

Rye inhibitors of animal α -amylases show different specificities, aggregative properties and IgE-binding capacities than their homologues from wheat and barley

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Three new members of the α -amylase/trypsin-inhibitor family of cereal endosperm have been isolated from rye. N-terminal amino acid sequence comparison revealed that two of the purified proteins were the rye homologues of the barley monomeric inhibitor (BMAI-1) previously described, while the other rye protein corresponded to one of the subunits of the barley and wheat heterotetrameric inhibitors. However, the inhibitory specificities (active against human salivary α -amylase), aggregative behaviours (mainly as dimeric forms) and IgE-binding capacities (not recognized by sera from allergic patients) of the rye inhibitors were clearly different from those of their wheat and barley counterparts. These results indicate that homologous inhibitors may have distinctive properties in different cereal species.

Plant proteins that inhibit α -amylases and proteinases from pests and pathogens are receiving considerable attention because of their potential role and use in plant protection [1, 2]. A further reason to study some of these proteins (i.e. those from cereal endosperm) comes from the recent finding that they are major allergens in the allergic diseases provoked by inhalation or ingestion of cereal flours [3, 4].

An inhibitor family of animal α -amylases and of trypsin, which is particularly abundant in wheat and barley endosperm, is among those that have been more actively studied (see [5, 6] for reviews). About 20 different members of this protein family, all of them with molecular masses in the 12–16-kDa range, have been characterized in these cereal species. The inhibitors of animal α -amylases can be either monomeric, homodimeric or heterotetrameric (three different subunits with one of them present in two copies), while those active against trypsin so far described are monomeric proteins. Joint consideration of the amino acid sequence similarities, the inhibitory activities and the chromosomal location of their coding genes, allows us to classify the inhibitor subunits into different subfamilies. In *Triticum* and *Hordeum* all subunits belonging to the same subfamily have similar inhibitory specificities and aggregative properties [6]. It should be mentioned that some subfamilies include components isolated from both wheat and barley, whereas others are represented by inhibitors detected in only one of these cereals [6].

Knowledge of this protein family in other plant species, even within the *Triticeae* tribe, is still very scarce. Thus in rye, and concerning components active towards animal α -amylases, although there are a few reports on their detection [7, 8], only the N-terminal amino acid sequences of two inhibitors have been reported. One of them shows a high degree of sequence similarity with a subunit of wheat and barley tetrameric inhibitors [9], but its aggregative behaviour and inhibitory activity has not been established. The second one is closely related with the subfamily of wheat homodimeric inhibitors and shows very similar properties to its *Triticum* counterparts [10].

We report here the characterization of three new members of the α -amylase-inhibitor family from rye. These proteins show quite different inhibitory and allergenic (IgE-binding) properties than their wheat and barley homologues.

MATERIALS AND METHODS

Plant material

Milled endosperms from rye (*Secale cereale*, genomes RR) INIA C/171-M, tetraploid wheat (*Triticum turgidum*, genomes AABB) cv. Enano de Andujar, hexaploid triticales (*Triticosecale*, genomes AABBRR) Cachirulo, and barley (*Hordeum vulgare*, genomes HH) cv. Bomi, were used in this study.

Extraction and gel-filtration of inhibitor preparations

Salt-soluble protein preparations enriched in inhibitors were obtained by 0.15 M NaCl extraction and $(\text{NH}_4)_2\text{SO}_4$ precipitation as in [11]. The crude inhibitor preparations were subjected to gel-filtration on Sephadex G-100 under non-dissociating conditions (80 cm $\times$ 2.5 cm column; 25 ml/h), using 0.1 M ammonium acetate, pH 6.8, as elution buffer. Fractions

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Abbreviations. BMAI, barley monomeric α -amylase inhibitor; BTAI, barley tetrameric α -amylase inhibitor; SGE, starch-gel electrophoresis; WTAI, wheat tetrameric α -amylase inhibitor; RAST, radioallergosorbent test.

Enzymes. α -amylase (EC 3.2.1.1.); trypsin (EC 3.4.21.4.).

corresponding to the different inhibitor types were pooled and freeze/dried.

