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Sequence and properties of HMW subunit 1Bx20 from pasta wheat (*Triticum durum*) which is associated with poor end use properties

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Abstract The gene encoding high-molecular-weight (HMW) subunit 1Bx20 was isolated from durum wheat cv. Lira. It encodes a mature protein of 774 amino acid residues with an M_r of 83,913. Comparison with the sequence of subunit 1Bx7 showed over 96% identity, the main difference being the substitution of two cysteine residues in the *N*-terminal domain of subunit 1Bx7 with tyrosine residues in 1Bx20. Comparison of the structures and stabilities of the two subunits purified from wheat using Fourier-transform infra-red and circular dichroism spectroscopy showed no significant differences. However, incorporation of subunit 1Bx7 into a base flour gave increased dough strength and stability measured by Mixograph analysis, while incorporation of subunit 1Bx20 resulted in small positive or negative effects on the parameters measured. It is concluded that the different effects of the two subunits could relate to the differences in their cysteine contents, thereby affecting the cross-linking and hence properties of the glutenin polymers.

Keywords Wheat · Gluten elasticity · Glutenin polymers · Disulphide bonds · HMW subunits

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Introduction

The demonstration that allelic variation in the composition of the high-molecular-weight (HMW) subunits of bread wheat glutenin is associated with differences in the visco-elasticity of gluten and dough and breadmaking quality (Payne 1987) has led to an interest in the identification of novel types of HMW subunits with unusual structures and properties which can be exploited in plant breeding programmes. These include novel HMW subunits with unusually high or low molecular masses from germplasm of Japanese or exotic origin (Payne et al. 1983; Tahir et al. 1996) and subunits with unusual properties from related species such as *Aegilops cylindrica* (Wan et al. 2000, 2002).

In contrast, subunit 1Bx20 is widely distributed in tetraploid pasta wheat, being present in 33.5% of the cultivars in a world collection of 502 cultivars and in 32.8% of 195 French cultivars (Branlard et al. 1989). Furthermore, its presence is associated with poor quality for pasta making when compared with other chromosome 1B-encoded subunits (Liu et al. 1996). Although it was initially considered to occur with a 1By null gene, analysis by reversed-phase-high performance liquid chromatography (HPLC) has shown the presence of a y-type subunit which accounts for about a third of the total protein and co-migrates with subunit 1Bx20 on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Margiotta et al. 1993). Subunit 1Bx occurs less widely in bread wheats but is again associated with poor end-use quality in this species (Lukow et al. 1989; Payne et al. 1989; Bianchi et al. 1993).

Preliminary characterization of subunit 1Bx20 by *N*-terminal amino acid sequencing showed that it dif-

fered from all other known x-type subunits in containing only one cysteine residue within the first 47 amino acids, with the cysteines present at positions 22 and 37 in subunit 1Bx7 being replaced by tyrosines (Tatham et al. 1991). Since x-type subunits typically contain three cysteines in their *N*-terminal domain and one in their *C*-terminal domain (Shewry et al. 1992), this suggested that subunit 1Bx20 contained only two cysteine residues. Indirect evidence in support of this conclusion came from comparison of the mobilities of "native" and reduced and alkylated subunits by acid-PAGE and reversed phase-HPLC (Morel and Bonicel 1996; Margiotta et al. 1993).

In addition to containing less cysteine residues, subunit 1Bx20 also appears to differ from all other HMW subunits in its conformational stability, showing an unusual concave pattern when unfolded on transverse urea gels in the presence of SDS (Lafiandra et al. 1999). It is possible, therefore, that the unusually low cysteine content and/or the low conformational stability could contribute to the association of subunit 1Bx20 with poor quality for pasta making. However, a contribution from the associated y-type subunit (1By20) cannot be ruled out.

In order to determine the precise molecular basis for the association of subunit 1Bx20 with poor quality, we have isolated the corresponding gene in order to determine its full amino acid sequence. In addition, we have compared the structures of subunits 1Bx20 and 1Bx7 (for which a full amino acid sequence is also known) using circular dichroism (CD) and Fourier-transform infrared (FT-IR) spectroscopy and their functional properties by incorporation into dough and Mixograph analysis.

Materials and methods

DNA extraction, polymerase chain reaction (PCR) amplification and cloning of the gene encoding HMW subunit 1Bx20

Genomic DNA was extracted from leaves of the durum wheat cv. Lira biotype 45 by following the procedure reported in D'Ovidio et al. (1992). PCR analysis to specifically amplify the gene encoding subunit 1Bx20 was carried out using the UTV2F/UTV2R primers as previously reported (D'Ovidio et al. 1997). The primers used span the complete coding region, including the signal peptide. Amplicons were recovered from agarose gel and inserted into the T/A site of the PCR-Xl-TOPO vector (Invitrogen, The Netherlands). One resulting clone, named pUTVBx20, containing the complete coding region encoding HMW subunit 1Bx20 was subjected to nucleotide sequencing analysis.

