Gluten hydrolysing proteases of Sunn pest *Eurygaster integriceps* Put. and related wheat bugs

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Key message. Wheat bug salivary gland proteases injected into grain damage gluten proteins responsible for bread quality. The restriction of the activity of these enzymes could be a safe for humans and the environment approach to reduce the detriment.

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Salivary gland (SG) proteases (Ps) from Sunn pest *Eurygaster integriceps* and related wheat bugs (WBs) are injected into grain during feeding and cause deterioration of the gluten proteins that are responsible for the most important bread-making qualities. Sunn bug Ps are also of interest as a diagnostic for wheat grain damage and can be used as modifiers of gluten for food technology or to destroy coeliac disease related epitopes in gluten proteins. The aim of the research was to reveal and study main bug Ps responsible for gluten degradation and find safe for humans approaches to limit their destructive activity or use them for practical needs. WBs Ps have been studied for many years, but owing to their complexity and absence of simple detection methods there is still no complete picture of them. *Methods.* The several substrate-containing replicas (with glutenin, gliadin, gelatin and other proteins) were developed for the detection of Sunn pest Ps after isoelectric focusing (IEF) of proteins extracted from bug's SGs or bug-damaged grains [1]. DNA encoding some Ps were cloned and expressed both in bacteria and yeasts [1, 2]. Several types of Ps were detected in wheat grains damaged by Sunn pest and related species from several regions of Russia and Turkey. Two P groups were identified in damaged grains based on pI: neutral Ps (NPs); with isoelectric points (pI) in the range 6.0-7.0 and alkaline Ps (APs), with the majority of pIs higher than 7.0. The NPs hydrolyse both glutenin and gliadin proteins and cause the most gluten deterioration. APs are capable of hydrolysing not only gluten but also animal proteins, gelatin and (or) casein. Analysis of APs showed that some of them were related to bovine trypsin or chymotrypsin. Both NPs and APs retain activity in dry matured grain for many years. Sunn bug Ps show a high degree of variability in terms of IEF patterns both between damaged grain samples and within one sample. IEF patterns of NPs and APs in combination with the micro gluten sedimentation method can be used as additional highly specific diagnostic criteria of wheat grain damage by WBs. NPs were isolated from bug-damaged wheat grain samples collected in Russia and Turkey and partially sequenced. Homologous to trypsins from arthropods and mammals 28 kDa Ps were capable of cleaving HMWGS specifically between the hexa- and nonapeptide repeats. They differed in cleavage site specificity from NPs of WB *Nysius huttoni* (Konarev et al., 2011). The recombinant forms of one of the Sunn pest SG NPs (rGHP3) hydrolysed gluten proteins and also their recombinant homologs. Using antibodies raised to rGHP3 it was found that in vivo GHP3 accumulated in SGs in secretory granules as zymogens which could be activated by trypsin-like Ps [1]. Results indicated that other NPs and also APs pass through the same path of accumulation and activation in SGs. In this research, proline specific endopeptidase, described earlier by Yandamuri et al. (2014), was also detected in bug's SG but damaged grains only contained weak activity of this enzyme. Sunn pest SGs produce a set of diverse GHPs which complicates the task of designing inhibitors to them. Most of APs from bug's SGs or bug-damaged wheat grains can be inhibited by some known inhibitors of plant or animal origin (e.g. STI, aprotinin) [1]. Since APs in some samples can play a significant role in the deterioration of the gluten quality so the search for more effective inhibitors of their activity may be justified. However, NPs, which are the main destructive factors in bug-damaged grains are almost insensitive to all known proteinaceous inhibitors. Only a few inhibitors including some members of potato chymotrypsin inhibitor I family can reduce the activity of these enzymes at high concentrations and can serve as a base for the design of effective inhibitors of Sunn pest NPs. Another approach to the design of specific NP inhibitors is based on the use of antibodies capable of linking to regions of active centre of the P to prevent its interaction with substrate. One of the fragments of polypeptide chain involved in substrate binding was inserted in a chimeric protein and used to produce antibodies in mice. The antibodies obtained inhibited GHP3 activity [3]. Such antibodies could be used in processing technologies or for the development of wheat forms resistant to WBs.

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