Purification of inhibitors

The rye inhibitors were isolated from the gel-filtration fractions corresponding to monomeric and homodimeric forms, by preparative reverse-phase HPLC. A Vydac-C4 column was used (22 mm×250 mm, particle size 10 µm), and elution was carried out by a three-step linear gradient of acetonitrile in 0.1% trifluoroacetic acid (from 10% to 20% in 45 min, from 20% to 35% in 140 min, from 35% to 50% in 100 min; 2 ml/min).

The tetrameric inhibitor subunits of tetraploid wheat (WTAI-CM2, WTAI-CM3 and WTAI-CM16) and barley (BTAI-CMa, BTAI-CMb and BTAI-CMd) were purified as in [3]. The barley monomeric α -amylase inhibitor BMAI-1 was obtained as previously described [12].

Protein concentration was quantified by the bicinchoninic acid assay [13].

Gel filtration of purified inhibitors

HPLC gel filtration of isolated proteins (40 µg) was performed on a Spherogel TSK-G300 column (21.5 mm ×300 mm) eluting with 0.1 M ammonium acetate, pH 6.8, at 0.5 ml/min.

Electrophoretic procedures

SDS/PAGE was carried out according to Laemmli [14] on Bio-Rad Miniprotein II system minigels. Two-dimensional electrophoresis (IEF and starch-gel electrophoresis, SGE) was performed as described previously [11].

Amino acid sequencing

N-terminal amino acid sequences were determined by standard methods using an Applied Biosystems 477A gas-phase sequencer.

Inhibition tests

Inhibition of animal α -amylases by the purified proteins was tested by the method of Bernfeld [15] with the modifications previously described [16]. The following buffers were used, according to the pH optima of the different enzymes: 20 mM sodium acetate, 100 mM NaCl, 0.1 mM CaCl_2 , pH 5.4, for *Tenebrio molitor* (Coleoptera) α -amylase; 25 mM glycine, 100 mM NaCl, 0.1 mM CaCl_2 , pH 9.5, for the enzyme from *Ephesia kuehniella* (Lepidoptera) and 20 mM potassium phosphate, 67 mM NaCl, 0.1 mM CaCl_2 , pH 6.9 for human salivary α -amylase. All tests were performed using approximately 1 U α -amylase, defined as the amount of enzyme required to produce the reducing equivalents of 1 µmol maltose in our experimental conditions.

Immunodetection

A pool of sera from five patients with baker's asthma (the gift of Dr A. Armentia, Hospital Rio Ortega, Valladolid, Spain) was used to test the IgE-binding capacity of the purified proteins in dot-blot assays. The pool was RAST (radioallergosorbent test) class 4 (the highest class of RAST indicating a high level of specific IgE in the sera) when tested

with a commercial wheat-flour and barley-flour disc (Phadebas-RAST Kit, Pharmacia).

Protein samples (1 µg) were solubilized in NaCl/Tris (20 mM Tris/HCl, 150 mM NaCl, pH 8.3) containing 0.001% (mass/vol.) SDS and 2% (by vol.) 2-mercaptoethanol, then heated at 100°C during 5 min. After their adsorption to poly(vinylidene difluoride) membranes, immunodetection was carried out by treatment with 1:2 sera dilutions and ^{125}I -labelled anti-human IgE as reported in [17].

Glycoprotein assay

Glycoproteins were identified after SDS/PAGE and electrotransfer to poly(vinylidene difluoride) membranes by the dansylhydrazine method [18] with minor modifications previously described [12], and by a glycan detection kit (DIG Glycan Detection Kit, Boehringer) as in [3].

