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Effects of specific domains of high-molecular-weight glutenin subunits on dough properties by an *in vitro* assay

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ABSTRACT

An *in vitro* system for incorporating bacterially produced high-molecular-weight glutenin subunits (HMW-GS) into doughs was used to study the effects of specific domains of the HMW-GS. Synergistic effects of incorporating into doughs both the Dx5 and Dy10 subunits are localized to the N-terminal domains. All single and pair-wise combinations of original subunits and hybrid subunits with their N-terminal domains exchanged between Dx5 and Dy10 finds three classes of respondents: the greatest response is when the N-termini of both Dx5 and Dy10 are present, followed by presence of the Dx5 N-terminus alone, and the least response by the presence of the Dy10 N-terminus alone. In addition, studies of Dx5 variants possessing repetitive domains of different length and composition find evidence that the length of the HMW-GS repetitive domain is important for dough properties and that the exact composition of the repeat domain has a detectible, though lesser contribution. Finally, in this experimental system, the *Glu-D1* x- and y-subunits function in the mixing experiments as if they were a fused dimer, although the exact molecular basis of the effect is not known.

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1. Introduction

The application of molecular techniques allows specific modification of HMW-GS genes. For example, a completely synthetic set of versions of the Dx5 subunit gene have been created with different repeat domain lengths based on a single repeat motif (Anderson et al., 1996b), and another set of Dx5 repeat versions were constructed by deleting or duplicating sections of the natural Dx5 repeat domain (D'Ovidio et al., 1997). The Dx5 variants were designed to express in a bacterial system from which natural or modified HMW-GS could then be extracted (Galili, 1989). A similar approach has been used to modify HMW-GS domain structure (Shimoni et al., 1997), to examine the effects of wild-type and modified C-hordeins (Greenfield et al., 1998; Tamas et al., 1998), and to study properties of a truncated fragment of the Dx5 repeat domain (Buonocore et al., 1998). What is still missing is a comprehensive molecular association of features of the intact HMW-GS and dough physical/chemical properties. While no one technique is likely to be sufficient, our intention is to carry out a detailed molecular dissection of the relationships between the primary structure of the HMW-GS polypeptides and dough functionality using multiple experimental systems. To accomplish this objective will require systematic modification of HMW-GS structure and the correlation of those changes with perturbations in dough parameters using both *in vitro* dough mixing experiments and transgenic wheats. We have previously reported using a procedure for incorporation of exogenous HMW-GS into the dough gluten matrix through a carefully controlled reduction/reoxidation cycle to mimic the behavior of untreated original dough (Bekes and Gras, 1992, 1999; Bekes et al., 1994c; Anderson and Bekes, in press). This procedure was used to show the similar behavior of HMW-GS isolated from the bacterial expression system and HMW-GS isolated from wheat flour, the relative effects of Dx versus Dy subunits, and a synergistic effect of incorporating Dx and Dy subunits together. We have also previously reported preliminary and partial results on effects on a single dough parameter (mixing time) for Dx5/Dy10 hybrid subunits and synthetic Dx5 genes (Anderson et al., 1996a; Bekes et al., 1998). We now report on complete details of seven mixing and two extension parameters of the full

Abbreviations: HMW-GS, high-molecular-weight glutenin subunit; BWPR, bandwidth at peak resistance; BWBD, band-width breakdown; Ext, extension; MBW, maximum band-width; MT, mixing time; PR, peak resistance; RBD, resistance breakdown; Rmax, maximum resistance; TMBW, time to maximum bandwidth.

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series of experiments using multiple flours with single and pairwise combinations of subunits Dx5, synthetic Dx5 variants, Dx5 variants with natural repetitive domains of different size, Dy10, and Dx5/Dy10 hybrids. The results show that the Dx5 and Dy10 subunits interact synergistically via their N-terminal domains, that there is a direct relationship between dough parameters and the repetitive domain length, and evidence that repetitive domain composition can affect dough properties. In addition, results suggest the Dx/Dy synergism may occur so rapidly upon incorporation that the pair behaves essentially as a dimer.

2. Experimental

2.1. Dx5 repetitive domain constructs

The general structure of the Dx5 and Dy10 HMW-GS are shown in Fig. 1a–b. For the natural subunits, common subunit names will be used; i.e., Dx5 will refer to the HMW-GS encoded by the *Glu-D1-1d* locus and will be understood to refer to the gene or subunit within the text containing the "Dx5" reference.

Three sets of genes were constructed based on these two naturally occurring HMW-GS genes. In the first set, two chimeric genes were constructed from the Dx5 and Dy10 genes as previously described (Shani et al., 1992). A *Hind*III restriction site is conserved among all known HMW-GS genes at the junction of the N-terminal non-repetitive coding domain and the central repetitive domain. This *Hind*III site was used to swap the N-terminal coding domains for Dx5 and Dy10 to produce two novel genes, i.e., a D10/5 chimera containing the N-terminal domain of Dy10 and the repetitive and C-terminal domains of Dx5 (Fig. 1c) and a D5/10 chimeric gene containing the N-terminal domain of Dx5 followed by the repetitive and C-terminal domains of Dy10 (Fig. 1d). These two genes and subunits will be referred to by 5/10 and 10/5.

For the second set of constructs, a Dx5 repeat length series was constructed by deleting or duplicating sections of the natural Dx5 gene repetitive region as described (D'Ovidio et al., 1997). These natural-repeat variants are named by the repetitive domain length in amino acids, i.e., Dx5-R853, Dx5-R576, and Dx5-R441 (Fig. 1e–g). The total amino acid lengths of these subunits are 985, 708, and 573 amino acids compared to 828 amino acids for the intact natural Dx5 subunit.

