GLUTEOSTOP®

ineo Pharma GmbH

PROFESSIONAL INFORMATION FOR HEALTHCARE EXPERTS ON
GLUTEOSTOP®

Current state-of-science on AN-PEP Enzyme

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GLUTEOSTOP®

NAHRUNGSERGÄNZUNGSMITTEL

GLUTEOSTOP®

HILFT GLUTEN ABZUBAUEN

30.000 PPI*

MINI-TABLETTEN

90

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INTENDED USE

GluteoStop® is a dietary supplement and is exclusively intended to support the break down of residual gluten remaining in a gluten-free diet. GluteoStop® is not intended to replace a gluten-free diet nor to prevent or treat celiac disease, wheat allergy or gluten hypersensitivity.

GluteoStop® contains a specific enzyme, prolyl oligopeptidase (AN-PEP), which cleaves the chain of amino acids (peptides) that forms gluten. Thereby, gluten can be degraded. Therefore, GluteoStop® is an ideal aid for people, who live on a gluten-free diet and want to avoid trouble caused by traces of gluten or hidden gluten in their food product. Several studies have shown that even with a supposedly gluten-free diet the accidental consumption of gluten can be as high as 150 to 400 mg per day.

ACTIVE INGREDIENT

The active ingredient in GluteoStop® is a natural enzyme, the so-called Aspergillus niger prolyl endopeptidase (AN-PEP). It is an enzyme that breaks down polypeptides by cleaving the peptide-chains specifically on the amino acid proline. This specific enzyme has been developed in a way that it shows its optimal performance under acidic conditions as present in the human stomach.

BACKGROUND

Gluten is a naturally occurring protein that is found in wholesome food, especially in those made from wheat, barley or rye. This type of food is the alimentary basis for a majority of people. Other products, like, for example, those based on oat, can contain small amounts of gluten if they are contaminated during the manufacturing process via cross-contamination.[1] But there are many more possible sources of gluten as shown in Table 1.

Gluten from wheat is a mixture of proteins. It consists approximately half-and-half of "gliadins" (prolamin fraction) and "glutenins" (glutelin fraction).[2] Both the gliadins and the glutenins are composed of several sub-types (α,ß,γ,Ω-gliadins and "low molecular weight"/"high molecular weight"- glutenins, respectively). Unfortunately, the digestive enzymes in the human gastrointestinal system are fairly ineffective, even in healthy persons, when digesting gluten proteins because of the unusual high content of the amino acid proline.[2-4] Many consumers avoid wheat and gluten due to the broad spectrum of health problems, including wheat allergy, intolerance of gluten like Coeliac Disease (CD) or Non Celiac Gluten Sensitivity (NCGS).[5]

TABLE 1: Sources for gluten are ubiquitous (adapted from the homepage of the German Celiac Disease Foundation).[18]

SOURCES OF GLUTEN	COMMON FOODS THAT CONTAIN GLUTEN	FOODS THAT MAY CONTAIN GLUTEN	OTHER ITEMS THAT MAY CONTAIN GLUTEN
● Wheat as well as derivatives of wheat –wheat berries - Durum - Emmer - Semolina - Spelt - Farina - Farro - Graham - Khorasan wheat - Einkorn wheat ● Sago of barley and wheat ● Dumplings and wheat noodles ● Taboilé (from bulgur or couscous) ● Kritharaki (greek rice noodles from whwat) ● Panko (Japanese breadcrumbs) ● Wheat starch ● Rye ● Barley ● Triticale (wheat/ rye hybrid)	● Pastas ● Noodles ● Breads and Pastries ● Crackers ● Baked Goods ● Cereal and granola ● Breakfast food ● Panncakes ● Breading and Coating Mixes ● Croutons ● Sauces and gravies -many use flours as thickeners -soy sauce -cream sauces made with a roux ● Flour tortillas ● Malt in various forms ● Beer ● Brewer's yeast	● Cornflakes, polenta, couscous ● French fries (treated) ● Potato products (puree, croquettes, gnocchi, Klossteig, ravioli, dumplings rösti) ● Potato chips (seasoned) ● Energy bar / muesli bar ● Processed meat dishes ● Sweets, candy bars and mints ● Finished soups with flour as thickener ● Light products ● Multigrain tortilla chips or tortillas ● Salad dressings / marinades ● Starch or dextrin ● Meat substitutes (seitan, vegetarian burgers, etc.) ● soy sauce	● Lipstick and other cosmetics ● Lip gloss and lip balm ● Personal care products ● Communion wafers ● Vitamin supplements ● Dietary supplements ● Medical Devices ● Drugs ● Over-the-counter medications ● Art and craft supplies - Modeling clay - Glue/adhesives