RESULTS

Isolation of putative rye inhibitor subunits

A crude inhibitor preparation from rye endosperm was obtained by NaCl extraction and $(\text{NH}_4)_2\text{SO}_4$ precipitation. When this protein preparation was analyzed by two-dimensional electrophoresis using the conditions employed to resolve the wheat and barley inhibitors [11], five major components were detected (Fig. 1A). According to their solubility properties, isoelectric points and electrophoretic mobility in SGE, these components were the main putative inhibitor subunits of rye endosperm. Two of them (Fig. 1A) will not be further considered in this study. RDAI-1 has been previously identified as a homodimeric inhibitor [10], and the spot marked with an asterisk in Fig. 1A corresponds to a protein without inhibitory activity and a low N-terminal sequence similarity with the known members of the inhibitor family (García-Casado, G., unpublished results).

To separate the three expected inhibitor types (tetrameric, dimeric and monomeric), as in wheat and barley [11, 19], the rye inhibitor preparation was fractionated by gel-filtration on Sephadex G-100 under non-dissociating conditions (Fig. 1B). No clear peaks appeared in the rye profile, in contrast with the results found in several *Triticum* and *Hordeum* species [11, 19, 20]. All five major components shown in Fig. 1A eluted chiefly in fractions that partially overlapped with those corresponding to both monomeric and homodimeric inhibitors from tetraploid wheat (Fig. 1B).

Rye components 1–3 were isolated from this gel-filtration fraction by preparative reverse-phase HPLC (Fig. 2A). Each purified protein appeared as a single spot when subjected to two-dimensional electrophoresis (IEF×SGE; data not shown) and as a band of about 13 kDa on SDS/PAGE (Fig. 2B).

N-terminal amino acid sequences of rye purified proteins indicate homologies with barley and wheat inhibitor subunits

To study the putative relationships with the previously characterized wheat and barley members of the inhibitor family [6], the N-terminal sequences of purified components 1–3 were determined (Fig. 3). No heterogeneity was detected at any point of the sequences. Comparison with known sequences of inhibitor subunits from *Triticeae* species allowed the inclusion of the rye proteins into specific subfami-

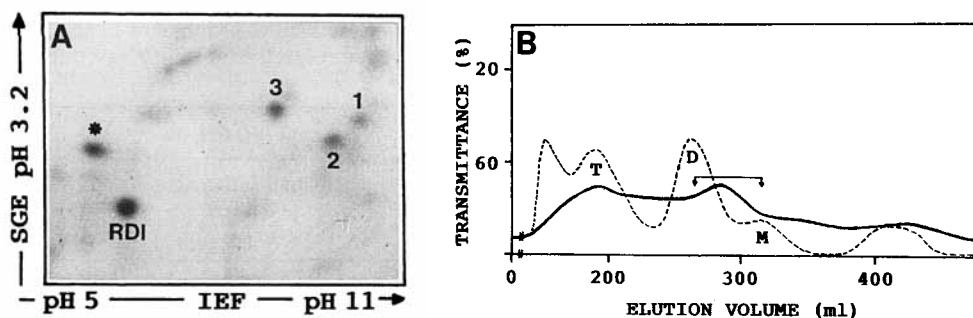


Fig. 1. Fractionation of α -amylase inhibitor subunits from rye. (A) Two-dimensional electrophoretic map (IEF $\times$ SGE) of the crude inhibitor preparation from rye endosperm. Positions of components 1–3 are indicated. RDI is a homodimeric inhibitor previously characterized (RDAI-1; [10]). The asterisk marks a component that is not further considered in this study. (B) Gel-filtration on Sephadex G-100 of the crude inhibitor preparations from rye (—) and tetraploid wheat (---). Peaks corresponding to tetrameric (T), dimeric (D) and monomeric (M) inhibitors are indicated in the wheat profile. The fractions of the rye inhibitor preparations used for further purification steps is marked by a horizontal bar.

