Blocking Peptides Decrease Tissue Transglutaminase Processing of Gliadin in Vitro

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Tissue transglutaminase (tTG) plays an important role in celiac disease pathology as it catalyzes deamidation and cross-linking of specific gluten peptides and converts them into potent epitopes recognized by intestinal T-cells. We investigated whether synthetic peptides with high affinity to gliadin could alter tTG activity on gliadin and whole gluten digest. The immobilized substrates were incubated with synthetic peptides identified by the phage display technique and a control peptide with no affinity to gliadin. Transglutaminase activity was measured with time resolved fluorescence. The mean tTG activity, compared to that of the control without the peptides, was reduced by 31, 33, and 36% for three selected gliadin-binding peptides, and 30% for the peptide pool ($P \leq 0.001-0.004$) when gliadin was the substrate. Finally, substrate specificity experiments suggested that avenin was processed in a manner similar that used for gliadin during in vitro assays with tTG. The results showed that the blocking peptides efficiently reduced tTG processing of gliadin in vitro, and this strategy will be further investigated as an alternative therapy for celiac disease.

KEYWORDS: Celiac disease; gliadin; synthetic peptides; time resolved fluorescence; tissue transglut-aminase

INTRODUCTION

Celiac disease (CD) is a complex inflammatory condition that is diagnosed in approximately 1% of European and American populations (*1, 2*). Clinical presentation varies from asymptomatic to severe gastrointestinal complaints including abdominal pain, diarrhea, weight loss, and malabsorption related conditions. Histologically, the disease is presented with infiltration of intraepithelial lymphocytes into the intestinal mucosa, which leads to lesions of the small intestine with villous atrophy and crypts hyperplasia. Symptoms develop because of an inadequate immune response and include antibody production and cytotoxic reaction to specific peptides originating from ingested wheat gluten as well as proteins from related cereals (*3−6*).

Wheat gluten proteins, gliadins and glutenins, have a unique amino acid composition with high content of proline ($\pm15\%$), hydrophobic amino acids ($\pm19\%$), and glutamine ($\pm35\%$). Moreover, they contain domains with numerous repetitive sequences rich in those amino acids (*7, 8*). Because of this glutamine- and proline-rich structure, gluten proteins are resistant to complete digestion by gastric and pancreatic human peptidases (*9, 10*).

Tissue transglutaminase (tTG) is an enzyme (EC 2.3.2.13) from the transglutaminase family of calcium dependent acyltransferases (*11*). It can be found in extracellular compartments of the subepithelial region of the small intestine where it catalyzes the formation of intermolecular, covalent cross-linking between primary amines or proteins (*12*). During the first stage of this reaction, a thiol ester is formed between a glutamine residue (an acyl-donor) from a gluten peptide and a cystine residue from the tTG active site, and ammonia is released as a reaction byproduct. During the second stage of the reaction, the acyl group is transferred to the acyl acceptor (often a lysine residue), and an isopeptide bond is formed. If the lysine residue is not present, tTG deamidates glutamine to glutamic acid by catalyzing only the first reaction stage after which the thiol ester is hydrolyzed (*12, 13*).

The tTG enzyme has been identified as an autoantigen in CD and is now considered to play an important role during disease pathology as it is targeted by highly specific antiendomysial antibodies, and is also involved in the generation of epitopes for intestinal T-cells (*12, 14−16*). Gluten peptides that cross the intestinal mucosa barrier are preferentially deamidated by tTG, which leads to enhanced stimulation of CD4+ T-cells and triggers a cascade of immune responses that result in mucosal inflammation (*12*). It has also been hypothesized that tTG can catalyze the cross-linking of gluten peptides with the mucosal matrix proteins, which could result in a longer exposure of these antigens in the mucosa of the small intestine. Moreover, high molecular weight

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complexes of tTG−gluten are targeted by disease specific IgA antibodies (*14*, *17*).

So far, the only treatment available for CD patients is a gluten-free diet. However, the abundance of gluten in several nonbakery food products as well as contamination of various foods with gluten during production processes makes the diet difficult to follow (*18*, *19*). This is why new complementary therapies allowing ingestion of low amounts of gluten are highly needed. Reduction of tTG-catalyzed gluten modification could possibly hinder the activation of the immune system and minimize the development of inflammation. To test this hypothesis, in the present work we asked whether the tTG activity on gluten could be changed by blocking gluten sequences with synthetic peptides selected for high affinity to wheat gliadin.

The effect of three blocking peptides on tTG-based intact gliadin modification was investigated in this study. Moreover, whole gluten digest obtained during simulated human gastro-intestinal digestion was incubated with the blocking peptides. Their potential to block gliadin sequences when interacting with gluten fragments was thereby investigated. Finally, an oat pro-lamin, avenin, was tested in a separate assay to examine whether it could act as a tTG substrate in vitro.