Coeliac Disease (CD) is an autoimmune disorder that is triggered by an improper T-cell mediated immune response to consumed gluten, particularly to α-gliadins. [4,6] It can result in the malabsorption of essential nutrients and chronic inflammation of the small intestine mucosa on both a macroscopic and microscopic level. [6,7] Typical symptoms of CD are gastro-intestinal troubles, but extra-intestinal symptoms can be found also in many other organ systems. Atypical manifestations of CD can be of neurologic, psychiatric, musculoskeletal, dermatologic or hematologic nature and/ or influence the endocrine system (including effects on fertility).[7,8]

Epidemiologic data provide evidence that CD prevalence has been doubling about every 20 years, and its present value is about 1-2% of the general population. However, the majority of those with CD have not been diagnosed and consequently not medicated.[9-11]

The cornerstone in the treatment of coeliac disease is to avoid the intake of gluten with the food.[12] A systematic review of 38 studies concerning adult patients with coeliac disease showed, however, that the strict compliance of a gluten-free diet was only in the range between 42% and 91%.[13] Moreover, 50 % of the patients, who comply with a strict gluten-free diet, report that they feel no improvement of the symptoms.[14] Their disorder is diagnosed as the so-called "non-responsive coeliac disease" (NRCD). The most probable cause is an unnoticed unintentionally gluten exposition or gluten intake due to contamination that occurs despite the attempt to live with a gluten-free diet.[15-17]

Gluten can provoke symptoms even in the absence of coeliac disease. This disorder is known as non-coeliac-non-wheat-allergy gluten sensitivity (NCGS). The symptoms resemble those of coeliac disease, but with an increased occurrence of extra-intestinal symptoms like behavioral changes, pain of bones and joints, muscular cramps, leg numbness, loss of weight and permanent fatigue.[5]

It is difficult to determine the prevalence of non-coeliac-non-wheat-allergy gluten sensitivity (NCGS) because its existence has been described in the medical literature only recently.[5] Initially, the prevalence was assumed to be as low as 0.55 %.[19,20] But population-based studies have shown that the prevalence of irritable bowel syndrome (IBS) in Northern Europe is 16 to 25 %, with coincident NCGS estimated to be 28 %.[21] Most likely, the actual prevalence of gluten sensitivity is much higher. Particularly for people with a non-coeliac-non-wheat-allergy gluten sensitivity (NCGS) it will be troublesome to accomplish a strictly gluten-free diet.

To comply with a strict gluten-free diet is a challenge for people of all age, but especially for teenagers and students.[7,22] The avoidance of gluten is difficult because of the abundance of gluten in basic staple like grain products, bread and other bakery products. Individuals, who try to avoid wheat and gluten, will often unintentionally consume gluten by eating products that contain hidden sources of gluten like e.g. soups thickened with wheat flower.[40]

ENZYMES THAT CLEAVE GLUTEN

The oral ingestion of specific proteolytic enzymes is suggested since many years as a promising strategy or adjuvant therapy, resp., for individuals who want to avoid the accidental intake of gluten from food in conjunction with a gluten-free diet.[23,24] In December 2006, the publication describing the Aspergillus niger-derived prolyl endoprotease (AN-PEP) from Stepniak D. et al[26] was distinguished as "Research Highlight" in the renowned international journal of science the "Nature Reviews".[24] The Aspergillus niger prolyl endopeptidase (AN-PEP) has been investigated in numerous in vitro and clinical studies.

ASPERGILLUS NIGER PROLYL ENDOPEPTIDASE (AN-PEP)

Aspergillus niger prolyl endopeptidase or Aspergillus niger prolyl endoprotease, resp., (AN-PEP) is a unique enzyme that is capable of breaking down gluten in smaller units. This enzyme is a naturally occurring enzyme and can be produced by fermentation with the help of Aspergillus niger, a microorganism that is widely used in bio-technology. Many well-known compounds, like citric acid, or numerous enzymes used in food technology are produced in biotechnological fermentation processes. The enzyme AN-PEP is a so-called endopeptidase that means it cleaves peptide bonds over the whole length of the peptide chain whenever a certain amino acid is present. In contrast, an exopeptidase splits the bond only if it is close to one end of the chain (N-terminus or C-terminus).[25] The enzyme AN-PEP works specifically for the amino acid proline, which occurs disproportionately often in gluten. Many of the enzymes present in the human intestinal tract, like pepsin, trypsin or chymotrypsin, are also endopeptidases, but cannot digesting gluten properly. Aspergillus niger prolyl endopeptidase has been assessed in vitro, as well as in several in vivo studies.