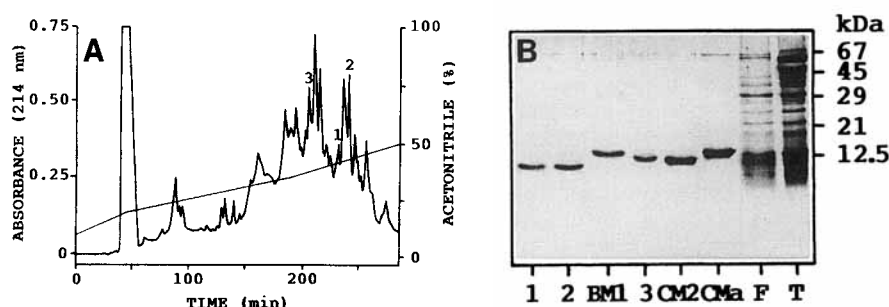


Fig. 2. Isolation of major α -amylase inhibitors from rye. (A) Reverse-phase HPLC separation of the gel-filtration fraction marked in Fig. 1B. Peaks corresponding to components 1–3 are indicated. (B) SDS/PAGE of purified components 1–3. Rye samples: T, crude inhibitor preparation; F, gel-filtration fraction marked in Fig. 1B; 1–3, purified components 1–3, respectively. Tetrameric inhibitor subunits from wheat (CM2) and barley (CMA) and the barley monomeric α -amylase inhibitor-1 (BM1) are included for comparison. The molecular masses of reference proteins appear on the right.

PROTEIN	N-TERMINAL SEQUENCE		
	1	10	20
COMP. 1	SPGEWCWPGMGHP	MY	PFPRCRAL
COMP. 2	SPGEWCWPGMGX	P	
BMAI-1	SPGEWCWPGMGYP	V	VPFPRCRAL
COMP. 3	TGPHYCYAGMGLPT	TK	PLEGCREYVAQQT
WTAI-CM2	TGPHYCYPGMGLP	SN	PLEGCREYVAQQT
BTAI-CMA	TGQYCYAGMGLP	SN	PLEGCREYVAQQT

Fig. 3. N-terminal sequences of the purified proteins from rye (components 1–3). The sequences of BMAI-1 (barley monomeric α -amylase inhibitor-1; [12]) and one of the subunits of the tetrameric α -amylase inhibitor from wheat (WTAI-CM2; [21]) and barley (BTAI-CMA; [22]) are included for comparison.

lies. Components 1 and 2, which showed identical sequences in the first 14 amino acids, were found to be closely related to the barley monomeric α -amylase inhibitor BMAI-1 (21 identical residues out of 23 in the case of component 1).

Component 3 was identified as the rye equivalent to one of the wheat, WTAI-CM2, and one of the barley, BTAI-CMA, subunits of the tetrameric inhibitors (25 identical residues out of 28 in either binary comparison). This protein is probably a genetic variant of the putative rye α -amylase inhibitor partially sequenced previously by Lyons et al. ([9]; 27 identical residues out of 28).

The purified rye components show different inhibitory activities than their wheat and barley homologues

The inhibitory properties against α -amylases from three different origins, *T. molitor* (Coleoptera), *E. kuehniella* (Lepidoptera) and human saliva, of the three proteins isolated from rye were tested. Neither rye components nor their equivalent wheat or barley inhibitor subunits showed activity towards the Lepidoptera enzyme. The effects observed against the amylases from *T. molitor* and human saliva are summarized in Table 1.

Component 1 behaved as component 2, as well as its barley homologue BMAI-1, with respect to *T. molitor* α -amylase, but, in contrast with them, also inhibited the human salivary enzyme.

Component 3 exerted a low effect against the Coleoptera enzyme, as BTAI-CMA, and was also active towards the human α -amylase. No inhibition of this amylase was found for its equivalent wheat and barley subunits (WTAI-CM2 and BTAI-CMA). Moreover, the tetrameric inhibitors from both species are also inactive against this enzyme [11, 19].