MATERIALS AND METHODS

Materials. Wheat gluten (G5004), pepsin from porcine gastric mucosa (P6887), pancreatin from porcine pancreas (P1750), porcine bile extract (B8631) *N,N*-dimethylcasein, bovine serum albumin (BSA), guinea pig liver transglutaminase (T5398), and Tween20 (T-20) were from Sigma Aldrich (Stockholm, Sweden). Oat meal *Ren Havre* (Semper AB; Sundbyberg, Sweden) was purchased in the local supermarket. All chemicals were purchased from Scharlau Chemie S.A., Sentmenat, Spain, except for CaCl$_2$·2H$_2$O (Merck Sharp & Dohme Sweden, Sollentuna, Sweden), polypropylene glycol P2000 (Fluka Chemie AG, Buchs, Switzerland), and Dulbeco's phosphate buffered saline (PBS) buffer without Ca and Mg (PAA Laboratories GmbH, Pasching, Austria). Microplates for fluorescence measurements *FluoroNunc MaxiSorp* were purchased from Nunc (Roskilde, Denmark). 5-(Biotinamido)Pentylamine was obtained from Pierce (Rockford, IL., USA). Eu-labeled streptavidin, DELFIA assay buffer, and DELFIA enhancement buffer were purchased from PerkinElmer, Wallac Oy (Åbo, Finland).

Blocking Peptide Synthesis. Gliadin binding peptide sequences were originally identified from a phage display library Ph.D.-12 Phage Display Peptide Library kit (New England BioLabs, Beverly, MA, USA). This library contains filamentous M13 phage derivatives carrying random 36 bp oligonucleotides cloned into the minor coat protein gene pIII. When expressed, a 12-mer peptide will be expressed at the N-terminus of pIII and exposed on the phage surface. The selection of phages carrying peptides binding to gliadin was carried out using microtiter plates coated with gliadin extracted from wheat gluten according to Mimouni (*20*). Candi-date phages were tested by ELISA, dot blot, and Western blot (Chen et al., unpublished results). Inserted DNAs from gliadin binding phages were isolated and sequenced, and the corresponding peptides were synthesized in vitro with 95% purity (obtained from Xaia Custom Peptides, Göteborg, Sweden). The sequences were as follows: peptide P64 WHWTWLSEYPPP; peptide P22 LETSKLPPPAFL; peptide P61 WHWRNPDFWYLK; control peptide PC31 with no affinity to gliadin AYYPQNHKSNAE (one-letter amino acid code; for three-letter amino acid codes, see **Table 1**).

Protein Extraction. Wheat prolamin (gliadin) was extracted from gluten during sequential extraction as described by Osborne (*21*) and modified by Weiss (*22*). Gluten (1 g) was mixed with 4 mL of Tris-HCl buffer (50 mM, pH 8.8), and the proteins were extracted for 1 h at 4 °C, on a shaker. The samples were centrifuged for 20 min, at 20000*g* at 4 °C, and the supernatant with salt soluble proteins was obtained. The extraction was repeated twice, and the supernatant was discarded. The pellet was washed with ultrapure water, 4 mL of 75% ethanol was added, and gliadins were extracted for 2 h at ambient temperature. Subsequently, the samples were centrifuged as described above, and clear supernatants were stored at −80 °C.

Table 1. Three-Letter Amino Acid Code Sequences of Gliadin Blocking Peptides and the Control Peptide Selected with the Phage Display Technique[a]

peptide	sequence
P64	Trp-His-Trp-Thr-Trp-Lys-Ser-Glu-Tyr-Pro-Pro-Pro
P22	Leu-Glu-Thr-Ser-Lys-Leu-Pro-Pro-Pro-Ala-Phe-Leu
P61	Trp-His-Trp-Arg-Asn-Pro-Asp-Phe-Trp-Tyr-Leu-Lys
PC31	Ala-Tyr-Tyr-Pro-Gln-Asn-His-Lys-Ser-Asn-Ala-Glu

[a] The peptides were incubated with coated wheat gliadin and gluten digest, and their ability to reduce tissue transglutaminase processing of these proteins was investigated with Time Resolved Fluoroscence.

Oatmeal was ground to fine flour and defatted as described by Lending et al. (*23*). Extraction of oat prolamin, avenin, was performed according to Kim et al. (*24*). The defatted flour was mixed with 52% ethanol (1:10 w/v), and avenin was extracted for 1 h at ambient temperature. The samples were centrifuged for 20 min at 10000*g*, and the supernatant was collected. Avenin was precipitated from solution with 2 volumes of cold NaCl (1.5%). Proteins were recovered by centrifugation for 30 min at 7000*g* and 4 °C. The pellet was washed with ultrapure water in order to remove the salt ions and dried. For further analyses, avenin was dissolved to the desired concentration in 52% ethanol.

