Materials and Methods

Expression and Purification of \( \alpha_2 \)-Gliadin
The \( \alpha_2 \)-gliadin gene (S1) was cloned in pET28a plasmid (Novagen) and transformed into the expression host BL21(DE3) (Novagen). The transformed cells were grown in 1-liter cultures of LB media containing 50 \( \mu \)g/ml of kanamycin at 37 °C until the OD\textsubscript{600} 0.6-1 was achieved. The expression of \( \alpha_2 \)-gliadin protein was induced with the addition of 0.4 mM isopropyl \( \beta \)-D-thiogalactoside (Sigma) and the cultures were further incubated at 37°C for 20 hours. The cells expressing the recombinant \( \alpha_2 \)-gliadin were centrifuged at 3600 rpm for 30 minutes. The pellet was resuspended in 15 ml of disruption buffer (200 mM sodium phosphate; 200 mM NaCl; 2.5 mM DTT (to reduce all disulfide linkages); 1.5 mM benzamidine; 2.5 mM EDTA; 2 mg/L pepstatin; 2 mg/L leupeptin; 30% v/v glycerol) and lysed by sonication (1 minute; output control set to 6). After centrifugation at 45000g for 45 min, the supernatant was discarded and the pellet containing gliadin protein was resuspended in 50 ml of 7M urea in 50 mM Tris (pH = 8.0). The suspension was again centrifuged at 45000g for 45 min and the supernatant was harvested for purification. The supernatant containing \( \alpha_2 \)-gliadin was incubated with 1 ml of nickel-nitrilotriacetic acid resin (Ni-NTA; Qiagen) overnight and then batch-loaded on a column with 2 ml of Ni-NTA. The column was washed with 7M urea in 50 mM Tris (pH = 8.0) and \( \alpha_2 \)-gliadin was eluted with 200 mM imidazole, 7 M urea in 50 mM Tris (pH = 4.5). The fractions containing \( \alpha_2 \)-gliadin were pooled into a final concentration of 70% ethanol solution and two volumes of 1.5M NaCl were added to precipitate the protein. The solution was incubated at 4 °C overnight and the final precipitate was collected by centrifugation at 45000 g for 30 min, rinsed in water, and re-centrifuged to remove the urea. The final purification step of the \( \alpha_2 \)-gliadin was developed with reverse-phase HPLC. The Ni-NTA purified protein fractions were pooled in 7 M urea buffer and injected to a Vydac (Hesperia, CA) polystyrene reverse-phase column (i.d. 4.6 mm \( \times \) 25 cm) with the starting solvent (30% of solvent B: 1:1 HPLC-grade acetonitrile/isopropanol : 0.1% TFA). Solvent A was an aqueous solution with 0.1% TFA. The separation gradient extended from 30–100% of solvent B over 120 min at a flow rate of 0.8 ml/min.

Enzymatic Digestion of Recombinant \( \alpha_2 \)-Gliadin
The purified recombinant \( \alpha_2 \)-gliadin in the lyophilized form was weighed and dissolved in 0.01M hydrochloric acid. The protein was incubated in a 37°C water bath with pepsin (1:100 protease to protein w/w ratio, Sigma) at pH 2.0 for 20 min. The reaction mixture was then pH adjusted to 7.0 in 50mM phosphate buffer and added with trypsin (1:100, Sigma), chymotrypsin (1:100, Sigma), elastase (1:500, Sigma) and carboxypeptidase (1:100, Cortex Biochem) at 37°C for various time periods. The above proteases were chosen because of their major roles in protein digestion based on their abundance and specificities (S2). The less abundant gastric protease, gastricsin (which prefers aromatic residues), was not used; however, since gastricsin does not cleave downstream of proline residues, it is unlikely to be effective on the 33-mer reported in this study.
In Vivo Rat Intestinal Peptide Perfusion Experiment

A female or male rat was anesthetized and maintained at 36-37°C anal temperature and the peritoneal cavity was cut open (S3). A small incision was made at the beginning and the end of a 15-20 cm jejunum segment. Polyethylene catheters were inserted and secured into the two ends. The input catheter was connected with a pump-driven syringe filled with a solution. The jejunum segment was rinsed with PBS buffer first to remove food and debris at a flow rate of 0.4 ml/min. Purified peptide solutions (peptide concentration ranges from 25-100 µM) were passed through the jejunum segment with a residence time of between 10-40 min. In the case of a co-perfusion, the input catheter is connected with two simultaneous syringes, one with a peptide solution and the other with the prolyl endopeptidase solution (concentration ranges from 50-500 µU/µl). Collections were made from the output catheter into small centrifuge tubes in dry ice and stored at -20°C for subsequent analysis. The collected digestive products were analyzed with LC-MS on a C18 column.

Figures and Legends

Figure S1. Alignment of representative gluten and non-gluten peptides homologous to LQLQPFPQPQQLYPYPQPQQLPYPQPF. The alignment was performed by BLASTP using the BLOSUM 45 matrix.
Figure S2. Circular dichroism evidence of the polyproline secondary structure of the 33-mer, pertactin and phosphatase peptides.

A. CD spectra of a standard poly-L-proline, the 33-mer, the pertactin peptide and the phosphatase peptide at the same concentration.

B. CD spectrum of the 33-mer peptide with and without 6M urea.

References and Notes