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ORIGINAL PAPER

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Comparative investigations of gluten proteins from different wheat species. III. N-terminal amino acid sequences of α-gliadins potentially toxic for coeliac patients

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Abstract Studies on the coeliac toxicity of wheat have been focused on common (bread) wheat. Other cultivated wheat species were tested in an inadequate manner or were tested not at all. Because in vivo testing by feeding to coeliac patients is out of the question for ethical reasons, the different species were compared by N-terminal sequences of α-gliadins including the potential toxic sequences. Flours of durum wheat, emmer, and einkorn were successively extracted with a salt solution and 60% ethanol. The alcoholic extracts (gliadins) were preparatively separated by reversed-phase HPLC using an elution system optimized for α-gliadins. Five to six different α-gliadin fractions were isolated from each species and were characterized by the determination of N-terminal amino acid sequences. The results for 30 steps of Edman degradation indicated a high degree of sequence homology within the N-terminal region and a close relationship with corresponding sequences of α-gliadin fractions from bread wheat and spelt wheat [12]. The α-gliadins from all wheat species investigated contained amino acid sequences potentially activating coeliac disease. For this reason, all cultivated wheat species are assumed to be coeliac toxic cereals and should be avoided by coeliac patients.

Keywords Wheat species · α-Gliadins · N-terminal sequences · Coeliac disease

Introduction

Coeliac disease (gluten-sensitive enteropathy) is known to be caused by the alcohol-soluble storage proteins (prolamins) of wheat (gliadins), rye (secalins), and barley (hordeins). There is still disagreement about oat prolamins (avenins), whereas prolamins from other cereals are not harmful to coeliac patients [1, 2, 3]. Investigations of the relationship between prolamin structure and toxicity have only be done with gliadin from common (bread) wheat. In vivo and in vitro studies indicated that all major gliadin types (α-, γ-, and ω-gliadins) produce toxic effects. Gliadins can be heated or digested into peptides without loss of toxicity. Thus, tertiary structures of the gliadins are not important, but small peptides arising from gliadins by gastrointestinal enzymes are responsible for the toxic effects. Experiments with peptides demonstrated that, in particular, the N-terminal domain of α-gliadins is involved in the activation of coeliac disease [3]. The tetrapeptide sequences PSQQ and QQQP (one-letter-code for amino acids) have been considered as key sequences for toxicity [4, 5]. Other cultivated wheat species were either tested in an inadequate manner (tetraploid durum wheat, diploid einkorn) [6, 7] or were not tested at all (hexaploid spelt wheat, tetraploid emmer). It is sometimes implied that "old" wheat species such as spelt wheat, emmer, and einkorn are safe for coeliac patients, but the literature does not present any rigorous scientific evaluation of such claims [8]. Their coeliac toxicity is, therefore, uncertain. Information of the coeliac toxicity of durum (spaghetti) wheat would be of particular interest because it is widely used for the production of noodles.

Most investigators would agree that in vivo testing such as direct instillation/biopsy is the "gold standard" for assessing the coeliac toxicity of proteins or peptides [9], but ethical reasons are the most crucial limiting factors. In vitro tests such as the culture of tissue from human small intestine are a valuable aid in the search for potentially toxic factors, but cannot substitute an ultimate in vivo test [2, 3]. Simple tests like skin tests were investigated for many years without success [1].

Based on the knowledge of coeliac toxic amino acid sequences of α-gliadins from bread wheat, a chemical approach was chosen to judge the toxic potential of the different wheat species. Previous papers have shown that hexa-, tetra-, and diploid species investigated contain all types of gliadin proteins (ω-, α-, γ-gliadins) [10] and

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their ω-gliadins were closely related in amino acid compositions and molecular masses [11]. In continuation of previous studies on the spelt wheat varieties "Roquin" and "Schwabenkorn" [12], α-gliadin fractions from durum wheat, emmer, and einkorn were isolated and N-terminal amino acid sequences were analyzed by Edman degradation.

Material and methods

Gliadin extraction. Defatted flours (1 g) of durum wheat "Biodur" (BIO), emmer (EMM), and einkorn (EIN) (cultivars unknown) were extracted stepwise with 2×10 ml of a salt solution (0.4 mol/l NaCl+0.067 mol/l $KHNaPO_4$, pH 7.6) and with 2×10 ml of 60 vol.% ethanol [10]. After centrifugation, both supernatants from the ethanol extraction (gliadins) were combined and filtered through a 0.45-mm membrane.