In Vitro Digestion. Wheat gluten digest was obtained during in vitro digestion in a fermenter by simulation of human physiological conditions and time dependent changes in pH in both gastric and intestinal phases (unpublished data). Shortly, the temperature was set to 37 °C, and wheat gluten was suspended in 500 mL water with 100 *μ*L of P2000 in a 2-L LABFORS fermenter (Infors, Switzerland). To mimic gastric conditions, pepsin in 500 mL of gastric solution prepared according to Matuschek et al. (*25*) and HCl were secreted into the fermenter vessel for 90 min to follow a designed pH gradient from pH 5.5 to pH 2.0. The intestinal phase was 60 min long, and pancreatin and bile salts in 150 mL of 0.1 M NaHCO$_3$ were pumped into the fermenter for 30 min to mimic the duodenal conditions. Bicarbonate was secreted to adjust the pH from 2.0 to 5.0 and subsequently to pH 6.8.

The concentration of all extracted proteins and gluten digest was measured with bicinchoninic acid (BCA)-based protein assay (Pierce; Rockford, IL., USA) with BSA used as a standard, and the results were expressed as *μ*g/mL.

Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electro-phoresis (SDS−PAGE) analysis of extracted prolamins from wheat gluten and oat was performed according to Laemmli (*26*). All the gels, molecular weight standards (MWS), low and broad range, and solutions were from BIO-RAD Laboratories (Sundbyberg, Sweden). Proteins were blended with the Laemmli sample buffer (+5% *β*-mercaptoethanol), heated for 5 min at 95 °C, cooled down to ambient temperature, and centrifuged 2 min at 4000*g* to remove insoluble material. Clear supernatants were loaded on 12%Tris-HCl gels. Analysis was performed for 75 min, at the constant voltage of 110 V in a vertical electrophoresis cell (BIO-RAD Laboratories). The gels were stained with Coomassie Blue R-250 as described in the BIO-RAD standard protocol. Images of the gels were taken with GS-800 Calibrated Image Densitometer (BIO-RAD Laboratories) and analyzed with the Quantity One Software (ver.4.5 BIO-RAD Laboratories). In order to exclude the contamination of oat flour with wheat gliadins, RIDASCREEN Gliadin Sandwich Enzyme Immunoassay (R-Biopharm AG; Darmstadt, Germany) was performed according to the producer's manual with wheat gliadin as a standard. The absorbance was measured at 450 nm with a microplate reader (Tecan Safire) with the operating Magellan software (Tecan Group LTD, Switzerland).

Transglutaminase Activity Assay. The transglutaminase activity assay with the guinea pig liver transglutaminase was performed accor-ding to Skovbjerg et al. (*27*). In this assay, active transglutaminase incorporates 5-(biotinamido)pentylamine to coated protein substrates. Subsequently, high biotin affinity Eu-labeled streptavidin is added to the wells, and time resolved fluorescence (TRF) of chelated Europium is measured.

Gliadin, avenin, and *N,N*-dimethylcasein were used as coated protein substrates in specificity experiments. The purpose was to investigate the specificity of the assay on the basis of a chosen protein substrate and the possibility that avenin could be a good substrate for tTG in vitro. Gliadin

and whole gluten digest were used in experiments with experimental blocking peptides.

Protein concentrations ranging from 500 ng to 5 mg/mL were tested in order to choose the lowest titration possible with reproducible results and high enzyme activity units that would be at least 1000 times higher than the background. For further experiments, 500 μg/mL was chosen. Proteins were diluted to 500 μg/mL in coating buffer (0.1 M carbonate buffer, pH 8.2) and filtered through a 0.22 μm MillexGS Filter (Millipore; USA). Microtiter plates were coated with a protein solution (150 μL/well) and incubated overnight at 4 °C. Blocking of the unbound sites was performed for 30 min at 37 °C with 0.1% BSA in coating buffer. Then, the plates were washed 3 times with 50 mM Tris-HCl buffer (pH 8.5, 0.05% T-20), and 50 μL of freshly prepared Tris-HCl (pH 8.5) with 1 mM 5-(biotinamido)-pentylamine, 10 mM $CaCl_2$, 20 mM DTT, and 50 μL of transglutaminase solution (50 ng/well) was added to the wells and incubated for 30 min at 37 °C. The wells were washed 6 times with washing buffer (PBS-0.05% T-20), and Eu-streptavidin (150 μL/well; 1 μg/mL) in Delfia assay buffer was added. The plates were incubated for 1 h at ambient temperature and washed with washing buffer. During the last stage of the analysis, samples were incubated with Delfia enhancement solution (180 μL/well) for 10 min at ambient temp and time resolved fluorescence (TRF) for Europium was measured with Tecan Safire.

During the experiments with blocking peptides, we made some protocol adjustments. After the BSA blocking and washing steps, we added the blocking peptides in PBS (500 μg/mL) to the wells and incubated them for 1 h at 37 °C to allow binding to the coated gliadin or whole gluten digest. The blocking peptides P22, P61, and P64, and the control peptide PC31 with no affinity to gliadin were tested separately and as a pool of peptides P22, P61, and P64, respectively, with 1:1:1 ratio. After the incubation, the unbound peptides were removed by washing 10 times with washing buffer. Further procedures were kept unchanged.