ASPERGILLUS NIGER PROLYL ENDOPEPTIDASE (AN-PEP) WITH-STANDS THE ACIDIC CONDITIONS IN THE STOMACH AND REMAINS ACTIVE IN THE GASTROINTESTINAL TRACT

Enzymes that are supposed to digest proteins in the human gastrointestinal tract must be stable under the highly acidic condition present in the stomach. Therefore, the activity of AN-PEP was measured in an in vitro experiment over a wide range of pH values with the result that the Aspergillus niger prolyl endopeptidase works optimally at pH 4-5, remains stable at pH 2 and is completely resistant to digestion with pepsin.(Figure 1).[26]

According to published data the acidity of the chyme in the stomach is changed within 30 min from originally pH = 5 to pH = 3.5, followed by a further decrease to pH = 2 measured about 60 min after intake of the meal. Assessment of gastric emptying showed that 45 min after intake nearly 90 % of the solid food still

exist in the stomach. Beyond the stomach, the pH of the chyme slowly rises and reaches about pH = 5 in the distal part of the duodenum, that is about 50 cm past the pylorus. Therefore, the optimization of the enzyme activity of AN-PEP to pH = 4.5 agrees best upon the physiological processes in the human digestive system.

FIGURE 1: The enzyme AN-PEP shows a high activity over a broad pH range with a maximum around pH = 4.5. Data adopted from Stepniak et al.[26]
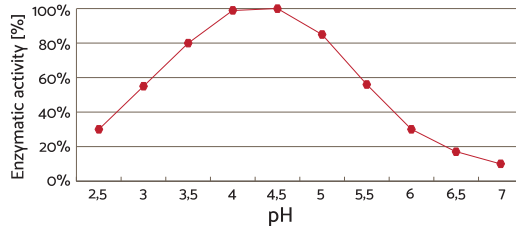
Oral administered enzymes must not only tolerate the low pH-values in the stomach but also withstand other naturally occurring enzymes (endoproteases) in the gastro-intestinal tract. To show its stability, the AN-PEP enzyme was incubated with pepsin at pH = 2 and after certain incubation time its enzyme activity was measured. It was found that the enzyme activity stayed unaltered high for an incubation period of up to 60 min (Figure 2).[26]

FIGURE 2: Resistance to low pH and pepsin digestion. The activity of AN-PEP is hardly reduced in strongly acidic environment (pH = 2) and the presence of pepsin. At each time point, the enzyme reaction of pepsin was stopped with pepstatin, and the activity of AN-PEP enzyme was measured at its pH-optimum. (NT = enzyme activity of AN-PEP at pH = 2 without pepsin). Data adopted from Stepniak et al[26]
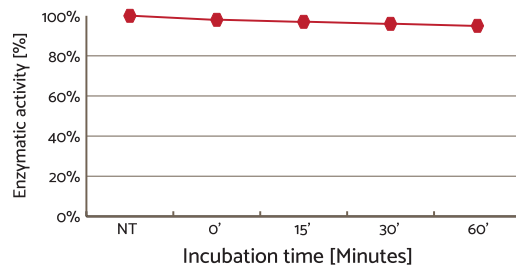
ASPERGILLUS NIGER PROLYL ENDOPEPTIDASE (AN-PEP) BREAKS DOWN GLUTEN

The results of in vitro experiments demonstrate that AN-PEP enzymes, which is the active ingredient in GluteoStop®, is highly efficient in degradation of both gliadin and glutenin molecules and that the AN-PEP treatment led to complete degradation of the T cell epitopes in almost all cases. In one test, 5 mg AN-PEP enzyme pro 1 g of gluten were incubated for 2 hours and then the mixture was analyzed by HPLC-MS. Mass spectrometric analysis of the digested gluten fractions revealed that AN-PEP treatment generated a large number of peptides; out of 152 identified peptides, 128 had a molecular mass 1 kDa. The amino acid sequences of 53 of these peptides were determined, showing that none of these contained the nine-amino acid core of the known T cell stimulatory gluten peptides.[26]