Reversed-phase HPLC. Preparative RP-HPLC was optimized for the separation of α-gliadins using a Beckman instrument (solvent module 126, system Gold software) and a Nucleosil 300 – 5 C_{18} column (Machery, Nagel; 4.6×240 mm, 50 °C). The elution system consisted of (A) 2-propanol (15 vol.%)+trifluoroacetic acid (TFA, 0.1 vol.%) and (B) acetonitrile (80 vol.%)+TFA (0.1 vol.%) [12]. The sample loop was 2.0 ml; injection volume was 500 µl of the gliadin extract (about 1.5 mg protein). Then 500 µl of TFA (0.1 vol.%) were injected before sample injection to prevent losses of hydrophilic gliadins [13]. Further HPLC conditions were a linear gradient of 0 min 30% B, 30 min 50%, a flow rate of 1.0 ml/min, and a UV detection at 220 nm. The eluates corresponding to the peaks of the chromatograms (Fig. 1) were collected and dried by means of a vacuum centrifuge C 412 (Jouan).

Protein analysis. The N-terminal amino acid sequences (30 cycles) of collected α-gliadins (≈50 µg) were analyzed by automatic Edman degradation using a pulsed-liquid protein sequence 471 A (PE biosystems).

Results and discussion

In a previous study [12] spelt wheat and common (bread) wheat were compared by the analysis of N-terminal sequences of α-gliadins, which have been proposed to be responsible for the activation of coeliac disease. The results did not reveal any significant difference between both wheat species within the first 25 positions of amino acid sequence. Recently, the complete amino acid sequence of an α-type gliadin (pTS 63) from spelt wheat was published by Kasarda and D'Ovidio [8]. The comparison of pTS 63 with α-gliadin sequences from bread wheat showed a high degree of homology. The N-terminal sequences were identical with those found in bread wheat cultivar "Rektor" (Table 1). Therefore, authors of both papers concluded that spelt wheat is a coeliac-toxic cereal. In accordance with the study on spelt wheat [12], the gliadins of durum wheat (BIO), emmer (EMM), and einkorn (EIN) were extracted from flours with 60% ethanol, after albumins and globulins had been removed. α-Gliadins were then preparatively separated by reversed-phase HPLC and five to six fractions of each wheat distributed over the whole elution region of α-gliadins were collected by means of several runs (Fig. 1). The N-termi-

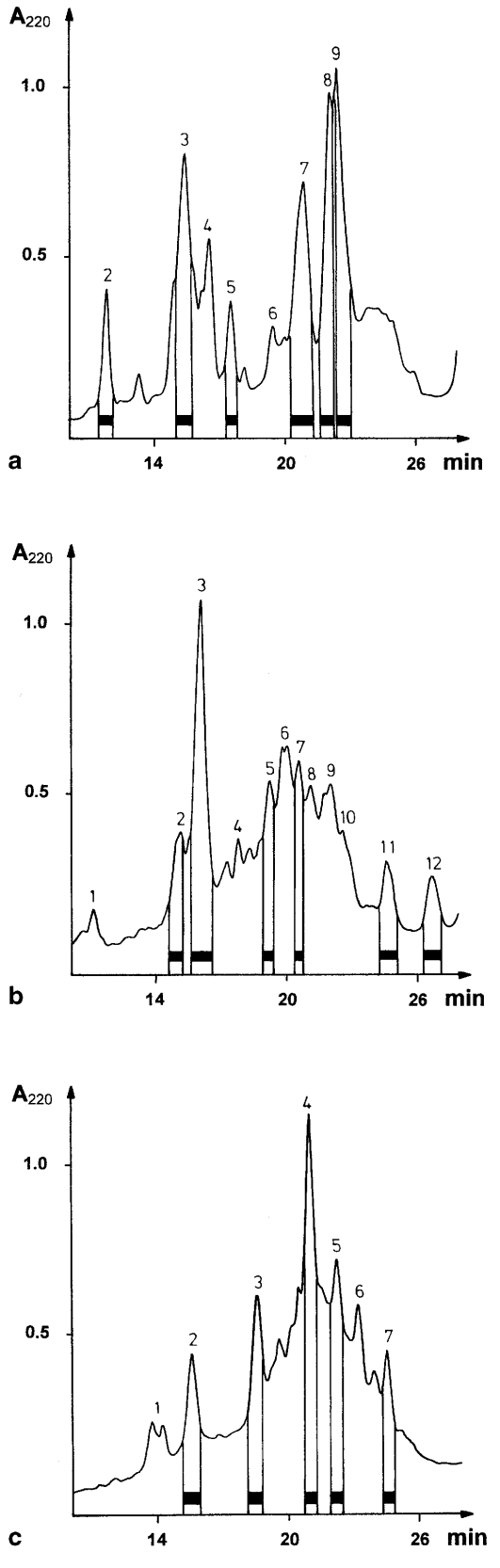

Fig. 1a–c Preparative RP-HPLC of α-gliadins from: **a** durum wheat "Biodur"; **b** emmer; **c** einkorn