Nucleotide sequence was obtained by primer walking. Chain-terminator sequencing reactions were performed with an ABI Prism dRhodamine Terminator Cycle Sequencing Reaction Kit, using a GeneAmp 9700 cycler (Perkin Elmer), with 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. The PCR products were precipitated with ethanol, dissolved in 3 µl formamide loading buffer, denatured by heating at 95 °C for 2 min and loaded onto a 4.45% (w/v) polyacrylamide gel in an ABI Prism 377XL (Perkin Elmer) sequencing machine.

Purification of HMW subunits 1Bx20 and 1Bx7

Flours from durum wheat cv. Bidi 17 and bread wheat cv. Longbow were defatted with chloroform, and monomeric proteins removed by extraction with 0.3 *M* sodium iodide and 7.5% (v/v) propan-*1*-ol (Fu and Kovacs 1999). The glutenin proteins were extracted with 50% (v/v) propan-*1*-ol containing 1% (w/v) DTT at 60 °C (based on Marchylo et al. 1989). The propan-*1*-ol concentration was increased to 60% (v/v) and, after 2 h at 4 °C, the precipitate was collected by centrifugation and discarded. The supernatant was allowed to stand overnight at 4 °C, and the precipitated protein was collected by centrifugation and dialysed against 1% (v/v) acetic acid for 30 h and freeze-dried.

Subunits 1Bx20 and 1Bx7 were isolated from the HMW subunit fractions by ion exchange chromatography using carboxymethyl cellulose (CMC) buffered with 50 m*M* glycine acetate, pH 4.6, containing 3 *M* urea and 3% (v/v) 2-mercaptoethanol. The subunits were eluted with a 0 to 100 m*M* linear gradient of NaCl in the same buffer (Tatham et al. 2000). Fractions were monitored using SDS-PAGE, and those containing subunits 1Bx20 or 1Bx7 were pooled, dialysed against 1% (v/v) acetic acid for 60 h at 4 °C and freeze-dried.

Structure prediction

The method of Chou and Fasman (1978) was used to predict the structure of the central repetitive domains of subunits 1Bx7 and 1Bx20, for α-helix, β-sheet, random coil and β-turn conformations.

CD Spectroscopy

The subunits were each dissolved in 8 *M* urea containing 1% (v/v) 2-mercaptoethanol and dialysed exhaustively against 1% (v/v) acetic acid or 20 m*M* CAPS buffer, pH 11.0. The protein concentrations of the dialysates were determined from the absorbances at 280 nm using molar extinction coefficients calculated from the contents of tryptophan, tyrosine and phenylalanine in the amino acid sequences (Gill and Hipple 1989). Protein concentrations were adjusted using the appropriate solvent giving four samples (1Bx7 and 1Bx20, each at low and high pH) of 35.8 µ*M*. Immediately prior to analysis, urea solution and buffer were added to give a range of urea concentrations from 0 *M* to 8 *M* and final protein concentrations of 7.16 µ*M*. Spectra were recorded at 25 °C on a Jobin-Yvon CD6 spectropolarimeter as the average of four scans and corrected for baseline changes in the presence or absence of urea as appropriate.

FT-IR spectroscopy

FT-IR spectra were recorded on a Bio-Rad FTS6000 spectrometer with a single reflection Diamond ATR accessory (SPECAC). The proteins were stirred in distilled water (10 mg/ml) using a Rotamixer for approximately 2 min and then left to stand overnight, all operations being carried out at 4 °C. The clear supernatant was used for IR experiments. The spectra were recorded at room temperature (approx. 20 °C) at 2 cm^{-1} resolution (256 scans, referenced against the empty crystal). Water spectra (liquid and vapour) were subtracted. Fourier deconvolution was performed with the spectrometer software using a half-width of 16 cm^{-1} and a resolution enhancement factor of 1.8. Secondary structures were estimated from the relative intensities of four peaks in the amide I band (height divided by total absorbance).

Mixograph analysis

Subunit 1Bx7 or 1Bx20 (10 mg) was incorporated into 2 g of base flour (bread wheat cv. Banks, protein content 10.9%) and the Mixograph properties determined as described by Békés et al. (1994).

Results and discussion

Isolation and sequence analysis of the subunit 1Bx20 gene

Genomic DNA from durum wheat cv. Lira biotype 45 was used as a template for the amplification of the *Glu-B1x* allele encoding HMW subunit 1Bx20. The PCR product of 2,408 bp was cloned and its nucleotide sequence obtained by primer walking. HMW subunit genes contain no introns, and the cloned PCR product contained an open reading frame of 2,398 bp encoding a peptide of 795 amino acid residues. The nucleotide sequence has been deposited in the EMBL databank under accession no. AJ437000.

