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Model gluten gels

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The origin of the unique rheological properties of wheat gluten, the water-insoluble protein fraction of wheat grain, questions scientists since the 18th century (Beccari, 1745). In 1907, Osborne (1924) investigated the biochemical composition of gluten proteins according to their solubility. The 70% ethanol-soluble fraction that mostly includes only individual polypeptides, with molecular weight (Mw) in the range [15–65] kg/mol, was named gliadin, whereas the poorly 70% ethanol-soluble fraction that shows an aggregative behaviour and consists of a concatenation of disulphide bonded polypeptides was named glutenin. Since then, various more or less denaturant fractionation methods, using different solvents and eventually sonication, have been used to isolate the different proteins of wheat gluten. But, whatever the experimental conditions, isolating all glutenin polymers in a single extract has appeared as a difficult, or even impossible, task. Two fractions, insoluble glutenin on one hand and soluble glutenin on the other hand, are generally obtained. Moreover, the soluble fraction is also rich in gliadin (Fu and Sapirstein, 1996), raising the question of interactions between gliadin and glutenin. It was shown that the flour content in sodium dodecyl sulfate (SDS)-insoluble glutenin is correlated to the dough strength (Southan and MacRitchie, 1999) and it is now generally admitted that glutenin polymers are responsible for the dough elasticity, whereas gliadin is associated to its extensibility [for a review, see Wrigley et al. (2006)]. However, the physical structure of gluten and the link with its rheological properties are still under debate (MacRitchie, 2014). Structural measurements on individual proteins or derived peptides (Thomson et al., 1999) miss the relevant supramolecular structure of the mixture of gluten proteins. MacRitchie argued that gluten behaves as an entangled polymer network, as a critical molecular weight of glutenin polymers, coinciding to the one that triggers glutenin insolubility in a SDS buffer, is required to greatly improve dough strength (MacRitchie, 2007). In addition, Belton suggested that the critical Mw could be due to a pre-existing protein polymeric network in the flour (Belton, 2007). On the other hand, based on the observation of micron sized particles in the SDS-insoluble gluten gel layer (glutenin macropolymer gel, GMP), whose quantity depends on the processing conditions (Don et al., 2005), van Vliet and Hamer proposed a hyper-aggregation model that implies covalent and non-covalent interactions between glutenin subunits and polymers at different scales (van Vliet and Hamer, 2007). Many studies principally focused on covalent bonds in gluten network (Johansson et al., 2013), while the role of hydrogen bonds appears crucial, as suggested by the “loop and train” model of Belton (1999). In addition, the existing supramolecular models often ignore gliadin while a recent study has evidenced numerous covalent interactions between gliadin variants carrying an uneven number of cysteine residues and SDS-soluble glutenin polymers (Schmid, 2016).

To better understand the supramolecular structure of gluten and its link to rheology, we have recently developed a model gluten system using a food-grade solvent, which allows quantitative comparisons with theoretical models for synthetic polymers. Here, we give a concise review of our previous results (Banc et al., 2016; Dahesh et al., 2014, 2016) and present new data that show the relevance of model gluten gels to understand native gluten in water and provide an evidence of non-covalent interactions between gliadin and glutenin polymers.

To develop a sound approach of gluten proteins, having a model system allowing the production of homogeneous suspensions and gels in a solvent that maintains the proteins in their native state and limits unfolding, is a prerequisite. Despite their ability to solubilize large amount of glutenin, chemicals (urea, SDS, dithioerythritol) susceptible to interact with the protein backbone should be disregarded. In this context, a binary mixture of alcohol and water, the typical good solvent of wheat prolamins, is an appropriate choice. We have therefore used ethanol-water (50-50 v-v) to fractionate