Table 1 N-terminal sequences of α-gliadins from different wheat species

Wheat[a]	Peak[b]	Sequence[c]
		5 10 15 20 25 30
REK[d]	–	VRVPVPQLQPQNPSQQQPQEQVPLV
		QA Q
SCH[d]	–	VRVPVPQLQPQNPSQQQPQEQVPLV
		QA P
Spelt[e]	–	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
BIO	2	VRFPVPQLQPQNPSQQQPQEQVPLVQXQQY
	3	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
	5	VRVPVPQLQPQNPSQQQPQEQVPLVQQQGF
		G
	7	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
		QAQGL P L LA
	8	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
	9	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
EMM	2	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
		Q
	3	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
		Q Q L L I
	5	VRVPVPQLQPQNPSQQQPQEQVPLVQQQXF
		Q LQQS YQLM EL Q L QIPE S
	7	VQVPVPQLYPQMPXQQQPQL
		ILQQ QV Q
	11	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
		Q L Q YQL Q QPQ QIPE
	12	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
		Q P
EIN	2	VRVPVPQLQPQNPSQQQPQEQVPLVQQQYF
		L
	3	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
		L P
	4	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
		Q S
	5	VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF
		Q
	7	VQVPVPQLQPQNPSQQQPQEQVXXVQQQQF

[a] REK=bread wheat "Rektor"; SCH=spelt wheat "Schwabenkorn"; BIO=durum wheat "Biodur"; EMM=emmer; EIN=einkorn
[b] See Fig. 1
[c] One-letter code for amino acids, X=not determined, upper line = major sequences, lower line = minor sequences
[d] From previous study [12]
[e] From Kasarda [14]

nal sequences of these fractions were then analyzed by 30 steps of Edman degradation; the results are shown in Table 1. For comparison, the previously determined sequences of α-gliadins from bread wheat "Rektor" (REK) and spelt wheat [8, 12] were included. The sequences demonstrated that most collected fractions were not pure proteins showing two amino acid residues in single positions. This could particularly be observed in the α-gliadin fractions from emmer (EMM 2–12). Nevertheless, the sequences of almost all fractions agreed with the main sequences of α-gliadins from REK and spelt wheat; only two residues of fraction BIO 2 (F in position 3 and Y in position 30), two residues of EMM 7 (Q in position 2 and M in position 12), and one residue of EIN 7 (Q in position 2) were different from the main sequences. The most frequent heterogeneities occurred in position 2 (R or Q) and in position 15 (Q or L). With respect to the potential coeliac toxic sequences PSQQQP (positions 13–18), they occurred in all analyzed α-gliadin fractions. Some minor sequences of BIO 7, EMM 3, EMM 5, EMM 11, EIN 2, EIN 3, and EIN 4 were modified to PSLQQP, PELQQP, QSQQQP, PSLQPP, and PSQQQS. Numerous studies on different bread wheat varieties indicated a close relationship between gliadins and a high degree of sequence homology. It can, therefore, be concluded that the results obtained for the selected samples of durum wheat, emmer, and einkorn are representative for each species. Thus, the probability that varieties without any potential toxic gliadin sequences might exist, appears to be very low.

Conclusion

Previous and present studies demonstrated that representatives of all cultivated hexa-, tetra-, and diploid wheat species contain all types of gliadin proteins (ω-, α-, γ-type). In the case of α-type gliadins known to be toxic for coeliac patients, the analyzed fractions show a high degree of homology within the N-terminal region and contain potentially toxic sequences. For these reasons all wheat species investigated are assumed to be coeliac-toxic cereals and should be avoided by coeliac patients. These conclusions are in accord with the relationship be-

tween cereal taxonomy and coeliac disease [14]. It is reasonable to suggest that any species of Triticum (bread, spelt and durum wheat, emmer, einkorn) is toxic to coeliac patients, when cereals considerably more distantly related to bread wheat – rye (*Secale cereale*) and barley (*Hordein vulgare*) – are toxic [8].

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