AN-PEP CLEAVES GLUTEN SPECIFICALLY AFTER THE AMINO ACID PROLINE (POST-PROLINE CUTTING ENZYME)

In order to prevent a stimulation of T-cells it is necessary that the used peptidase degrades in particular those gluten epitopes (fragments) that are known to trigger the T-cell mediated immune response. In vitro experiments showed that the enzyme AN-PEP used in GluteoStop® has degraded every tested epitope that is capable of stimulating T-cells. The test included as well the immunogenic alpha-gliadin-peptides AA31-49 (glia31-49), which are considered as toxic epitopes for patients with CD.[27-29]

In another study, 13 different gluten peptides, which were known to trigger a T-cell mediated immune response, were investigated. The results of mass spectrometry confirmed that the AN-PEP enzyme degrades each of the 13 peptides and that that AN-PEP cleaved the peptides specifically after the amino acid proline (Table 2).[26]

TABLE 2: Gluten peptides were incubated with the Aspergillus niger prolyl endoprotease (AN-PEP) and the digestion products were identified by MALDI-TOF-mass spectrometry. Minimal T cell stimulatory sequences are given in bold. Arrows (↔) indicate cleavage sites. (‡) Less efficiently cleaved peptide bonds. (Adapted from Stepniak et al.[26])

GLUTEN EPITOP	MAJOR CLEAVAGE SITES
Glia 31–43	**L G Q Q Q P**↔**F P P Q Q P**↔**Y** P↔Q P Q P F
Glia-α2	**P**↔**Q P Q L P**↔**Y P Q** P Q L P Y
Glia-α9	Q L Q **P**↔**F P**↔**Q P Q L P**↔**Y**
Glia-α20	P **F R P**↔**Q Q P**↔**Y P‡Q** P Q P Q
Glia-γ1	Q P Q Q **P**↔**Q Q S F P**↔**Q Q Q** R P↔F
Glia-γ2	Q Q **P**↔**Y P Q Q P**↔**Q Q P F** P Q
Glia-γ30	V Q G Q G I **I Q P**↔**Q Q P A Q** L
Glt-17	Q Q P **P**↔**F S Q Q Q Q Q P**↔L P Q
Glt-156	Q Q P **P**↔**F S Q Q Q Q S P‡F** S Q
Glu-5	Q Q U S Q P↔Q U P↔Q Q Q **Q U P**↔**Q Q P Q Q F**
Glu-21	Q P Q P↔F P↔Q **Q S E Q S Q Q P**↔**F Q P Q** P F
DQ8-Glt	Q **Q G Y Y P**↔**T S P**↔**Q** Q S
DQ8-Glia	S G **Q G S F Q P**↔**S Q Q** N

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THE AN-PEP ENZYME RAPIDLY BREAKS DOWN GLUTEN

The ideal case is that gluten is cleaved efficiently already in the stomach so that gluten peptides cannot trigger the T-cell mediated immune responses in the small intestine. In vitro investigations have shown that the AN-PEP enzyme can break down gluten peptides within a very short period of time.

To measure the rate and efficiency of the enzyme's activity, the AN-PEP enzyme was incubated at pH = 4.5 with four different gluten peptides with high and low molecular weight, resp.: gliadin (Glia-α-9), gliadin (Glia-γ-1), LMW-glutenin (Glt-156) und HMW-glutenin (Glt). Then, the enzyme reaction was stopped at time points 0, 5, 10, 30, 60 and 120 min after start and the concentration of the remaining intact, undegraded peptides were measured via Q-TOF-mass spectrometry. The Half-Life of the reactions determined from the corresponding plots (concentration versus reaction time) ranges between 2.4 and 6.2 min. (Table 3).[26] This implies that the degradation of the selected gluten peptides by the AN-PEP enzyme is in average by a factor of 60 faster than with a comparable prolyl oligopeptidase derived from Flavobacterium meningosepticum (FM-POP).[26]

TABLE 3: At pH = 4.5 AN-PEP degrades fast Gluten-peptides with high and low molecular weight, resp. (adapted from Stepniak et al.[26]).