The aggregative behaviour of components 1–3 can be studied in Triticale

The crude inhibitor preparations from Triticale (genomes AABBRR; an allohexaploid from *Triticum turgidum*, tetra-

Table 1. Activity towards *T. molitor* and human salivary α -amylases of components 1–3 from rye. Values are means $\pm$ SE ($n = 4$). All tests were carried out using approximately 1 U α -amylase. BMAI-1 (barley monomeric α -amylase inhibitor-1) and WTAI-CM2 and BTAI-CMa (CM2 and CMa subunits of the wheat and barley α -amylase tetrameric inhibitor, respectively) were assayed for comparisons. (–), no inhibition

Protein	Inhibition of α -amylase from					
	<i>T. molitor</i> at			human saliva at		
	0.5 μ g/assay	1 μ g/assay	3 μ g/assay	0.5 μ g/assay	1 μ g/assay	3 μ g/assay
	%					
Component 1	51 $\pm$ 2	60 $\pm$ 1	76 $\pm$ 4	11 $\pm$ 3	15 $\pm$ 3	41 $\pm$ 5
Component 2	62 $\pm$ 3	70 $\pm$ 1	85 $\pm$ 2	–	–	–
BMAI-1	61 $\pm$ 2	69 $\pm$ 2	79 $\pm$ 1	–	–	–
Component 3	–	12 $\pm$ 3	27 $\pm$ 1	17 $\pm$ 5	23 $\pm$ 5	40 $\pm$ 2
WTAI-CM2	–	–	–	–	–	–
BTAI-CMa	–	–	24 $\pm$ 5	–	–	–