The reduction of enzymatic activity was calculated by subtracting the values achieved in the presence of experimental blocking peptides from the values achieved when the peptides were absent. Subsequently, the values were presented as the percentage of activity reduction.

Statistics. Results obtained from the transglutaminase activity assay were evaluated with the paired *t*-test (SPSS software, version 15.0 for Windows), with 95% significance level. Differences were considered significant when $P < 0.05$. The tTG activity counts obtained for each peptide were compared with that of the control (no peptide present). All of the peptides were tested at least in triplicate, during three experiments; during the avenin assay, we used samples in quadruplicate.

RESULTS

Gel Analysis of Extracted Prolamins and Whole Gluten Digest. SDS−PAGE analysis of extracted wheat gliadins (**Figure 1a**) showed the presence of major protein bands approximately between 28 and 55 kDa. Moreover, two bands at around 19 and 20 kDa and a thick band between 13 and 15 kDa were visible. Electrophoretic analysis of oat avenins (**Figure 1a**) showed distinct bands between 22 and 34 kDa and a weak band around 15 kDa. The SDS−PAGE of whole gluten digest showed a mixture of peptides in several molecular sizes ranging below 18 kDa (**Figure 1b**).

The oat flour samples were not contaminated with gliadin, indicated by RIDASCREEN sandwich ELISA. Gliadin amount, if any in oat flour, was below the assay detection limit (1.5 ppm gliadin).

Transglutaminase Activity Assay. *Blocking Peptides.* The TRF based microplate assay was used to study tissue transglutaminase catalyzed cross-linking in the presence of blocking peptides. The method described by Skovbjerg (*27*) allows fast and sensitive measurements and results in high fluorescence values already at low concentrations of tissue transglutaminase.

Gliadin and whole gluten digest were used as tTG substrates during incubation with the blocking peptides. Results from the gliadin−peptide incubations showed that the blocking peptides significantly reduced the tTG activity detected as reduced ability to incorporate 5-(biotinamido)pentylamine to coated protein. In
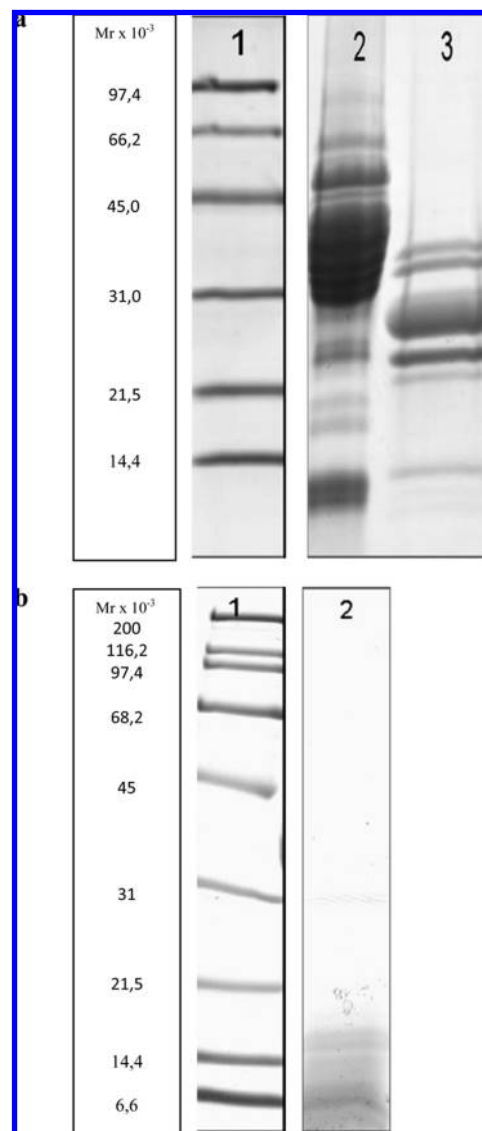

Figure 1. Electrophoretic image of extracted wheat (gliadins) and oat (avenins) prolamins, and whole gluten digest used in the transglutaminase activity assay. (**a**) Lane 1, MWS; lane 2, gliadins; lane 3, avenins (**b**). Lane 1, MWS; lane 2, whole gluten digest.

all cases, the tTG activity reduction compared with activity in the absence of peptides ranged from ∼30 to 36.1% (**Figure 2a**). The highest reduction of enzymatic activity was observed for peptide P22 with ∼36% ($P < 0.001$); followed by ∼33% ($P < 0.001$) reduction for peptide P64; ∼31.4% ($P < 0.001$) reduction for peptide P61; and ∼30% ($P < 0.004$) for the peptide pool. The results differed when in vitro digested gluten was used as a coated tTG substrate (**Figure 2b**). Levels of the measured tTG activity reduction were between ∼5.5−28.4%. The highest activity reduction, ∼28%, was observed for peptide P61, ∼20.1% for the peptide pool, and ∼5.5% for peptide P64. Peptide P22 enhanced the tTG activity by ∼9.4%. However, because of the high level of variance within the groups, these results were statistically insignificant at the 95% significance level. The control peptide PC31 did not cause any significant changes in tTG activity with either gliadin ($P < 0.11$) or gluten digest ($P < 0.59$) as substrates, which demonstrated the absence of unspecific blocking by the peptide without gliadin affinity.