Comparison of the amino acid sequences of subunits 1Bx7 and 1Bx20

The protein encoded by the DNA amplified from the *Glu-B1x* gene had a similar structure to previously characterized HMW subunits. It comprised a 21 amino acid signal peptide and short *N*- and *C*-terminal domains flanking a more extensive repetitive domain. The predicted mature protein consisted of 774 amino acid residues with a predicted M_r of 83,913. The identity was confirmed by comparison with the previously reported *N*-terminal sequence of subunit 1Bx20 determined by direct analysis of the purified protein (Tatham et al. 1991). The *N*- and *C*-terminal domain sequences were used to produce a dendrogram of 1Bx20 and HMW subunits encoded by the other genomes of bread wheat (1Ax1, EMBL accession no. X61009; 1Ay silent, accession no. X03042; 1Bx7, accession no. X13927; 1By9, accession no. X61026; 1Dx5, accession no. X12928; 1Dy10, accession no. X12929). This analysis showed that the sequence was most closely related to that of subunit 1Bx7.

The amino acid sequence of HMW 1Bx20 is aligned with that of HMW 1Bx7 in Fig. 1. The sequences differ by only 29 amino acid substitutions (over 96% identity) and three deletions/insertions, resulting in 1Bx7 being slightly smaller than 1Bx20. Subunit 1Bx7 comprises 768 amino acid residues with a predicted M_r of 82,528. Although subunit 1Bx20 is slightly larger than subunit 1Bx7, it migrates faster on SDS-PAGE (Fig. 2). Similar anomalous mobility has been noted previously for other allelic pairs of HMW subunits; for example, subunit 1Dy10 (M_r of 67,495) migrates slower than subunit 1Dy12 (M_r of 68,696). There is currently no explanation for this behaviour, but it could result from differences in the extent of residual secondary structure in the allelic proteins.

The *N*-terminal domain of 1Bx7 contains a deletion of five residues (residues 12–16 of 1Bx20) in a region that is also variable in other x-type HMW subunits (Wan et al. 2002). There are also three amino acid substitutions in the *N*-terminal domain, two of which result in a cys-

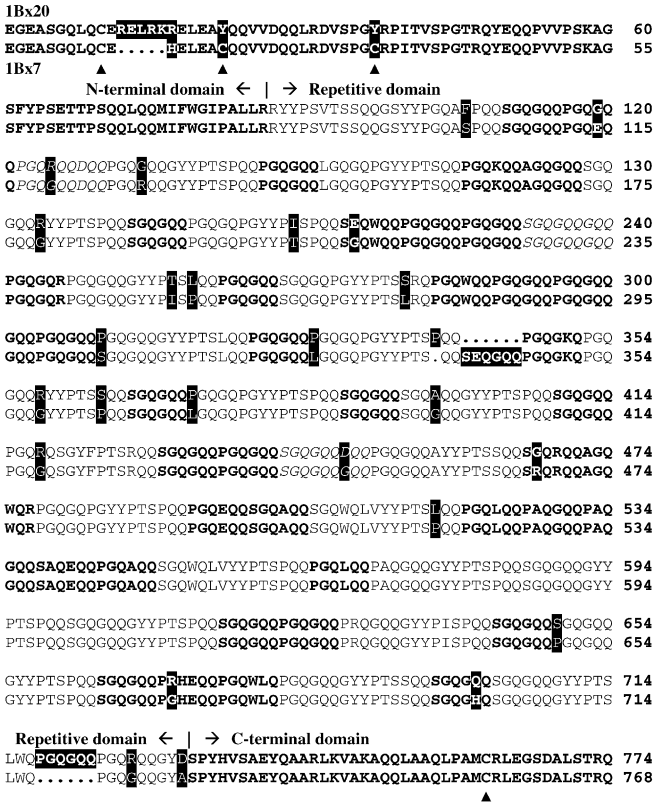


Fig. 1 Alignment of the amino acid sequences of HMW subunits 1Bx20 and 1Bx7. The *N*- and *C*-terminal domains and blocks of hexapeptides in the repetitive domain are shown in *bold*. Nine amino acid repeats (hexapeptide plus tripeptide) are shown in *italics*, 15 amino acid repeats (hexapeptide plus nonapeptide) are in *normal font*, *not bold*. Cysteines are indicated by *arrows*. Differences between the subunits are shown in *black boxes*

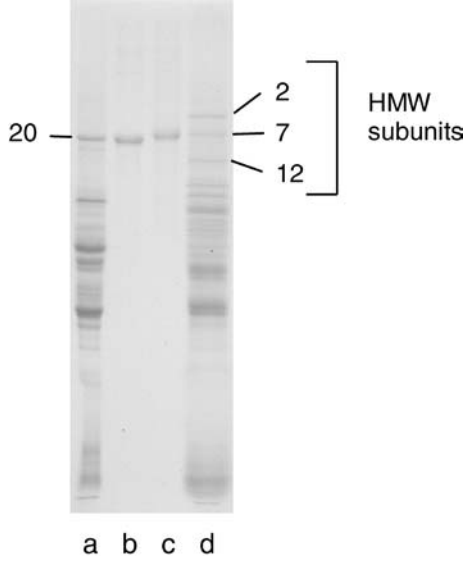


Fig. 2 SDS-PAGE of the purified subunits 1Bx20 and 1Bx7. *a* Total seed proteins from durum wheat cv. Bidi 17, *b* subunit 1Bx20 purified from cv. Bidi 17, *c* subunit 1Bx7 purified from bread wheat cv. Longbow, *d* total seed proteins from cv. Longbow