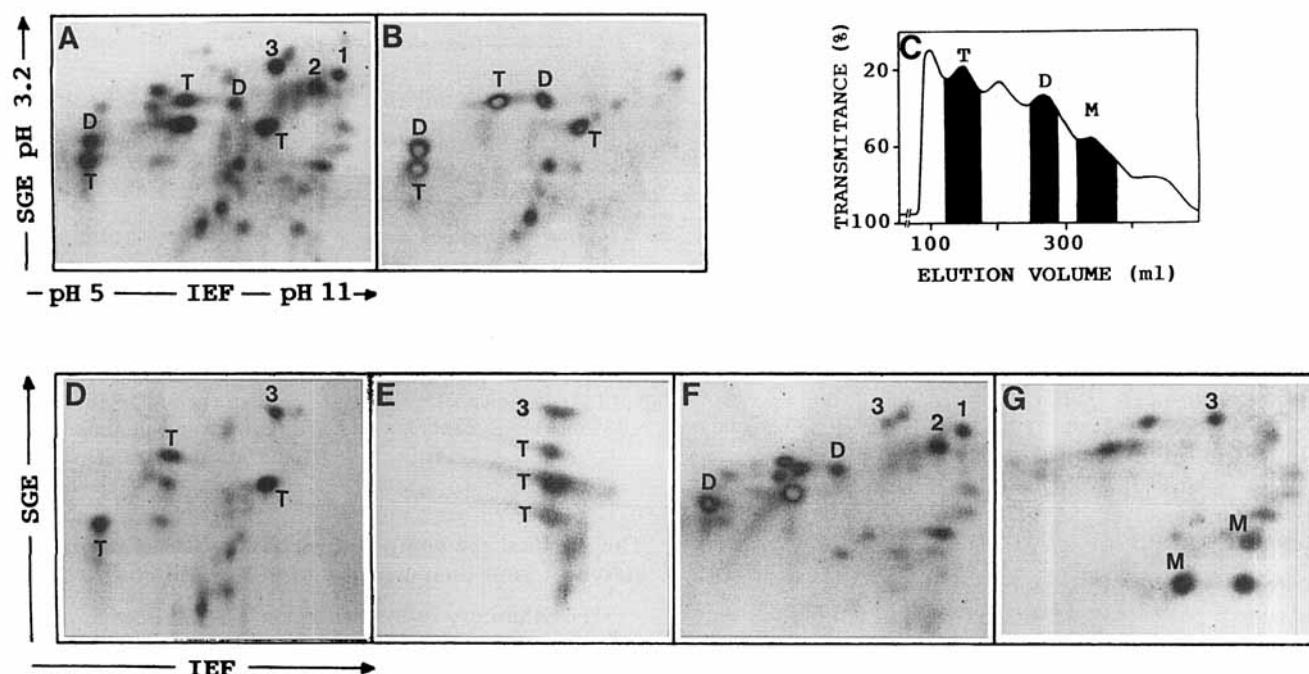


Fig. 4. Fractionation of α -amylase inhibitors from Triticale. (A, B) Two-dimensional electrophoresis maps of the crude inhibitor preparations from Triticale (A) and *T. turgidum* (B). Positions of rye components 1–3 and of wheat tetrameric (T) and dimeric (D) inhibitor subunits are indicated (no monomeric inhibitors, M, can be detected in the crude preparation from *T. turgidum*; [23]). (C) Gel-filtration on Sephadex G-100 under non-dissociating conditions of the crude inhibitor preparation from Triticale. Fractions corresponding to wheat tetramers (T), dimers (D) and monomers (M) are marked. (D–G) Two-dimensional electrophoretic maps of the gel-filtration fractions from Triticale shown in (C). (D, E) Tetrameric, (F) dimeric and (G) monomeric inhibitors. (D) and (E) are the same sample analyzed under dissociating (with 6 M urea) and non-dissociating (without 6 M urea) conditions in the first dimension (IEF). Positions of the rye and wheat inhibitors are indicated as in (A).

ploid wheat and rye) and *T. turgidum* (genomes AABB) were obtained using the same strategy as in the case of rye. The two-dimensional electrophoretic maps of these proteins extracts appear in Fig. 4A and B, respectively. All three components purified from rye could be detected in the Triticale preparation. When this preparation was fractionated by gel filtration under non-dissociating conditions, an elution profile with defined peaks corresponding to the three different inhibitor types from *T. turgidum* was obtained (Fig. 4C). This approach allows us to use the wheat inhibitor subunits as internal molecular markers to study the aggregative properties of rye components 1–3.

Gel-filtration fractions of Triticale with elution volumes similar to those of wheat tetrameric, dimeric and monomeric inhibitors (Fig. 4C), were analyzed by two-dimensional electrophoresis (Fig. 4D–G). Component 3 eluted with the wheat tetrameric inhibitor, but also with the dimeric and monomeric ones. In contrast to these results, components 1 and 2 were found only in fractions where dimeric inhibitors from *T. turgidum* appeared.

The association of component 3 with the subunits of the wheat tetramer was confirmed by using non-dissociating conditions in the first dimension (IEF) of the electrophoretic procedure. The rye protein was aligned with the wheat sub-

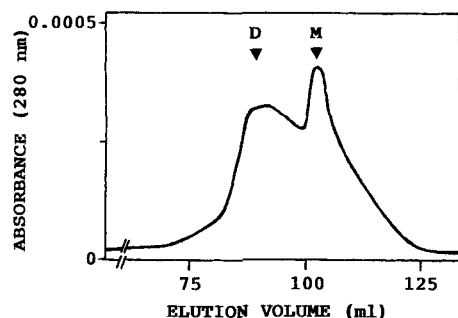


Fig. 5. Gel filtration on HPLC of purified component 3 from rye. Arrowheads point to the elution volumes of two purified proteins from *T. turgidum* that behave as dimeric (D; WDAI-1) and monomeric (M, WTAI-CM2) proteins.

units (conforming the putative oligomers) in these conditions (Fig. 4E). However, component 3 was shown to form only dimers and monomers in the absence of the *T. turgidum* inhibitor subunits, when the purified protein was analyzed by gel filtration on a HPLC column (Fig. 5).

Rye component 3 can interact with wheat and barley subunits to form functional tetrameric inhibitors *in vitro*

The results presented above suggest that component 3 associates with wheat subunits to form tetramers in Triticale. This was further confirmed by the *in vitro* reconstitution of the tetramers from the appropriate mixtures of the purified wheat subunits and rye component 3 (Table 2). The rye protein was able to replace its homologous wheat subunit, WTAI-CM2, in the tetramers. The mixture with the stoichiometry of purified proteins 1 component 3: 2 WTAI-CM3: 1 WTAI-CM16 was active both against Coleoptera (*T. molitor*) and Lepidoptera (*E. kuehniella*) α -amylases, although a slight decrease in the inhibitory effects was observed in both cases in comparison with the *T. turgidum* tetramer (stoichiometry of subunits, 1 WTAI-CM2: 2 WTAI-CM3: 1 WTAI-CM16). As expected, the mixtures where component 3 replaced the wheat subunits WTAI-CM3 and WTAI-CM16, which belong