Substrate Specificity in the tTG Assay. Gliadin, avenin, and *N,N*-dimethylcasein were used to examine the enzyme specificity

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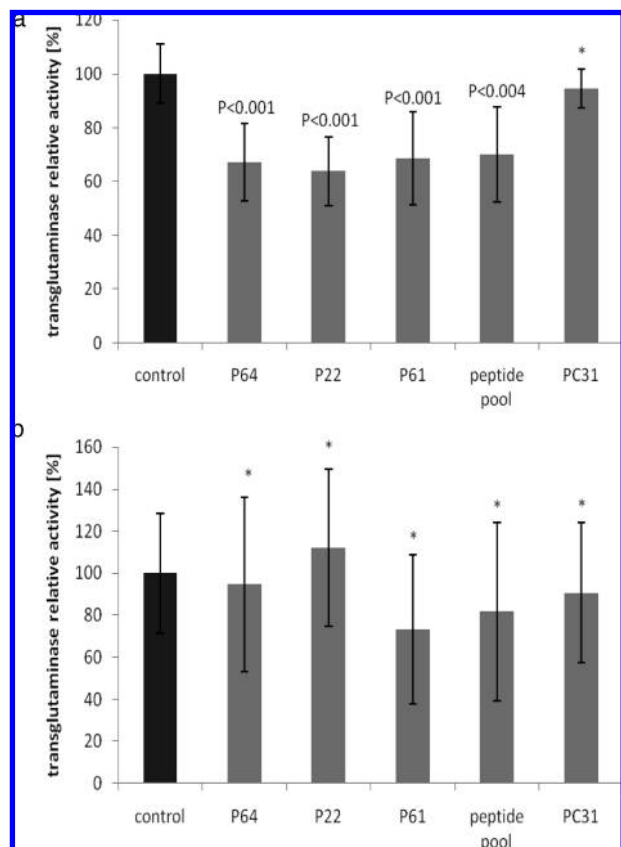*J. Agric. Food Chem.*, Vol. 57, No. 21, 2009 **10153**

Figure 2. Tissue transglutaminase activity in the presence of blocking peptides. Peptides P64, P22, P61, a peptide pool (P64, P22, and P61 in equal proportions), and a control peptide PC31 without gliadin affinity as compared to a control without peptides. Values obtained for all tested peptides are presented as the percentage ($\pm$SD) of transglutaminase activity in relation to control values obtained when the blocking peptides were absent. * indicates statistically insignificant results. (**a**) Gliadin used as the immobilized substrate. (**b**) Whole gluten digest used as the immobilized substrate.
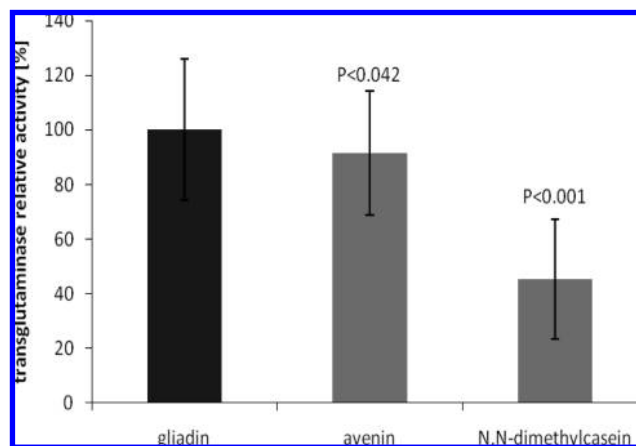

Figure 3. Comparison of tissue transglutaminase activity in the presence of wheat gliadin, oat avenin, and *N,N*-dimethylcasein. Relative values are expressed as the percentage of activity ($\pm$SD) in comparison to the activity values obtained when gliadin was used as an immobilized substrate.

for the relevant substrates and investigate whether avenin could function as a substrate for tissue transglutaminase in vitro. *N,N*-Dimethylcasein has been used as a standard substrate in several enzymatic in vitro assays for transglutaminase (tTG) activity (*28, 29*) and was also used by Skovbjerg et al. (*27*) who described this assay for the first time. With a coating substrate concentration of 500 μg/mL, the activity with *N,N*-dimethylcasein was on average $\sim$45.2% ($P < 0.001$) of the activity for gliadin. Transglutaminase activity on avenin was higher than that on *N,N*-dimethylcasein. The enzyme activity values for avenin were $\sim$91.5% ($P < 0.042$) of the activity with gliadin as a substrate (**Figure 3**), showing that avenin may function as a tTG substrate in vitro.

