THE MECHANISM OF ACTION OF PROTEOLYTIC ENZYMES ON PROTEINS IN WHEAT GRAINS DURING GERMINATION

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RESEARCH ARTICLE

Abstract

Proteolytic activities during germination of wheat grains were monitored using solution methods and one- and two-dimensional PAGE with gels that contained embedded substrate proteins. Total proteolytic activity increased during the first three days of germination, but not after that. Proteinase activity was measured at pH 3.8, 6.0, and 8.0 in the presence and absence of class-specific proteinase inhibitors. This indicated that enzymes from all four proteinase classes were present during the germination process. Germinated wheat grains mainly contained aspartic and cysteine-proteinase activities which are particularly active at pH 3.8. In general, the hydrolysis pattern was very similar to that observed during germination and in other cereals.

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INTRODUCTION

The life cycle of a grain of wheat has two phases, germination and development, which are separated by a rest period. During seed germination, a large part of the supply of amino acids needed to grow the seedling as quickly as possible comes from the degradation of proteins from the protein storage inside the seeds. The latter are synthesized during seed maturation and deposited as protein, which are specialized vacuoles. During germination, the mobilization of the seed takes place when, under the action of specific proteases, amino acids are synthesized sequentially and are transported to the germ. The aim of this study was to initiate the characterization of malt proteinases wheat proteinases by the method in solution with different substrates to quantitatively measure the activities of different proteinase groups.

MATERIAL AND METHOD

Wheat grains were germinated on a pilot-scale system under optimal conditions of temperature, humidity and aeration. The grains were soaked (soaked in water with alternate air rest of 4 hours) at 18°C up to 45%
humidity (16 hours) and germinated at 18°C for five days. During germination, seeds were slowly rotated in the dark at 100% humidity. During this process, grain samples were collected before soaking (ungerminated or dormant seeds), at the end of soaking (from steep), and every 24 h thereafter for an additional five days, and the collected samples were immediately frozen in liquid nitrogen and stored at -20°C. Before use, the wheat samples were lyophilized and were not cut after germination to avoid loss of activity due to high oven temperatures during the lyophilization period.
Germinated wheat milling fractions were obtained by grinding three-day germinated and lyophilized samples (5.0 kg) at 14.5% moisture on a laboratory stone mill to obtain eight streams: bran, bran and six flour fractions (B1, B2, B3, C1, C2, and C3). B fractions come from successive breaking rolls and C fractions come from successive reducing rolls. Germinated whole wheat flour or milling fractions were extracted by mechanical agitation with 0.05 M sodium acetate buffer solutions (pH 5.0) containing 2.0 mM cysteine (1:10, w/v) for 30 min at 7°C. After centrifugation (15,000 × g, 4°C, 15 min), the supernatants were paper-filtered sprouted whole wheat flour extracts sprouted wheat extracts from different milling fractions were used to analyze proteolytic activities. In some cases, grains were treated with 20% sodium hypochlorite bleach to kill or wash away contaminating microbes.
Proteolytic activities

Hemoglobin hydrolyzing activity. Hemoglobin solution (0.25 ml, 1.0%, w/v, in 0.2 M sodium acetate buffer, pH 4.0), 0.2 ml of sodium acetate buffer (0.2 M, pH 4.0) and 0.05 mL of GRWME or GREs were mixed. After incubation (150 min, 40°C), the reaction was stopped by adding 0.4 ml of cold (7°C) 10.0%, w/v,trichloroacetic acid (TCA);the precipitated proteins were removed by centrifugation (10,000 × g, 10 min). Levels of free α-amino nitrogen of the supernatants were tested with trinitrobenzenesulfonic acid reagent (TNBS) (0.3%, v/v, in 0.2M sodium phosphate buffer, pH 8.0) using L-leucine as standard. For this purpose, the supernatant (0.025 mL) and TNBS reagent (0.225 mL) were incubated for 20 min at 50°C, after which the reaction was quenched with 0.2 M HCl (0.75 mL). The absorbance of the solution was measured at 340 nm. Under the such conditions, the plot of absorbance versus time was linear for at least 210 min of incubation. The standard reaction the mixture was prepared by mixing 0.35 mL of azocasein (1.4%, w/v,
in 0.05 M McIlvaine buffer, pH 5.5) and 0.25 mL of GRWME or HARD. After incubation (4 hours, 40°C), the reaction was stopped addition of 0.5 ml of cold (7°C) 10% TCA; precipitated proteins were removed by centrifugation (10,000 × g, 10 min). Dilute
Sodium hydroxide (0.5 M) was added to an equal volume of the supernatant. The mixture was allowed to stand for 20 min and absorbance

RESULTS AND DISCUSSIONS

Characteristics of wheat extracts from wholemeal flour During germination sodium acetate buffer (0.05 M, pH 5.0) is an effective extraction buffer for the solubilization of proteolytic enzymes from whole mass ungerminated wheat (Brijs et al 1999). Addition of 2.0 mM cysteine to this buffer strongly increased the proteolytic activity extracted from sprouted wheat. Therefore, it was added to all extraction buffers. It could be observed that the global proteolytic activity of wheat grain increased during the first three days of germination and remained constant thereafter. The hemoglobin hydrolyzing activity after three days of germination was ≈ 7.5× higher than in non-germinated ones. Similar results were found when proteolytic activity in germinated wheat grain samples it was measured with azocasein. When exoproteolytic activities were measured as a substrate, the increase in activity was not as pronounced as in the case of hemoglobin and azocasein. However, there was still more exoproteolytic activity in three-day-germinated grainsthan in ungerminated grains. In contrast, BAPA-ase activity remained almost constant during the germination process. To guarantee that the activities detected were not due to microbial contamination, samples treated with bleach were analyzed for proteinase activity well. No decrease in protease activity was observed.
Data on laboratory milling of three-day-germinated wheat are as follows: The yield of flour was 67%, which was significantly higher than that of ungerminated wheat grains. This is probably due to an increased degradation of the inner cell wall components of the grain
Assays of the grinding fractions indicated that the proteolytic activities increased steadily as the nitrogen and ash content of the fractions increased. Hemoglobin and azocasein hydrolyzing activities in bran and sputum were higher than those of different flour fractions .

CONCLUSIONS

The bran fraction had the highest proteolytic activity, but the high activity of the C1 fraction, compared to that of the ungerminated wheat sample, is remarkable. The relative amount of the C1 milling fraction to the germinated wheat increased, while the bran and bran fractions decreased. Proteolytic activities of different milling fractions of germinated wheat the grains were much larger than those of ungerminated wheat grains.

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