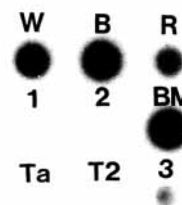


Fig. 6. IgE immunodetection by dot blotting of components 1–3 from rye. Protein samples (1 μ g) were adsorbed on poly(vinylidene difluoride) membranes, which were subsequently treated with a pool of sera from baker's asthma patients and ^{125} I-labelled anti-human IgE antibody. The following samples were tested: crude inhibitor preparations from wheat (W), barley (B) and rye (R) and purified rye components 1–3 (1–3), barley monomeric α -amylase inhibitor-1, BMAI-1 (BM) and wheat WTAI-CM2 (T2) and barley BTAI-CMa (Ta) subunits of tetrameric inhibitors.

to different subfamilies to that of component 3, were not effective (Table 2). It has been shown previously [11] that neither the individual *T. turgidum* subunits nor their binary mixtures (such as WTAI-CM16: WTAI-CM3) were active towards the insect amylases used. These results were confirmed by the appropriate replacements in the barley tetrameric inhibitor (Table 2).

Rye component 1 and 2 have very different human IgE-binding capacities than their barley homologues

Several members of the α -amylase/trypsin inhibitor family are major allergens associated with the occupational allergy baker's asthma [3, 12]. The IgE-binding capacity of the three proteins purified from rye were tested by a dot-blot assay, and compared with those of their wheat and barley homologues (Fig. 6). No reaction was found in the case of component 1 and 2, in contrast with the strong one shown by their barley equivalent, BMAI-1. Component 3 and their homologous BTAI-CMa and WTAI-CM2 did not give a significant response.

As BMAI-1 and two other major allergens of the inhibitor family are glycosylated, and the attached sugars seem to

Table 2. Activity towards *T. molitor* and *E. kuehniella* α -amylases of tetrameric inhibitors reconstituted from purified subunits from rye, wheat and barley. Values are means $\pm$ SE ($n = 4$). All tests were carried out using approximately 1 U α -amylase. WTAI-CM2, WTAI-CM3 and WTAI-CM16 are the subunits of the tetrameric inhibitor from *T. turgidum* cv. Enano de Andujar (stoichiometry 1 WTAI-CM2: 2 WTAI-CM3: 1 WTAI-CM16), and BTAI-CMa, BTAI-CMb and BTAI-CMd are those of the tetrameric inhibitor from *H. vulgare* cv. Bomi (stoichiometry 1 BTAI-CMa: 2 BTAI-CMd: 1 BTAI-CMb). (–), no inhibition

Subunits	Inhibition of α -amylase from			
	<i>T. molitor</i>		<i>E. kuehniella</i>	
	0.5 μ g/assay	1 μ g/assay	1 μ g/assay	3 μ g/assay
	%			
WTAI-CM2+2 WTAI-CM3+WTAI-CM16	82 $\pm$ 2	84 $\pm$ 2	82 $\pm$ 1	87 $\pm$ 1
Component 3+2 WTAI-CM3+WTAI-CM16	69 $\pm$ 1	77 $\pm$ 2	33 $\pm$ 8	54 $\pm$ 4
WTAI-CM2+2 component 3+WTAI-CM16	–	–	–	–
WTAI-CM2+2 WTAI-CM3+component 3	–	13 $\pm$ 2	–	–
BTAI-CMa+2 BTAI-CMd+BTAI-CMb	26 $\pm$ 2	57 $\pm$ 2	–	–
Component 3+2 BTAI-CMd+BTAI-CMb	–	21 $\pm$ 1	–	–
BTAI-CMa+2 component 3+BTAI-CMb	–	–	–	–
BTAI-CMa+2 BTAI-CMd+component 3	–	–	–	–

be important in IgE recognition [3, 12], the proteins purified from rye were analyzed in two different glycoprotein assays, the dansylhydrazine method [18] and the DIG glycan detection kit [3]. None of the three rye components showed a positive staining in conditions where BMAI-1 did (data not shown).