DISCUSSION

So far, the only available therapy for gluten intolerant patients is a strict avoidance of wheat and other gluten containing cereals. However, because of the abundance of gluten in several non-bakery food products (e.g., vegetarian meat replacement products) where it is used to improve the mouth-feel and chewiness as well as the risk of contamination of, e.g., cereal based products with gluten during production and processing, there is a need for new technological solutions that would allow CD patients to consume low amounts of wheat prolamins without risk for developing the symptoms (*18*).

Tissue transglutaminase is considered an important factor in celiac disease pathology, and transglutaminase catalyzed

deamidation and cross-linking reactions are crucial for the production of epitopes for intestinal T-cells (*4*). In the present work, we investigated the influence of gliadin blocking peptides on the ability of tissue transglutaminase to actively modify relevant protein substrates.

Three blocking peptides used in the experiment were randomly chosen from groups that consisted of peptides with very high affinity to gliadin, selected using the phage display technique. Peptides P22, P61, and P64 but not the control peptide PC31 had the ability to reduce the action of the enzyme on wheat gliadin. Most likely, during the incubation with substrate proteins, the blocking peptides covered their glutamine rich regions and reduced the number of available glutamine residues. They could also hinder the modification of glutamine residues nearby the already covered regions. Moreover, the blocking peptides themselves did not contain glutamine residues that could be selected by tissue transglutaminase during the enzymatic reaction.

Two different gluten derived substrates were tested in our experiments: intact gliadin and a heterogeneous mix of hydrolyzed gluten derived peptides, in order to investigate the ability of blocking peptides to interact with gliadin after simulated gastrointestinal digestion. While the blocking peptides strongly blocked gliadin, they were not as effective when gluten digest was used, and relatively high variance was observed during the measurements. Gluten digest is a heterogeneous mix of polypeptides originating from several protein groups present in wheat gluten, with molecular sizes ranging from 18 kDa and below (**Figure 1b**). The limited efficiency of the blocking peptides with gluten digest was most probably caused by the fact that they were only selected for high affinity to intact gliadin. The electrophoretic pattern of gliadin showed typical distribution with proteins that belong to ω-gliadins (around 40−50 kDa), and $\alpha/\beta/\gamma$ gliadins (30−45 and 28 kDa). Moreover, the bands at 13−15 kDa probably showed the presence of α-amylase/trypsin inhibitors (*30, 31*). Gliadin sequences preferred by the blocking peptides could be cleaved by proteases during the in vitro digestion procedure, thus reducing the binding efficiency of the blocking peptides. Yet, despite the high variance of the obtained results from the incubation of gluten digest with the blocking peptides, peptide P61 could still be considered a candidate for decreasing enzymatic activity, which should be further evaluated. These results indicate that the most probable way for a future application could be the incubation of peptides with gliadin prior to ingestion.

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Currently, the role of oats in the gluten-free diet is rather controversial. Contradicting reports have been published stating either the safety of oats during patient trials or the induction of inflammatory reaction when oats are consumed in gluten-free diet (*32, 33*). Oats are recognized as safe for gluten intolerant patients in some countries; however, they tend to be contaminated by wheat both in the field and in milling, which may be responsible for the development of symptoms (*34, 35*). However, Arentz-Hansen et al. (*36*) recently isolated oat-specific T-cells from some CD patients, showing that avenin is a recognized substrate for the immune system cells for at least some patients. It was, however, not known to what extent avenin could function as a substrate for tTG. Our data from substrate specificity experiments are in general agreement with those of Skovbjerg (*27*) showing that wheat gliadin is a better substrate for tissue transglutaminase than *N,N*-dimethylcasein. Our results suggest that avenin may be processed similarly to gliadin during in vitro assays with tTG. This is most probably due to the slight differences in the amount of glutamine residues in these two proteins. Gliadin is particularly rich in this amino acid. Glutamine contributes to approximately 37.1% of the total amino acid content in this protein, whereas in avenin, it contributes to $\pm34.1\%$ (*7*). Although the results are interesting, they are not sufficient to draw any conclusions regarding the role of avenin in CD, but they could serve as the starting part for further investigations.

Transglutaminase catalyzed deamidation of gluten peptides plays a crucial role in CD pathology; therefore, preventing this event could be of importance in possible future therapies. There have been several attempts to develop therapies based on tissue transglutaminase. So far, only the enzyme inhibitor approach has been tested in a number of studies. Among others, cystamine was used during human transglutaminase 2 (TG2) treatment of gliadin. This inhibitor binds covalently to TG2 and prevents it from catalyzing gliadin deamidation. TG2 inhibition results in a reduced proliferation of T-cells derived from intestinal biopsies from CD patients during incubation with gliadin (*37*). Moreover, synthetic compounds, for example, dihydroisoxasole derivatives have shown to be efficient active-site inhibitors of TG2 (*38*). During oral application in mice, these compounds were shown to have good bioavailability and were able to inhibit TG2 in the small intestine. Their short serum half-life prevented the distribution to other tissues. However, the detoxification of proteins without inhibiting tissue transglutaminase could also be considered a possible solution. In our study, we showed that the gliadin blocking peptides had this potential.

