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Review

The endogenous endoprotease inhibitors of barley and malt and their roles in malting and brewing

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Abstract

Endoproteases play an important role in barley germination by controlling the hydrolysis of the grain's storage proteins into peptides and amino acids that are needed by the young plant. During malting, the commercial version of this process, many high M_r barley biopolymers are converted into malt nutrients that can be utilized by yeasts during brewing. However, barley and malt both contain endogenous proteins that inhibit the enzymatic activities of these proteases. High levels of these inhibitors can cause brewing problems by preventing the proteases from producing optimal levels of soluble proteins and amino acids. Both high and low M_r inhibitors of cysteine proteases occur in barley and malt. Two of the high M_r inhibitors, lipid transfer protein 1 (LTP1) and LTP2, have been purified and studied. Recently, members of the trypsin/*alpha*-amylase inhibitor protein family (CM proteins) have been shown to inhibit the activity of SEP-1, a purified serine class barley protease. No inhibitors of aspartic proteases or metalloproteases have yet been purified, but it has been reported that endogenous metalloprotease inhibitors do exist. The inhibitors of the cysteine proteases and metalloproteases are probably the ones most important for brewing, because members of these two protease classes apparently catalyse most of the protein hydrolysis that occurs during malt mashing and, presumably, also during malting. More biochemical studies are needed to clarify how these proteins interact with the proteases to control protein hydrolysis during germination.

Keywords: Protease; Inhibitors; Protein solubilization; Malting; Barley; Germination

1. Introduction

The endoproteases of germinating seeds are important for seedling development because some of them degrade storage proteins to supply the amino acids that are required for the growth of the new plant. These hydrolases are also commercially important because they are critical for making malt, which is widely used in food processing and forms the basis of beer production (Jones, 2005). Malt contains many proteases, including multiple representatives from each of the four common protease classes, the aspartic, serine, cysteine and metalloproteases (Zhang and Jones, 1995). Some members of the cysteine, aspartic and metalloprotease classes are apparently directly involved in solubilizing barley storage proteins during malting and during the first (mashing) phase of brewing (Jones and Budde, 2005). If any serine proteases affect protein solubilization, they do so indirectly.

However, it is not possible to define the protein solubilization (hydrolysis) that occurs in germinating barley simply by studying its endoproteases, because there are proteinaceous (and non-protein) compounds in barley and malt that strongly inhibit some of these enzymes and thereby affect the process. In this review, the endogenous protease inhibitors of barley and malt are discussed, with seeds and malt being considered as two aspects of a single system. Thus, an inhibitor from ungerminated seed that inhibits a protease from malt will be considered an endogenous inhibitor. However, the protease and inhibitor complements of barley seeds and of malt are quite different

Abbreviations: 2-D, two dimensional; CMC, carboxymethylcellulose ion exchanger; E, uncomplexed endoprotease enzyme molecules; E-I, an enzyme-inhibitor complex; FAN, free amino nitrogen; HPLC, high performance liquid chromatography; I, uncomplexed endogenous protease inhibitor molecules; IEF, isoelectric focusing; LTP, lipid transfer protein; M_r, molecular mass; PAGE, polyacrylamide gel electrophoresis; SP, soluble protein.

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(Jones, 2005). In addition to the endogenous protease inhibitors, barley also contains proteins that inhibit proteases from microorganisms and animals, but these inhibitors and their complementary enzymes have been reviewed previously (e.g. Murdock and Shade, 2002; Shewry, 1999) and therefore are not discussed in detail in this review.

The processes and terminologies involved in malting and mashing are described in Jones (2005) and an in-depth description of the processes can be found in Hardwick (1994).

