Studies of the Bifunctional Alpha-Amylase/Subtilisin Inhibitor from Barley

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Introduction

The bifunctional alpha-amylase/subtilisin inhibitor (BASI) from barley was first characterised in the 1980s. Related proteins are found in other cereals (Fig. 1). This endosperm protein is relatively abundant suggesting a role as a storage protein. The BASI protein binds to the high pI alpha-amylases from barley and wheat (Battershell and Henry 1990) and apparently has a role in the regulation of endogenous alpha-amylase action (Henry *et al.*,1992,1993). The inhibition of bacterial subtilisin by the same protein may be associated with a defense role for this protein. These properties suggest that this seed storage protein has evolved multiple functions. Analysis of a wide range of cereal and grass seeds demonstrated that proteins with action against cereal alpha-amylases are restricted to wheat, barley and rye. The barley protein is more effective in alpha-amylase inhibition than the related proteins from wheat and rye (Henry *et al.*,1991). An ELISA test based upon a monoclonal antibody to the protein has been used to show that the level of the protein was remarkably constant in barley samples from a wide range of environments in Australia but did show genetic variation (Jarrett *et al.*, 1997). The gene from barley has been characterised (Henry and Oono, 1991) and the protein expressed in bacteria (Jones *et al.*,1997). The promoter of the gene encoding the BASI protein was recently isolated and is being characterised in transgenic plants. The properties of this promoter suggest a role in the production of transgenic cereals with altered endosperm protein composition.

Biological and Industrial Applications

The following options have been proposed as roles for this protein or modified versions of the protein.
Improving the quality of flavour for wheat damaged by pre-harvest sprouting.
    (protein expressed in micro-organisms)

Breeding sprouting tolerant wheat varieties
    (trangenic wheat and conventional introgression)

Plant defense
    (transgenic plants with pest and disease resistance)

Cereals with more consistent processing quality in variable environments
(exploits stable expression pattern)

Cereals with improved nutritional quality
(exploits high level of expression)

Promoter Analysis

The BASI promoter was isolated by inverse PCR. Functional analysis of progressive deletions were conducted using the green fluorescent protein (*gfp*) reporter gene. Motifs confining seed specific expression and hormonal regulation were identified. The expression of *gfp* in transgenic barley and rice was compared to that with constitutive promoters.

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         1          10         20          30         40         50         60
Rice      APPPVYDTEGHELSARDGSYYVLPASPGHGGLTMAPRRVIPCPLLVAQETDERRKGFPVR
Wheat     DPPPVHDTDGNELRADANYYVLPANRAHGGLTMAPGHGRRCPLFVSQEADGQRDGLPVR
Barley   ADPPPVHDTDGHELRADANYYVLSANRAHGGGLTMAPGHGRHCPLFVSQOPNGQHDGFPVR
           ****   **   * **   **   *****  *    *********          ***  *  *       * *  *

                   70         80         90         100        110        120
Rice     FTPWGGAASPRTLRVSTDVRIRFNAATLCVQSTEWHVGDEPLTGARRVVTGPLIGPSPSGR
Wheat    IAPHGGAPSDKIIRLSTDVRISFRAYTTCVQSTEWHIDSELVSGRRHVITGPVRDPSPSGRRR
Barley   ITPYGVAPSDKIIRLSTOVRISFRAYTTCLQSTEWHIDSELAAGRRHYVITGPVKDPSPSGRRR
          *   * * *        ******  * *  * * ******   *    * *    * **          ******

                  130        140        150        160        170
Rice     ENAFRVEKYGG- - - - - YKLVSCRDSCQDLGVSRDGAGAAW – IGASQPPHVVVFKKARPSPP
Wheat    ENAFRIEKYSGAEVHEYKLMACGDSCQDLGVFRDLKGGAWFLGATEPYHVVVFKKAPPA A
Barley   ENAFRIEKYHGAEVSEYKLMSCGDWCQDLGVFRDLKGGAWFLGATEPYHVVVFKKAPPA
         ***** ***  *        ***  *  *  ******  **   * **   **  *  **********
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Figure 1 . Comparison of bifunctional and alpha-amylase/subtilisin inhibitors from rice, wheat and barley (Ohtsubo and Richardson, 1992).

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