Inactivation of Alpha-Amylase Activity by Purothionins

To the Editor:

Cereal Chem. 59(4): 321

In 1943, Kneen and Sandstedt discovered that wheat grains contain inhibitors of α-amylase enzymes (Kneen and Sandstedt 1943). The inhibitors from wheat were soon shown to suppress the activities of α-amylase enzymes from many nonwheat sources, but only recently were proteins isolated from hexaploid wheats reported to have the ability to inhibit α-amylases from malted wheat (Warchalewski 1977) and wheat germinated 48 hr (Peruanskii and Gabsattarova 1979).

We tested α- and β-purothionins as possible inhibitors of the activity of wheat α-amylase. We used the amylase assay method of Mathewson et al (1982), which involves the digestion of a covalently-dyed amylose substrate, with subsequent measurement of the color released into solution under specified conditions. The method is said to be relatively specific for α-amylase, being only slightly affected by even high concentrations of β-amylase. A crude water-extract of field-sprouted wheat flour was therefore used as an enzyme source. The purothionins were extracted from durum wheat flour and purified by a method previously described (Mak and Jones 1976).

We used a simple technique in which 0.5 mg of purothionin was incubated with 1 ml of flour extract (65 mDU enzyme activity, aqueous extract from 60 mg of flour) for 10 min at 27°C. The enzyme activity was then determined by reaction with one substrate tablet in a total volume of 10 ml at 60°C for 3 min. By this crude technique we found that α_1-purothionin inhibited 45% of the amylase activity, whereas β-purothionin inhibited 39% of the activity found when no inhibitor was present.

A fully controlled experiment, including controls in which the amylase activity was destroyed by acid treatment and subsequent neutralization (Meredith 1970), showed that β-purothionin did not interfere with the assay method. However, when calcium chloride (10^{-5} mol) was included in the inhibition-incubation mixture containing β-purothionin, the amylase activity was not inhibited. In the absence of calcium chloride, inhibition (34%) occurred as before. Addition of calcium chloride to an enzyme assay without purothionin did not result in augmentation of activity, indicating that calcium was not a limiting factor in the enzyme assay.

The amount of purothionin used in each of these experiments was greater than that normally extractable from the amount of flour (60 mg) used as amylase source. However, if the purothionins in the seed are compartmentalized around the amylase, the effective concentration of purothionin in that small area might be comparable to that used in the experiments. In addition, the reported results were obtained in vitro under conditions that probably differed greatly from those inside a germinating seed and that were not adjusted to allow optimal enzyme inhibition. We concluded that purothionins can inhibit the activity of wheat α-amylase under the conditions of this assay. They may act by controlling the availability of calcium to serve as a cofactor. This may have some importance in the control of dormancy or germination of wheat.

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