To our knowledge, this is the first study that uses a blocking peptide approach to reduce the processing of gliadin by tissue transglutaminase. The results show that these peptides have a potential for gluten detoxification and could be evaluated as an alternative in designing new food products for gluten intolerant patients provided that both peptides and their complexes with gliadin are stable during the passage through the GI tract, which will be further investigated in future work.

ABBREVIATIONS USED

T-20, Tween-20; PBS, phosphate buffered saline; P2000, polypropylene glycol; MWS, molecular weight standard; tTG, tissue transglutaminase.

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LITERATURE CITED

(1) Fasano, A.; Berti, I.; Gerarduzzi, T.; Not, T.; Colletti, R. B.; Drago, S.; Elitsur, Y.; Green, P. H.; Guandalini, S.; Hill, I. D.; Pietzak, M.; Ventura, A.; Thorpe, M.; Kryszak, D.; Fornaroli, F.; Wasserman, S. S.; Murray, J. A.; Horvath, K. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch. Intern. Med.* **2003**, *163*, 286–92.

(2) van Heel, D. A.; West, J. Recent advances in coeliac disease. *Gut* **2006**, *55*, 1037–46.

(3) Sollid, L. M. Coeliac disease: dissecting a complex inflammatory disorder. *Nat. Rev. Immunol.* **2002**, *2*, 647–55.

(4) Dieterich, W.; Esslinger, B.; Schuppan, D. Pathomechanisms in celiac disease. *Int. Arch. Allergy Immunol.* **2003**, *132*, 98–108.

(5) Fasano, A.; Catassi, C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology* **2001**, *120*, 636–51.

(6) Maki, M.; Collin, P. Coeliac disease. *Lancet* **1997**, *349*, 1755–9.

(7) Belitz, H.-D.; Grosch, W.; Schieberle, P. Cereals and Cereal Products. In *Food Chemistry*, 3rd revised ed.; Springer-Verlag: Berlin, Germany, 2004; pp 681−692.

(8) Stern, M.; Ciclitira, P. J.; Van Eckert, R.; Feighery, C.; Janssen, F. W.; Mendez, E.; Mothes, T.; Troncone, R.; Wieser, H. Analysis and clinical effects of gluten in coeliac disease. *Eur. J. Gastroent. Hepat.* **2001**, *13*, 741–747.

(9) Hausch, F.; Shan, L.; Santiago, N. A.; Gray, G. M.; Khosla, C. Intestinal digestive resistance of immunodominant gliadin peptides. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2002**, *283*, G996–G1003.

(10) Shan, L.; Molberg, O.; Parrot, I.; Hausch, F.; Filiz, F.; Gray, G. M.; Sollid, L. M.; Khosla, C. Structural basis for gluten intolerance in celiac sprue. *Science* **2002**, *297*, 2275–9.

(11) Ichinose, A.; Bottenus, R. E.; Davie, E. W. Structure of transglutaminases. *J. Biol. Chem.* **1990**, *265*, 13411–4.

(12) Fleckenstein, B.; Molberg, O.; Qiao, S. W.; Schmid, D. G.; von der Mulbe, F.; Elgstoen, K.; Jung, G.; Sollid, L. M. Gliadin T cell epitope selection by tissue transglutaminase in celiac disease. Role of enzyme specificity and pH influence on the transamidation versus deamidation process. *J. Biol. Chem.* **2002**, *277*, 34109–16.

(13) Folk, J. E.; Chung, S. I. Transglutaminases. *Methods Enzymol.* **1985**, *113*, 358–75.

(14) Dieterich, W.; Ehnis, T.; Bauer, M.; Donner, P.; Volta, U.; Riecken, E. O.; Schuppan, D. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat. Med.* **1997**, *3*, 797–801.

(15) Maki, M. Tissue transglutaminase as the autoantigen of coeliac disease. *Gut* **1997**, *41*, 565–6.

(16) Sjöström, H.; Lundin, K. E. A.; Molberg, O.; Körner, R.; Mcadam, S. N.; Anthonsen, D.; Quarsten, H.; Norén, O.; Roepstorff, P.; Thorsby, E.; Sollid, L. M. Identification of a gliadin T-cell epitope in coeliac disease: general importance of gliadin deamidation for intestinal T-cell recognition. *Scand. J. Immunol.* **1998**, *48*, 111–115.

(17) Szabolcs, M.; Sipka, S.; Csorba, S. In vitro cross-linking of gluten into high-molecular-weight polymers with transglutaminase. *Acta Paediatr. Hung.* **1987**, *28*, 215–27.

(18) Day, L.; Augustin, M. A.; Batey, I. L.; Wrigley, C. W. Wheat-gluten uses and industry needs. *Trends Food Sci. Technol.* **2006**, *17*, 82–90.