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industrial gluten (Dahesh et al., 2014). About 50% of the initial gluten proteins are solubilized in this solvent. The soluble fraction is divided into two extracts thanks to a low temperature liquid-liquid phase separation that was first reported by Dill and Alsberg in 1925 but largely ignored since (Dill and Alsberg, 1925). The light phase is enriched in gliadin while the dense phase is enriched in glutenins. Both high-Mw and low-Mw subunits compose the glutenin polymers of the dense phase (Dahesh et al., 2014), which contains 34% of the SDS-soluble glutenin polymers present in the initial gluten. Due to its glutenin/gliadin mass ratio (glu/gli = 1.1) similar to that of flour, the dense phase can be regarded as an adequate model system of gluten, with the remarkable advantage that all its glutenin polymers are fully soluble in the ethanol-water solvent and remains so even after freeze-drying. The protein powder can be homogeneously re-dispersed in ethanol-water (50-50 v-v) in a very large range of protein concentrations, from typically 2–600 mg/ml. For concentrated suspensions (between 200 and 600 mg/ml) a time- and concentration-dependent evolution of the linear rheological properties are measured, disclosing a spontaneous sol-to-gel transition (Dahesh et al., 2016). Model gluten gels with elastic moduli spanning 6 orders of magnitude can be produced by tuning protein concentration. The gels display the typical features of fractal polymer gels in good solvent conditions, as probed by small angle X ray scattering (SAXS) (Dahesh et al., 2014), with a fractal dimension directly correlated to the linear viscoelastic properties following percolation theory of critical gels (Dahesh et al., 2016). Notably, at large concentrations, the elastic moduli of the model gels are similar to those of gluten in water (Fig. 1A). Even so, one can raise the question of their structural homology. Fig. 1B displays SAXS spectra of gluten and model gels both prepared in water or in ethanol-water at the typical gluten concentration (400 mg/ml). At low scattering vectors q, different power law evolutions of the scattered intensity with q ($I \sim q^{-n}$) are measured for the model extract in ethanol-water ($n = 1.9$) and for other samples ($n \approx 3$). This reflects the more compact structure of the proteins at large length scale (in the range of hundreds of nm) for gluten and for the model extract in water, due to attractive interactions between the proteins, which would cause phase separation at lower protein concentrations. In addition, whatever the solvent, the low q intensity is higher for gluten than for the model extract, suggesting a higher content of large scale heterogeneities, consistent with the higher content of large polymers of glutenin in gluten than in the extract. Interestingly, however, comparable structural features are measured for all samples at the nanometer length scale (at high q). Here, the structure of individual polypeptidic chains is in all cases measured, whose statistics is quantified by the exponent m in the power law evolution of the scattered intensity ($I \sim q^{-m}$). We find however that locally the polypeptidic chains are slightly more expanded in ethanol-water ($m = 1.5–1.6$, typical of polymer chains in good solvent) than in water ($m = 2$, typical of polymer chains in theta solvent), reflecting different interactions between protein and solvent. Hence, proteins are not globular or aggregated and rather behave like intrinsically disordered proteins in water (Pomposo et al., 2014). Therefore, based on the protein composition and on the structural features, we believe that model gluten gels in water-ethanol are relevant to study the assembly processes of native gluten proteins.

In a dilute regime, the model extract comprising gliadin and glutenin is initially composed of proteins assemblies that swell in ethanol-water to form microgel-like 150 nm large objects (Dahesh et al., 2014). In a more concentrated regime, this size is irrelevant due to the interpenetration of the objects, but a comparable size (60 nm) is measured by small angle neutron scattering for samples prepared with the same but deuterated solvent (Banc et al., 2016). This characteristic size has been attributed to the heterogeneity of the H/D exchanges between the initially protonated proteins and the deuterated solvent in which they are dispersed. Our interpretation is that within the proteins assemblies, a core would gather strongly H-bonded interacting protein chains from which H/D exchanges are hindered. Hence, a H/D contrast is established between these cores and the remaining protein matrix. At this stage whether these cores mainly consist of glutenin polymers remains a matter for conjecture. Upon ageing, protein interactions develop as proven by the spontaneous sol-to-gel transition. In the presence of a maleimide thiol blockers, spontaneous gelation of the model extract suspension still occurs with a minor effect on the evolution of the elastic modulus, indicating that thiol/disulfide interchanges is not the main trigger of gelation. By contrast, the total solubilization of the gel in an ethanol-water solvent comprising 6 M urea highlights the importance of hydrogen bonds in the gelation processes.

Gel swelling/release experiments are performed to further evaluate interactions between gliadin and glutenin polymers. The model gluten fraction, initially soluble in ethanol-water, cannot

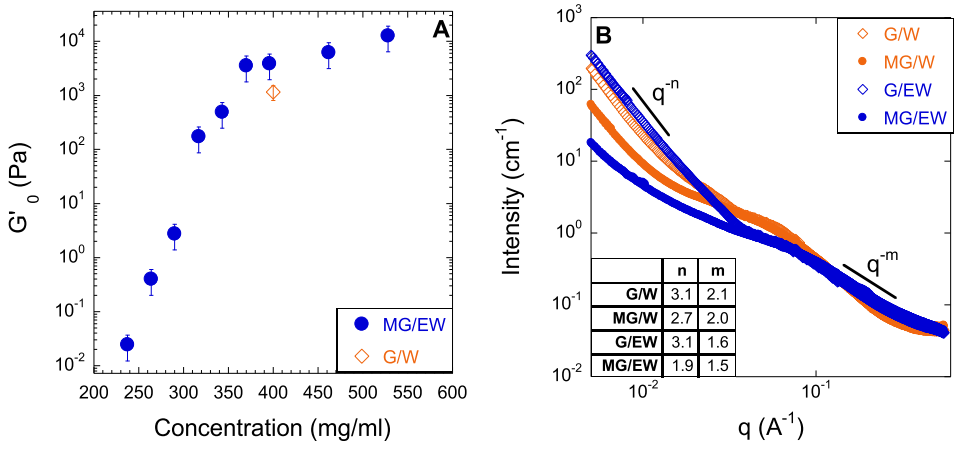

Fig. 1. A- Low frequency elastic moduli of model gluten fraction (MG) in ethanol-water (EW) solvent as a function of protein concentration and compared to gluten (G) in water (W) (data extracted from Dahesh et al. (2016)). B- SAXS spectra of G and MG in W and in EW. The protein concentration is 400 mg/ml. Experimental conditions are similar to those used in Banc et al. (2016).