Gluten Peptides	Half-Life [minutes]
α-Gliadin (Glia-α-9)	3.87
γ-Gliadin (Glia-γ-1)	2.36
LMW-Glutenin (Glt-156)	5.80
HMW-Glutenin	6.19

In another study, degradation of gliadin peptides by AN-PEP under acidic conditions could be visualized by Western blotting within 30 minutes. The equivalent of 33,000 PPI (Protease Picomol International) AN-PEP per gram of gluten was sufficient. The results obtained in this study demonstrated that the addition of a carbonated drink increases the efficiency of gluten degradation by AN-PEP suggests that any dietary component that reduces chyme pH could improve AN-PEP activity and, thus, reduce gluten detoxification time.[30]

THE AN-PEP ENZYME PREVENTS STIMULATION OF T-CELLS BY GLUTEN-PEPTIDES

Part of the pathophysiology of gluten-induced autoimmune diseases involves the stimulation of T cells by gluten peptides. In vitro studies have shown that AN-PEP enzyme can prevent this stimulation:

Stepniak et al.[26] incubated the AN-PEP enzyme with gluten and the digestive enzymes pepsin or trypsin. After 5, 10, 15, 30, 60, and 120 minutes, as well as on the next day, the enzyme reaction was stopped and the degradation of gluten epitopes (fragments) that stimulate T-cells was measured by competitive ELISA technique. To this end, antibodies were chosen that act specifically on four different gluten epitopes (α-gliadin, γ-gliadin, and glutenin with high and low molecular weight). These epitopes are known to trigger T-cell mediated immune responses. In accordance with expectation the AN-PEP enzyme very efficiently cleaves gliadin epitopes, whereas glutenins show a higher resistance towards proteolysis and are therefore degraded much slower. Already after 30 minutes two gluten epitopes, α-gliadin, γ-gliadin, were below detection limit. Although glutenins are degraded slower after 120 minutes all of the glutenins with low molecular weight were degraded and 90% of the glutenins with high molecular weight. In the sample that was incubated overnight none of the gluten epitopes could be detected.[26]

In an in vitro TIM Modell (TNO gastro-intestinal model), a multi-compartmental model, designed to realistically simulate conditions in the lumen of the gastro-intestinal tract, the ability of AN-PEP enzyme to degrade T-cell stimulatory gluten epitopes was studied. Two experiments were performed. In the first experiment, a slice of white bread (70g bread with 5g gluten) was tested in the described TIM system with or without the co-administration of AN-PEP (200 mg enzyme pro g protein). For the second experiment, a standard fast food menu was chosen. Samples of the digested meals were taken from the stomach, duodenum, jejunum and ileum compartments at time zero (beginning of the experiment) and 15, 30, 45, 60, 120, 150, 180, and 240 minutes after the start of the experiment. In these samples the levels of immunogenic peptides from gliadins and glutenins (Glia-α-9, Glia-γ-1, glutenin with high (HMW glutenin) and low molecular weight (LMW glutenin) were assessed by monoclonal antibody-based competition assays, Western blot analysis and proliferation T-cell assays. Whereas in the control sample gluten epitopes could be detected even after 120 minutes by the highly specific ELISA technique, no gluten epitope could be detected after 90 minutes in the stomach compartment, if the enzyme AN-PEP was added. Likewise, none of the investigated gluten epitopes could be detected in the following compartments (duodenum, jejunum and ileum). These results prove that within this period of time, which the food usually needs to pass the stomach, the simultaneous intake of the enzyme AN-PEP results in a complete disappearance of T-cell stimulating peptides (gliadins and glutenins).[31]

THE AN-PEP ENZYME IN CLINICAL STUDIES

The ability of the AN-PEP enzyme to degrade gluten was also investigated and verified in human clinical studies.

In a study by Salden et al.[35], healthy subjects were given over 4 days of testing in a randomized, double-blind, placebo-controlled, cross-over study either a low calorie (143 kcal) or high calorie (405 kcal) gluten containing diet via a nasoduodenal catheter. The gluten content was 4 grams. After administration of a placebo or the AN-PEP enzyme (1.6 Mio PPI) gastric and duodenal content was sampled at baseline (t = 0 min), and after start of meal infusion at t = 15, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 min. The gastric and duodenal samples were used to calculate 240-min area under the curve (AUC0–240 min) of α-gliadin concentrations. The amount of α-gliadin epitope DQ2.5-glia-α3 was examined by ELISA (Gluten-Tec® ELISA). The results were in addition verified via western blotting (DQ2.5-glia-α1 Epitope). To assess the presence of AN-PEP enzyme (protein) in gastric and duodenal samples, the protein in the samples was separated on SDS-PAGE followed by Coomassie Blue staining.