2. Early studies

2.1. The effect of unmalted barley on mashes

In 1962, Birtwhistle and colleagues (Birtwistle et al., 1962) tested the effect of using unmalted wheat as an adjunct (a cheap source of starch) during brewing, using it to replace 25% of the normal barley malt complement. Good brews were obtained but the nitrogen (soluble protein, SP) contents of the worts were low. With brews made with 50% wheat the fermentations were often slow and this was attributed to the fact that their wort nitrogen contents were only about half those of all-malt brews. The same phenomenon was even more evident when unmalted barley was used as an adjunct (Hudson, 1963). Building on and extending these observations, Enari et al. (1964) showed that ungerminated barley contained compounds that inhibited malt endoproteases and that both high and low molecular mass inhibitors were present. The addition of 20% unmalted barley had little effect on the wort SP or free amino nitrogen (FAN) levels of infusion mashes, but when the proportion of barley was raised to 60% both the wort SP and free amino acid levels were strongly depressed (Linko et al., 1966). The addition of either unmalted maize or wheat to mashes gave similar but smaller effects, showing that these cereals also contained endoprotease inhibitors. It was also noted that the inhibitors were very stable, remaining unaffected by heating at 100 °C for at least 3 h.

2.2. Studies with partially purified inhibitors

Enari and Mikola (1968) prepared crude inhibitor solutions from ungerminated barley and tested their effects on the endoproteases present in a green malt extract. They reported that 85% of the green malt endoproteolytic activity could be inhibited and that both cysteine and metalloproteases were inactivated. They noted that the inhibitory activity fell sharply as germination progressed, and proposed that the inhibitors were being destroyed. These experiments were performed at pH 5.4 in the presence of 2-mercaptoethanol (2-ME), a strong reducing agent. These conditions are favorable for the action of the malt cysteine proteases but they strongly impede the activities of the serine and metalloproteases (Jones, 2005; Jones and Budde, 2003; Zhang and Jones, 1995). The observed inhibition was thus probably different from that would occur a real mash, at pH 6.0.

The endoprotease inhibition activities of two barley cultivars disappeared just prior to the time during malting at which the endoproteolytic activities dramatically increased (Mikola and Enari, 1970), raising the possibility that the increase in endoproteolytic activity was due to the destruction of the inhibitors. However, this was not the case, since calculations showed that there was insufficient inhibitor present in barley for its destruction to account for the large increase in enzymatic activity observed. There was, however, sufficient inhibitor in resting grain to inhibit the small amount of protease that was present prior to germination (Mikola and Enari, 1970). The authors rationalized that the endoproteinase inhibition that had been seen earlier in some malts (Enari et al., 1964) was due to the presence of certain previously reported low M_r (i.e. non-protein) inhibitors (Mikola and Enari, 1970). This is a moot point now, because Jones and Marinac (1991) have shown that the high M_r (i.e. protein) inhibitors are present in large amounts in malt, as well as barley. Mikola and Enari did not detect the malt inhibitors because during germination the amounts of cysteine proteases increased greatly. The newly formed enzyme molecules formed complexes with the inhibitor molecules already present in the grain, thereby rendering them undetectable (See Section 3.1). In resting grain the inhibitors of the endogenous malt proteases are located in the embryo and endosperm, with most occurring in the embryo (Kirsi and Mikola, 1971).

3. Recent studies

For nearly twenty years, there was no further research on endogenous malt/barley inhibitors, until Jones and Marinac (1991) attempted to extract and characterize the barley protease inhibitors. Their initial extracts showed little inhibitory activity. However, after the samples were boiled to ensure that the activities of co-extracted proteases were not masking the inhibitory activities, the heated fractions strongly inhibited endoprotease preparations (Jones and Marinac, 1991). This observation made it possible to study the barley and malt inhibitors in depth.

3.1. Interactions between the endogenous inhibitors and barley/malt endoproteases

The interactions that occur between barley/malt proteases and their inhibitors was studied by Jones (2001). This work showed that as soon as malt or ground barley seeds were put into water (or possibly in the barley or malt grains themselves), the soluble inhibitors (I) and the soluble proteases (enzymes, E) formed tight, soluble, complexes (E-I). The E-I complexes were readily dissociated by boiling