The third set of constructs is a series of synthetic Dx5 genes with the Dx5 terminal domains and repetitive domains encoding multiples of a single 15 amino acid repeat motif (PGQGQQGYYPTSPQQ) as previously described (Anderson et al., 1996b). This construct series includes genes encoding 48, 32, and 16 copies of the single repeat motif, and a termini fusion construct (no repetitive domain), for total repeat domain lengths of 720, 480, 240, and zero amino acids, respectively. The total lengths of the subunits are 856, 616, 376, and 136 amino acids. These genes and corresponding subunits are named the, Dx5-48x, Dx5-32x, and Dx5-16x, and Dx5-term (Fig. 1h–k).

For easier reading, both sets of Dx5 repeat variant constructs will be referred to without the "Dx5", i.e., Dx5-48x = 48x, and Dx5-R853 = R853.

2.2. Mixing experiments

In vitro incorporation and mixing experiments were carried out at the former CSIRO Grain Quality Research Laboratory, North Ryde, NSW as outlined and referenced previously (Anderson and Bekes, in press). Five different flours were used in those experiments, including a medium-protein commercial bakers flour (MPF; 10.3% protein), a high-protein commercial baker's flour (HPF; 13.0% protein), a commercial flour blend (Imperial brand name), flour from cultivar Supreme, and a wheat line null for the D-genome

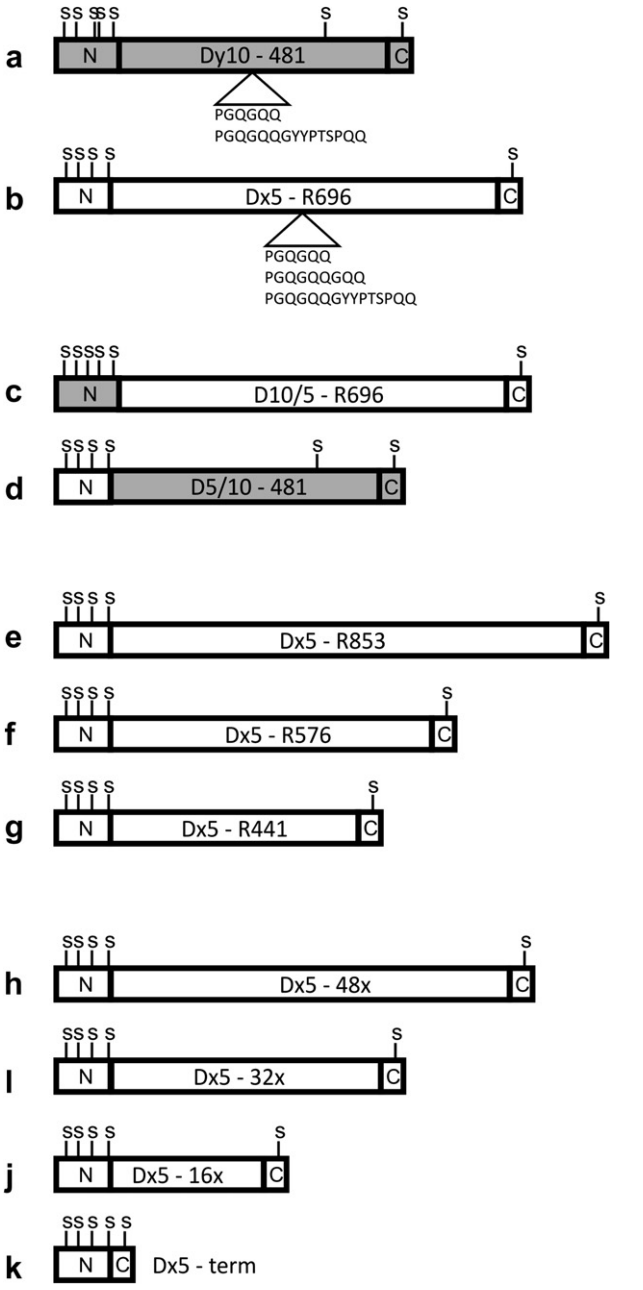

Fig. 1. Modified HMW-GS. Native and modified HMW-GS are diagrammed. (a) Dy10. (b) Dx5. (c) D10/5 hybrid. (d) D5/10 hybrid. (e) Dx5-R853. (f) Dx5-R576. (g) Dx5-R441. h) Dx5-48x. i) Dx5-32x. j) Dx5-16x. k) Dx5-term. Domains from Dx5 are in white and domains from Dy10 are in gray. Construct details are described in the Experimental section. Basic repeat motif patterns are show below the Dx5 and Dy10 diagrams. Positions of each cysteine residue is indicated by an S.

HMW-glutenin genes and subunits and symbolized by "(++−)" to indicate presence of the A- and B-genome HMW-GSs and lack of D-genome subunits (Lawrence et al., 1988). The *Glu-D1* null line is a result of a cross between parents Gabo (null at *Glu-A1* and *Glu-D1*) and Olympic (null at *Glu-B1*).

The measured mixing curve parameters included mixing time (MT), peak dough resistance (PR), bandwidth at peak dough resistance (BWPR), resistance breakdown (RBD), breakdown in peak bandwidth (BWBD), time to maximum bandwidth (TMBW), and maximum bandwidth (MBW), and extensibility parameters Rmax (maximum resistance to extension) and Ext (extension before