The AN-PEP enzyme has significantly increased the degradation of gluten in the stomach. An increase of the caloric density extends the retention time of the food in the stomach from 1.5 h to 3 h. Because the AN-PEP enzyme degraded the gluten added to the low caloric meal already in the stomach no incremental effect was observed when the energy density of the meal was increased. Already after 60 minutes the total amount of gluten was degraded in the stomach below the limit of detection. In the group that received the meal with placebo gluten could be detected in the stomach still 3 hours after administering. Salden et al. could prove without doubt the ability of the AN-PEP enzyme to efficiently degrade gluten and identify the stomach as the primary site of action for gluten digestion.[35]

In another randomized, double-blind, placebo-controlled pilot study evaluating the safety and efficacy of AN-PEP enzyme in celiac disease, Tack et al. show that the intake of AN-PEP enzyme is safe and is well tolerated by the subjects. In trial phase 1, the participants received for 2 weeks every day bread (with 7g gluten) together with AN-PEP. In phase 2, the participants consumed every day bread (with 7g gluten) together with AN-PEP or placebo. No serious adverse events occurred during the trial and no patients withdrew during the trial. The mean score for the gastrointestinal subcategory of the celiac disease quality (CDQ) was relatively high throughout the study, indicating that AN-PEP was well tolerated and appeared to be safe in celiac patients. Because of the short period of gluten intake no significant histological changes could be observed. However, the primary endpoint was not met due to lack of clinical deterioration upon placebo, impeding an effect of AN-PEP.[33]

The efficacy of the AN-PEP enzyme in a complex physiological meal setting was investigated in a further randomized, placebo-controlled crossover study by König et al.[36]. 18 gluten-sensitive individuals consumed a porridge containing 0,5 g gluten together with two tablets either containing a high or low dose of the AN-PEP enzyme, or with placebo. Gastric and duodenal content was sampled via a multi-lumen-nasoduodenal catheter 5, 15, 30, 45, 60, 75, 90, 105, 120, 150, and 180 minutes after intake, and areas under the curve (AUC) of gluten concentrations were calculated. By means of a Gluten-Tec ELISA assay (EuroProxima B.V., Arnhem, The Netherlands) the amount of the gluten epitope DQ2.5-glia-α3 (formerly known as glia-α 20) was quantified and, using the manufacturer's information, the equivalent amount of gluten calculated. In both the high and the low dose the AN-PEP enzyme significantly reduced the gluten concentration (AUC 0 - 180 min) in the stomach and in the duodenum in comparison to the placebo. In the stomach, gluten levels were reduced from a median with placebo of 176.9 µg × min/ml (interquartile range 73.5–357.8) by 88 % to 22.0 µg × min/ml (10.6–50.8, p = 0.001) with the high dose and by 86 % to 25.4 µg × min/ml (16.4–43.7, p = 0.001) with the low dose of the AN-PEP enzyme. In the duodenum, gluten levels were reduced by a comparable extent, namely from 14.1 µg × min/ml (8.3-124.7) with placebo by 56% to 6.3 µg × min/ml (3.5-19.8, p = 0.019) with the higher dose and by 48% to 7.4 µg × min/ml (3.8-12.0, p = 0.015) with the lower dose of the AN-PEP enzyme.

The results of this randomized, double-blind, placebo-controlled pilot study showed that a high as well as a low dose of the AN-PEP enzyme administered in form of tablets significantly reduced the gluten concentration in the stomach and in the duodenum of gluten sensitive persons. These results also confirm the findings by Salden et al.[35] who investigated the efficacy of gluten degradation by the AN-PEP enzyme in the stomach of healthy volunteers. In the study of König et al.[36] about 0.5 g of gluten was added to a complex, solid meal. This rather low amount of gluten was chosen, to represent the amount of gluten described by literature that gluten-intolerant subjects might unintentionally consume even when trying to avoid gluten.[37,38]

Whereas Salden et al. administered the AN-PEP enzyme in liquid form, König et al. investigated the efficacy of this enzyme in form of tablets added to a solid meal because this reflects better the daily practice. Thereby it could be shown that administration of the AN-PEP enzyme in form of tablets to a conventional, complex solid meal setting results in a significant degradation of most of the gluten already in the stomach before it entered the duodenum.