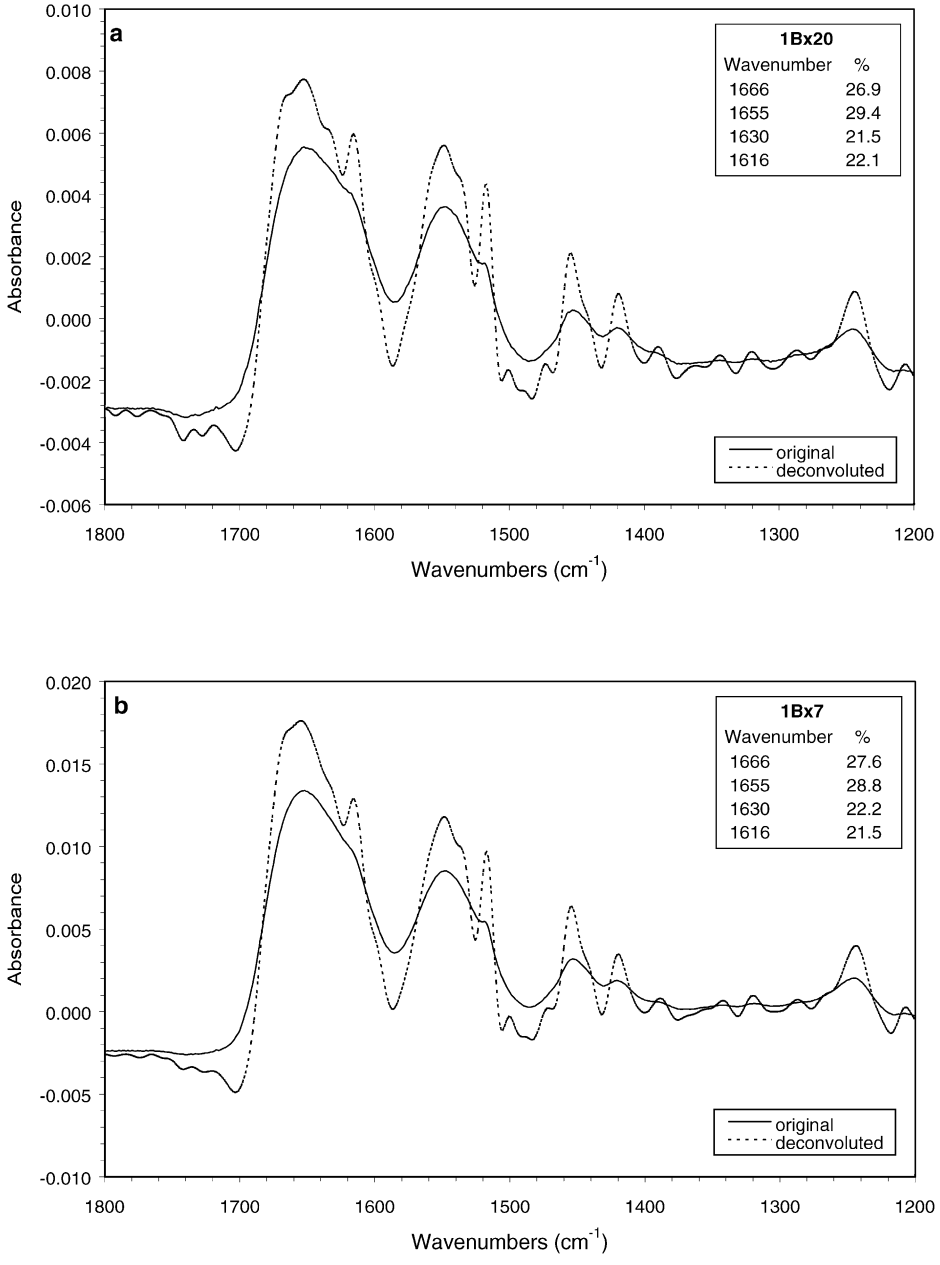


Fig. 3 Original and deconvoluted FT-IR spectra of subunit 1Bx20 (**a**) and subunit 1Bx7 (**b**) in the hydrated solid state (water subtracted). *Insets* show the relative absorbances for the major amide I band components, calculated from the deconvoluted spectra. The standard error for these measurements was ±0.7%

teine residue present in 1Bx7 being replaced with a tyrosine residue. This means that 1Bx20 has only a single cysteine residue in the *N*-terminal domain and only two in total. All other x-type HMW subunits have three cysteines in the *N*-terminal domain. It is intriguing that 2 of 29 amino acid substitutions (7%) have replaced cysteine residues when cysteines represent only 0.5% by number of the amino acids present. The *C*-terminal domains of the two proteins are identical.

