A Relaxed Specificity in Interchain Disulfide Bond Formation Characterizes the Assembly of a Low-Molecular-Weight Glutenin Subunit in the Endoplasmic Reticulum[1][W][OA]

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Wheat (*Triticum* spp.) grains contain large protein polymers constituted by two main classes of polypeptides: the high-molecular-weight glutenin subunits and the low-molecular-weight glutenin subunits (LMW-GS). These polymers are among the largest protein molecules known in nature and are the main determinants of the superior technological properties of wheat flours. However, little is known about the mechanisms controlling the assembly of the different subunits and the way they are arranged in the final polymer. Here, we have addressed these issues by analyzing the formation of interchain disulfide bonds between identical and different LMW-GS and by studying the assembly of mutants lacking individual intrachain disulfides. Our results indicate that individual cysteine residues that remain available for disulfide bond formation in the folded monomer can form interchain disulfide bonds with a variety of different cysteine residues present in a companion subunit. These results imply that the coordinated expression of many different LMW-GS in wheat endosperm cells can potentially lead to the formation of a large set of distinct polymeric structures, in which subunits can be arranged in different configurations. In addition, we show that not all intrachain disulfide bonds are necessary for the generation of an assembly-competent structure and that the retention of a LMW-GS in the early secretory pathway is not dependent on polymer formation.

The unique ability of wheat (*Triticum* spp.) flour to form a dough that has the rheological properties required for the production of leavened bread and other foods is largely due to the characteristics of the proteins that accumulate in wheat endosperm cells during seed development (Gianibelli et al., 2001). Among these endosperm proteins, a major role is played by prolamines, a large group of structurally different proteins sharing the characteristic of being particularly high in Pro and Gln.

On the basis of their polymerization status, wheat prolamines can be subdivided into two groups, the gliadins and the glutenins. While gliadins are monomeric, glutenins are heterogeneous mixtures of polymers where individual subunits are held together by interchain disulfide bonds (Galili et al., 1996; Tatham and Shewry, 1998). The subunits participating to the formation of these large polymers have been classified into four groups according to their electrophoretic mobility (Gianibelli et al., 2001). The A group is constituted by the so-called high-molecular-weight glutenin subunits (HMW-GS), while polypeptides in groups B, C, and D are collectively termed low-molecular-weight glutenin subunits (LMW-GS). While only three to five HMW-GS are expressed in common wheat endosperm, LMW-GS include a very large number of different polypeptides.

Different models of glutenin assembly have been proposed (see Gianibelli et al., 2001 for a review), but the determination of their precise structure and M_r distribution has been hampered by their large size and complex subunit composition. Crucially, because disulfide bonds appear to be the major factor affecting polymer stability, it would be very useful to know whether the pairing between specific Cys residues, rather than random assembly, controls glutenin polymer formation. Indeed, data obtained with HMW-GS indicate that the formation of certain types of intermolecular disulfide bonds is particularly favored (Tao et al., 1992; Shimoni et al., 1997). In the case of LMW-GS, at least two functionally distinct types of subunits can be distinguished. Subunits of the first type, to

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