DISCUSSION

Three major components of the rye α -amylase-inhibitor family have been isolated and characterized. Comparison of their N-terminal sequences with those of wheat and barley members of the same inhibitor group, has allowed the classification of the rye components into two different subfamilies previously established [5, 6].

Two of the purified proteins, components 1 and 2, have an identical N-terminal sequence in the first 14 residues. Whether they are encoded by the same gene or by different genes remains to be shown. Both components are rye members of the BMAI-1 (a barley monomeric inhibitor which does not seem to have the corresponding wheat homologue) subfamily [6]. However, components 1 and 2 behave as homodimeric proteins and, unlike BMAI-1 [12], are not glycosylated. This last difference is likely to be responsible for the strong IgE-binding of BMAI-1 when tested with sera of allergic patients, in contrast with the lack of response of the rye components. Concerning their inhibitory properties, the three proteins show similar activities towards *T. molitor* α -amylase, but only component 1 is effective against the human salivary enzyme. This case shows (together with that of component 3 also presented in this study) two members of the same cereal α -amylase inhibitor subfamily with different specificities towards α -amylases of different origins.

The third protein purified from rye, component 3, belongs to another inhibitor subfamily, which also includes the tetrameric inhibitor subunits WTAI-CM2 from wheat and BTAI-CMa from barley [6]. Tetrameric inhibitors composed of subunits which are representative of three different highly conserved subfamilies have been characterized in several species of *Triticum* and *Hordeum* [6, 11, 19, 20]. No members of two of these subfamilies have been detected in rye, even when specific extraction solvents for the tetrameric subunits, such as chloroform/methanol mixtures, are employed. This suggests that no heterotetrameric inhibitor is present in rye endosperm.

Component 3 shows significantly different inhibitory and aggregative properties than the tetrameric inhibitor subunits from wheat and barley [11, 19]. It is active towards *T. molitor* and human salivary α -amylases, whereas the *Triticum* and *Hordeum* subunits are not inhibitory by themselves, with the exception of BTAI-CMa which weakly affects the insect enzyme. With regard to the aggregative properties, component 3 appears in fractions corresponding to dimers and monomers, unlike its wheat counterpart, WTAI-CM2, which behaves as a monomeric protein in isolation, or eluted with the tetramers when the other two wheat subunits are present. However, in spite of the differences discussed above, the rye protein has retained the ability to interact with the subunits of wheat and barley tetramers both *in vivo* (Triticale) and *in vitro* (effectively replaces its wheat and barley homologues in reconstitution experiments of tetrameric inhibitors).

The differential behaviour of rye inhibitors with respect to their homologues from other *Triticeae* species should not be the rule. Recently a homodimeric inhibitor has been char-

acterized which shows similar aggregative and inhibitory properties to its equivalent wheat proteins [10].

The information so far available indicates a high degree of conservation among the genomes of wheat, barley and rye, both in gene order and composition [24]. However, joint consideration of the results presented in this study and those of García-Casado et al. [10], concerning major inhibitor subunits, show that in rye, components included in inhibitor subfamilies which seem to be specific to wheat or barley, are present. In contrast, subunit inhibitor types highly conserved in these two cereals (such as two components of the tetramers) are not detected in rye. These observations suggest that there are important changes among closely related species in the array of inhibitor subunits expressed. Furthermore, post-translational modifications, such as glycosylation, of homologous proteins (i.e. the case of BMAI-1 versus components 1 and 2) can also be different from one species to another.

Taking these facts all together with regard to the expression of the α -amylase inhibitor multigene family in various cereals, the *Triticeae* species can be considered as different manifestations of one manageable genome. This situation is fully in accord with the proposal of Bennetzen and Freeling [25] that grasses be considered as a single genetic system.

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