In x-type HMW subunits, the repetitive domain consists of three types of repeat motif (Shewry et al. 1992): a hexapeptide repeat with the consensus sequence Pro Gly Gln Gly Gln Gln, a nonapeptide repeat consisting of the hexapeptide plus a tripeptide with the consensus sequence Gly Gln Gln, and a 15 amino acid repeat consisting of the hexapeptide plus a nonapeptide with the consensus sequence Gly Tyr Tyr Pro Thr Ser Pro Gln Gln. Both subunits 1Bx7 and 1Bx20 fit this pattern (Fig. 1). There are 26 single amino acid substitutions between the two proteins in this domain, but the repeat motifs line up exactly except for a single additional hexapeptide in 1Bx7 at position 340–345 and an additional hexapeptide in 1Bx20 at position 718–723.

Purification and characterization of subunits 1Bx7 and 1Bx20

Subunits 1Bx7 and 1Bx20 were purified from flour of bread wheat cv. Longbow and pasta wheat cv. Bidi 17, re-

spectively, using differential precipitation followed by ion exchange chromatography on CM cellulose. SDS-PAGE of the preparations (Fig. 2) showed a high level of purity.

Previous studies using structural prediction and direct spectroscopic analysis of whole proteins and recombinant and synthetic peptides have demonstrated that the central repetitive domains of the HMW subunits are rich in β-reverse turns which are arranged in a regular fashion to reflect the repetitive primary structure (Tatham et al. 1985, 1990; Field et al. 1987; van Dijk et al. 1997a, b; Gilbert et al. 2000).

Structure prediction was, therefore, initially used to compare the sequences of the repetitive domains of subunits 1Bx7 and 1Bx20 using the method of Chou and Fasman (1978). The results did indeed indicate that both proteins formed regularly repeated β-reverse turns, but the turns predicted were similar in total number and position (data not shown) in the two subunits. A direct comparison of the secondary structure contents of the two subunits was also made using FT-IR spectroscopy of protein in the fully hydrated state. The infrared spectra of the two subunits were almost identical (Fig. 3). Fourier-deconvolution resolved four major components in the amide I band at about 1,666, 1,655, 1,630 and 1,616 cm^{-1}. Previous studies of HMW subunits have indicated that the absorbance at 1,666 cm^{-1} can be assigned to β-turns and the component at 1,655 cm^{-1} to a mixture of glutamine side chains and α-helix. The absorbances at 1,616 cm^{-1} and 1,630 cm^{-1} can be assigned to inter- and intramolecular β-sheet based on analogy with model proteins and peptides and, in the case of the HMW subunits, may reflect the formation of an "inter-chain" β-sheet-like structure by adjacent β-turn-rich domains (Belton et al. 1995; Gilbert et al. 2000). The approximate proportions of these four structural components, determined from the relative absorbance at these wavelengths in the deconvoluted spectra, were similar for the two subunits (see insets in Fig. 3a, b). The small differences between the values of the two samples were within the standard error of ±0.7% which has been determined for this method (unpublished results of N. Wellner, K. Feeney, A.S. Tatham, P.R. Shewry and P.S. Belton). It can be concluded, therefore, that subunits 1Bx7 and 1Bx20 have essentially identical secondary structure contents.

The conformational stability of the two subunits was compared using a further spectroscopic approach, circular dichroism. CD spectroscopy of a recombinant peptide from the repetitive domain of subunit 1Dx5 has demonstrated that the β-turn conformation is in fact in equilibrium with the poly-L-proline II (PPII) structure, the latter being favoured at lower temperatures (Gilbert et al. 2000). In the present study, CD spectroscopy was used to determine the effects of urea (a chaotropic agent that denatures proteins by disruption of hydrogen bonds) at concentrations up to 8 *M*. Spectra could not be recorded below 205 nm due to the strong absorption of urea at low wavelengths but were measured up to 260 nm (i.e. in the far uv region). Previous studies and analyses of model peptides and proteins indicate the presence of various competing signals from

