated and **2,** immune serum treated, extracts of germinated cereal grains. A pH gradient of 3.5–9.5 was used.

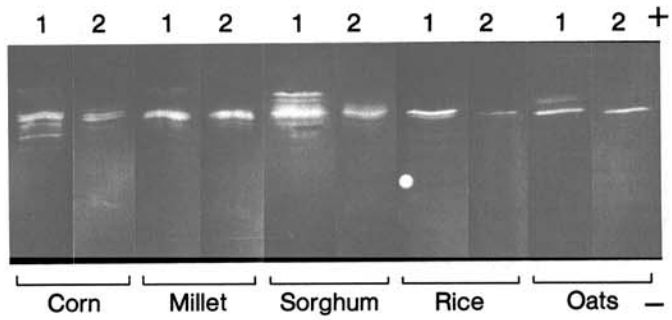

Fig. 6. Zymogram of α-amylases in **1,** untreated and **2,** immune serum treated, extracts of germinated cereal grains. A pH gradient of 3.5–9.5 was used.

low-pI α-amylase, lead to the attractive idea that cereals in which the aleurone is the major site of α-amylase synthesis produce mainly high-pI α-amylases, whereas low-pI α-amylases predominate in cereals in which the scutellum is the major site of synthesis. This hypothesis must be treated with some caution, however. No published information is available on the site of synthesis of millet α-amylase, but there is evidence of α-amylase synthesis in embryos (Cairns and de Villiers 1983) and aleurones (Hooley 1982, Zwar and Hooley 1986) of wild oats and also in embryos (Dure 1960, Okamoto et al 1980) and aleurone cells (Harvey and Oaks 1974a,b; Goldstein and Jennings 1975, 1978; Vianello and Macri 1982) of maize. There are no reports, however, on the relative contributions of embryo and aleurone to α-amylase synthesis in these two cereals. This information as well as data on the types of α-amylase isozymes synthesized by both tissues would increase significantly our understanding of α-amylase synthesis in cereal grains.

CONCLUSIONS

Germinated samples of all the cereals included in this study contained multiple forms of α-amylase. The enzymes from barley, wheat, triticale, and rye could be divided into low- and high-pI groups, with the high-pI groups containing a high proportion of the total enzyme activity in each cereal. No high-pI enzymes were detected in germinated samples of oats, corn, millet, sorghum, or rice, but some of the corn α-amylase components formed an intermediate pI group.

The results of this study showed clearly that visual assessment of the proportion of α-amylase components detected on an isoelectric focusing gel could be misleading. More reliable, quantitative data on major α-amylase components can be obtained using chromatofocusing techniques.

Immunochemical relationships were found to exist among the low-pI α-amylase components of barley, wheat, rye, and triticale and some of the lower pI components of corn, millet, sorghum, and oats, but not with those from rice.

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