(19) Thompson, T.; Méndez, E. Commercial assays to assess gluten content of gluten-free foods: why they are not created equal. *J. Am. Diet. Assoc.* **2008**, *108*, 1682–1687.

(20) Mimouni, B.; Azanza, J.-L.; Raymond, J. Wheat flour proteins: isolation and functionality of gliadin and HMW-glutenin enriched fractions. *J. Sci. Food Agric.* **1998**, *78*, 423–428.

(21) Osborne, T. B. *The Proteins of the Wheat Kernel*; Carnegie Institution: Washington DC, 1907.

(22) Weiss, W. C. V.; Görg, A. Electrophoretic characterization of wheat grain allergens from different cultivars involved in bakers' asthma. *Electrophoresis* **1993**, *14*, 805–816.

(23) Lending, C. R.; Chesnut, R. S.; Shaw, K. L.; Larkins, B. A. Immunolocalization of avenin and globulin storage proteins in developing endosperm of *Avena sativa* L. *Planta* **1989**, *178*, 315–324.

(24) Kim, S. I.; Charbonnier, L.; Mossé, J. Heterogeneity of avenin, the oat prolamin: fractionation, molecular weight and amino acid composition. *Biochim. Biophys. Acta* **1978**, *537*, 22–30.

(25) Matuschek, E.; Towo, E.; Svanberg, U. Oxidation of polyphenols in phytate-reduced high-tannin cereals: effect on different phenolic groups and on in vitro accessible iron. *J. Agric. Food Chem.* **2001**, *49*, 5630–8.

(26) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680–5.

(27) Skovbjerg, H.; Noren, O.; Anthonsen, D.; Moller, J.; Sjostrom, H. Gliadin is a good substrate of several transglutaminases: possible implication in the pathogenesis of coeliac disease. *Scand. J. Gastroenterol.* **2002**, *37*, 812–7.

(28) Jeon, W. M.; Lee, K. N.; Birckbichler, P. J.; Conway, E.; Patterson, M. K., Jr. Colorimetric assay for cellular transglutaminase. *Anal. Biochem.* **1989**, *182*, 170–175.

(29) Slaughter, T. F.; Achyuthan, K. E.; Lai, T. S.; Greenberg, C. S. A microtiter plate transglutaminase assay utilizing 5- (biotinamido)-pentylamine as substrate. *Anal. Biochem.* **1992**, *205*, 166–171.

(30) Gómez, L.; Martin, E.; Hernández, D.; Sánchez-Monge, R.; Barber, D.; del Pozo, V.; de Andres, B.; Armentia, A.; Lahoz, C.; Salced, G.; Palomino, F. Members of the [alpha]-amylase inhibitors family from wheat endosperm are major allergens associated with baker's asthma. *FEBS Lett.* **1990**, *261*, 85–88.

(31) Wieser, H. Chemistry of gluten proteins. *Food Microbiol.* **2007**, *24*, 115–119.

(32) Janatuinen, E. K.; Kemppainen, T. A.; Julkunen, R. J.; Kosma, V. M.; Maki, M.; Heikkinen, M.; Uusitupa, M. I. No harm from five year ingestion of oats in coeliac disease. *Gut* **2002**, *50*, 332–5.

(33) Lundin, K. E.; Nilsen, E. M.; Scott, H. G.; Loberg, E. M.; Gjoen, A.; Bratlie, J.; Skar, V.; Mendez, E.; Lovik, A.; Kett, K. Oats induced villous atrophy in coeliac disease. *Gut* **2003**, *52*, 1649–52.

(34) Thompson, T. Do oats belong in a gluten-free diet? *J. Am. Diet. Assoc.* **1997**, *97*, 1413–1416.

(35) Thompson, T. Gluten contamination of commercial oat products in the United States. *N. Engl. J. Med.* **2004**, *351*, 2021–2.

(36) Arentz-Hansen, H.; Fleckenstein, B.; Molberg, O.; Scott, H.; Koning, F.; Jung, G.; Roepstorff, P.; Lundin, K. E.; Sollid, L. M. The molecular basis for oat intolerance in patients with celiac disease. *PLoS. Med.* **2004**, *1* (1), e1.

(37) Molberg, O.; McAdam, S. N.; Lundin, K. E.; Kristiansen, C.; Arentz-Hansen, H.; Kett, K.; Sollid, L. M. T cells from celiac disease lesions recognize gliadin epitopes deamidated in situ by endogenous tissue transglutaminase. *Eur. J. Immunol.* **2001**, *31*, 1317–23.

(38) Choi, K.; Siegel, M.; Piper, J. L.; Yuan, L.; Cho, E.; Strnad, P.; Omary, B.; Rich, K. M.; Khosla, C. Chemistry and biology of dihydroisoxazole derivatives: selective inhibitors of human transglutaminase 2. *Chem. Biol.* **2005**, *12*, 469–475.