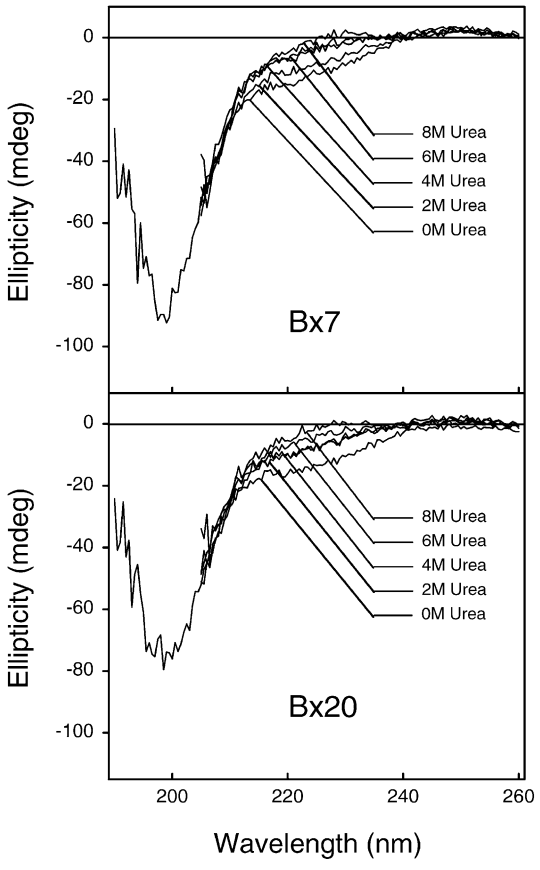
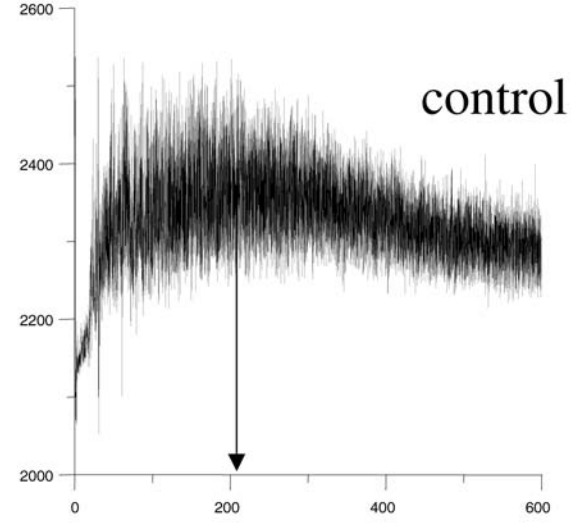


Fig. 4 Far UV CD spectra of subunits 1Bx7 and 1Bx20 dissolved in CAPS buffer at pH 11 with increasing concentrations (0–8 *M*) of urea

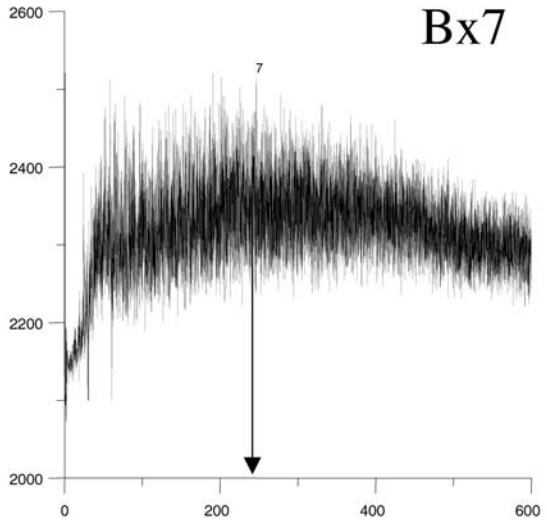
the α-helical domains (minima at 208 and 222 nm), βI/III turns (200–220 nm), PPII structure (217–228 nm) and aromatic residues, in particular tyrosine (226 nm or 245 nm). Furthermore, while a high concentration of urea destabilises α-helical structure, it may promote the formation of PPII (Drake et al. 1988). Consequently, interpretation of the spectra is not straightforward. However, comparisons at pH 4.0 and 11.0 showed similar behaviour for the two subunits. The spectra at pH 11.0 (Fig. 4) are readily interpreted as the conversion of tyrosines from the phenolic to phenolate form resulting in a "redshift" of their absorbance (from 226–245 nm) revealing the PPII absorbance at 228 nm. The increased absorbances of the two proteins at 217–228 nm with increasing concentrations of urea therefore reflect similar increases in their PPII contents. It is concluded, therefore, that subunits 1Bx7 and 1Bx20 are almost indistinguishable in their behaviour in the presence of increasing concentrations of urea.

Mixograph analysis

The effects of the subunits on the dough mixing properties of wheat were compared by incorporation into a base flour using a 2 g Mixograph with a reduction/re-oxidation procedure that has been demonstrated to result in the



	MT	PR	BWPR	RBD	BWBD
cntrl	216	238	166	17	37
Bx7	259	243	177	8	35
Bx20	238	223	172	11	29
LSD	*19*	*21*	*8*	*3*	*5*