OTHER ENZYMES THAT ARE BELIEVED TO DEGRADE GLUTEN, E.G. DPP-IV

Dipeptidyl peptidase IV (DPP-IV) is one of the most commonly used enzymes in dietary supplements aimed to support the digestion of gluten. Like the AN-PEP enzyme the DPP-IV is a naturally occurring enzyme and very often is also produced by means of an Aspergillus species. In contrast to the AN-PEP enzyme the DPP-IV is an "exo protease" with a special property: it cleaves exclusively proteins, which contain the amino acid proline in the second to last positon counting from the N-terminus of the protein or peptide.[34] It has been shown that because of this high specificity the DPP-IV enzyme is not capable of degrading gliadin peptides except if other proteases are present.[4]

In contrast, the AN-PEP enzyme is able to digest gliadin peptides and gluten without additional enzymes.[26,30,31,32] In addition, DPP-IV loses its activity and functionality to degrade gluten at pH values below pH = 4, whereas the AN-PEP enzyme is very well able to efficiently cleave gluten even at significantly lower pH's around pH = 2.[25,26,34] In a recent study by Janssen et al. the very limited efficacy of various commercial available enzyme products compared to AN-PEP enzyme was shown. For this purpose, five different enzyme preparations, mainly with DPP-IV enzyme and AN-PEP were incubated with gliadin for a maximum of 30 minutes at 37°C and then measured by means of R5 monoclonal antibody-based ELISA to determine the remaining gluten. While the investigated AN-PEP enzyme completely degraded the immunogenic peptides at pH 4.5 after only 10 minutes of incubation, the other enzyme preparations investigated were unable to completely break down the R5-reactive peptides neither at pH 4.5 nor at pH 6.2. Since the R5-ELISA test cannot specifically detect all immunogenic epitopes an additional investigation of the degradation products was performed by mass spectroscopy to detect the presence of known immunogenic peptide sequences. In particular, the degradation products of the gluten epitopes of α-gliadin, the 33-mer peptide and the γ-gliadin, the 26-mer peptide were investigated. While long, immunogenic peptide sequences could still be measured in DPP-IV preparations even for a long incubation time, the measurable fragments during treatment with AN-PEP enzyme were for the most part only eight amino acids long or smaller and thus too small to contain immunogenic epitopes. Finally, the T-cell proliferation triggered by the digested fragments of the 33-mer was measured using a gluten-specific T-cell clone (T-cell clone N10 specific for the DQ2-glia- α1 epitope). It could be shown that the AN-PEP enzyme, in contrast to the other enzyme preparations investigated, was able to neutralize the T-cell-stimulating properties of the 33-mer.[39]

SUMMARY

The oral ingestion of gluten-degrading enzymes is a very promising additional possibility (adjunctive therapy) for all those individuals who follow a gluten-free diet and want to avoid disorders due to the unintended intake of gluten, e.g. through contamination. The enzyme used in GluteoStop® is a so-called Aspergillus Niger prolyl endopeptidase (AN-PEP) and is a digestive enzyme specific for the amino acid proline. The naturally occurring AN-PEP enzyme has been extensively investigated in numerous in vitro and in vivo studies. It is resistant to the harsh conditions of the gastrointestinal tract, including its extreme pH-values and endogenous digestive enzymes (pepsin / trypsin).

Numerous studies have shown that the AN-PEP enzyme can rapidly and efficiently degrade gluten peptides and intact gluten protein, thereby helping to prevent that gluten peptides stimulate T cells reaction. The increasing number of scientific studies and extensive investigations on the AN-PEP enzyme suggest that this enzyme is clearly superior to other common, commercially available enzyme preparations.

GluteoStop® with AN-PEP enzyme can not be used as a substitute for a gluten-free diet or for the treatment and prevention of celiac disease, wheat allergy or gluten hypersensitivity. But many people who want or need to eat a gluten-free diet can benefit from products with AN-PEP enzyme as a supplement in addition to a gluten-free diet.

Recent clinical studies have shown that the administration of the AN-PEP enzyme in form of tablets with a dose of 83'300 PPI (Protease Picomol International) can almost completely degrade 500 mg gluten in a complex physiological meal setting. In latest scientific studies it has been found that even celiac disease patients who follow a strict gluten-free diet unintentionally, inadvertently ingests an average of 150-400 mg of gluten daily.[40]

Therefore, GluteoStop® is highly recommended as additional adjuvant / adjunctive therapy to reduce unintentionally accidental exposure to dietary gluten in addition to a strict gluten-free diet.

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