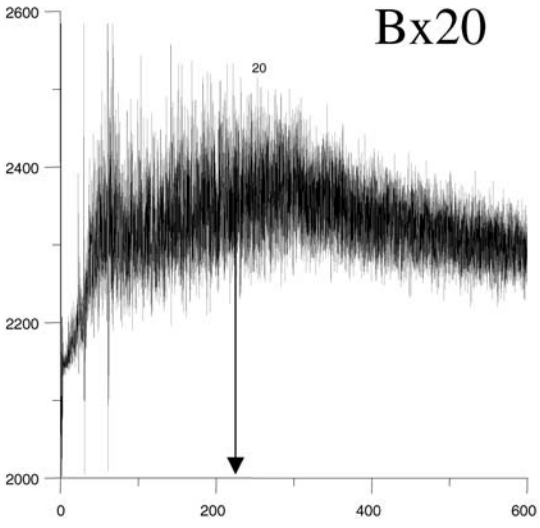


Fig. 5 Mixograph profiles of the base wheat flour cv. Banks (control, *cntrl*) without and with the incoporation of subunits 1Bx20 and 1Bx7. *Arrows* indicate the time of peak resistance. LSD values are based on three replicate analyses. The *inset table* summarises the parameters measured: mixing time (*MT*), peak resistance (*PR*), band width at peak resistance (*BWPR*), resistance breakdown (*RBD*) and breakdown in band width (*BWBD*)

incorporation of exogenous subunits into glutenin polymers (Békés et al. 1994). A 10-mg aliquot of each subunit was incorporated into 2 g of base flour, which resulted in an increase in the flour protein content from about 10.9 to 11.4%. The curves for the three base flours with and without the two subunits and the parameters measured are summarised in Fig. 5. Incorporation of subunit 1Bx7 resulted in increased mixing requirement (i.e., dough strength) (measured as increases in mixing time, MT) and increased stability to overmixing (indicated by a reduced value for resistance breakdown, RBD). These results are consistent with the increased contents of total protein and HMW subunits. In contrast, incorporation of subunit 1Bx20 gave clearly different results, with only in a small increase in MT and a small effect on RBD. It is of interest that incorporation of the subunits did not result in an increase in peak resistance, which is also a measure of dough strength. Instead, this did not change significantly with subunit 1Bx7 and decreased with subunit 1Bx20. It is possible that this parameter was affected by the imbalance in the ratio of x-type to y-type subunits resulting from the incorporation as well as the total amounts of HMW subunit in polymers.

As shown recently (Békés et al. 2001), the extentional properties of the dough can be estimated by the protein content, the width at peak resistance (BWPR) and the breakdown in band width (BWBD). Incorporation of either subunit resulted in small increases in BWPR while incorporation of subunit 1Bx20 also gave a reduction in BWPR, indicating a decrease in extensibility.

It can be concluded, therefore, that the incorporation of 1Bx7 into flour had a much greater impact on dough strength than did the incorporation of subunit 1Bx20.

Conclusions

It can be concluded that subunit 1Bx20 has a detrimental effect on dough strength when compared with subunit 1Bx7. These subunits are highly similar in their sequence, structure and stability to denaturation, the only major difference between them being the substitution of two cysteine residues in the *N*-terminal domain of subunit 1Bx20 by tyrosines. This substitution could be responsible for the negative effect on dough strength by decreasing the number and affecting the pattern of disulphide cross-links in the glutenin polymers. It can also be noted that this explanation is not consistent with the recent suggestion by Tilley et al. (2001) that dityrosine cross-links are important in determining glutenin structure and functionality.

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References

Békés F, Gras PW, Gupta RB (1994) Mixing properties as a measure of reversible reduction/oxidation of doughs. Cereal Chem 71:44–50

Békés F, Gras PW, Anderssen RS, Appels R (2001) Quality traits of wheat determined by small-scale dough testing methods. Aust J Agric Res 52:1325–1338

Belton PS, Colquhoun IJ, Grant A, Wellner N, Field JM, Shewry PR, Tatham AS (1995) FTIR and NMR studies on the hydration of a high-M_r subunit of glutenin. Int J Biol Macromol 17:74–80

Bianchi A, Pogna NE, Borghi B, Zheng DS, Giorgi B (1993) The past and the possible future impact of the Italian germplasm for the improvement of wheat in China. V.2. In: Zi XS, Li ZA (eds) Proc 8th Int Wheat Genet Symp. Scientech Press, Beijing, pp 1235–1240

Branlard G, Autran J-C, Monneveux P (1989) High molecular weight glutenin subunit in durum wheat (*T. durum*). Theor Appl Genet 78:353–358

Chou PY, Fasman GD (1978) Empirical predictions of protein conformations. Annu Rev Biochem 47:251–276

D'Ovidio R, Tanzarella OA, Porceddu E (1992) Isolation of an alpha-type gliadin gene from *Triticum durum* Desf. and genetic polymorphism at the *Gli*-2 locus. Genet Breed 46:1–48

D'Ovidio R, Masci S, Porceddu E, Kasarda DD (1997) Duplication of the Bx7 high-molecular-weight glutenin subunit gene in bread (*Triticum aestivum* L.) cultivar "Red River 68". Plant Breed 116:525–531

Drake AF, Siligardi P, Gibbons WA (1988) Reassessment of the electronic circular dichroism criteria for random coil conformations of poly(L-lysine) and the implications for protein folding and denaturation studies. Biophys Chem 31:143–146

Field JM, Tatham AS, Shewry PR (1987) The structure of a high M_r subunit of durum wheat (*T. durum*) gluten. Biochem J 247:215–221

Fu BX, Kovacs MIP (1999) Rapid single step procedure for isolating total glutenin proteins from wheat flour. J Cereal Sci 29:113–116

Gilbert SM, Wellner N, Belton PS, Greenfield JA, Siligardi G, Shewry PR, Tatham AS (2000) Expression and characterisation of a highly repetitive peptide derived from a wheat seed storage protein. Biochim Biophys Acta 1479:135–146

Gill SC, von Hipple PH (1989) Calculation of protein extinction coefficients from amino acid sequence data. Anal Biochem 182:319–326

Lafiandra D, Turchetta T, D'Ovidio R, Anderson OD, Facchiano AM, Colonna G (1999) Conformational polymorphism of high Mr glutenin subunits detected by transverse urea gradient gel electrophoresis. J Cereal Sci 30:97–104

Liu C-Y, Shepherd KW, Rathjen AJ (1996) Improvement of durum wheat pastamaking and breadmaking qualities. Cereal Chem 73:155–166

Lukow OM, Payne PI, Tkachuk R (1989) The HMW glutenin subunit composition of Canadian wheat cultivars and their association with breadmaking quality. J Sci Food Agric 46:451–460

Marchylo BA, Kruger JE, Hatcher DW (1989) Quantitative reversed-phase high-performance liquid chromatographic analysis of wheat storage proteins as a potential quality prediction tool. J Cereal Sci 9:113–130

Margiotta B, Colaprico G, D'Ovidio R, Lafiandra D (1993) Characterization of high M_r subunits of glutenin by combined chromatographic (RP-HPLC) and electrophoretic separations and restriction fragment length polymorphism (RFLP) analyses of their encoding genes. J Cereal Sci 17:221–236

Morel M-H, Bonicel J (1996) Determination of the number of cysteine residues in high molecular weight subunits of wheat glutenin. Electrophoresis 17:493–496

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annu Rev Plant Physiol 38:141–153

Payne PI, Holt LM, Lawrence GJ (1983) Detection of a novel high molecular weight subunit of glutenin in some Japanese hexaploid wheats. J Cereal Sci 1:3–8

Shewry PR, Halford NG, Tatham AS (1992) The high molecular weight subunits of wheat glutenin. J Cereal Sci 15:105–120

Tahir M, Pavoni A, Tucci GF, Turchetta T, Lafiandra D (1996) Detection and characterization of a glutenin subunit with unusual high M_r at the Glu-A1 locus in hexaploid wheat. Theor Appl Genet 92:654–659

Tatham AS, Miflin BJ, Shewry PR (1985) The β-turn conformation in wheat gluten proteins: relationship to gluten elasticity. Cereal Chem 62:405–412

Tatham AS, Drake AF, Shewry PR (1990) Conformational studies of synthetic peptides corresponding to the repetitive region of the high molecular weight (HMW) glutenin subunits of wheat. J Cereal Sci 11:189–200

Tatham AS, Field JM, Keen JN, Jackson PJ, Shewry PR (1991) Purification and characterization of HMW glutenin subunits encoded by chromosome 1B of durum wheat (*Triticum durum*). J Cereal Sci 14:111–116

Tatham AS, Gilbert SM, Fido RJ, Shewry PR (2000) Extraction, separation, and purification of wheat gluten proteins and related proteins of barley, rye and oats. In: Marsh MN (ed) Methods in molecular medicine, vol 41. Celiac disease: methods and protocols. Humana Press, Totowa, pp 55–73

Tilley KA, Benjamin RE, Bagorogoza KE, Okot-Kotber BM, Prakash O, Kwen H (2001) Tyrosine cross-links: molecular basis of gluten structure and function. J Agric Food Chem 49:2627–2632

van Dijk AA, de Boef E, Bekkers A, van Wijk LL, van Swieten E, Hamer RJ, Robillard GT (1997a) Structure characterization of the central repetitive domain of high molecular weight gluten proteins. II. Characterization in solution and the dry state. Protein Sci 6:649–656

van Dijk AA, van Wijk LL, van Vliet A, Harris P, van Swieten E, Tesser GI, Robillard GT (1997b) Structure characterization of the central repetitive domain of high molecular weight gluten proteins. I. Model studies using cyclic and linear peptides. Protein Sci 6:637–648

Wan Y, Wang D, Shewry PR (2000) High molecular weight glutenin subunits in the *Cylindropyrum* and *Vertebrata* section of *Aegilops* and identification of *Aegilops* subunits related to those encoded by the Dx alleles of common wheat. Theor Appl Genet 101:879–884

Wan Y, Wang D, Shewry PR, Halford NG (2002) Isolation and characterization of five novel high molecular weight subunit genes from *Triticum timopheevi* and *Aegilops cylindrica*. Theor Appl Genet 104:828–839