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Research note

Selection of a monoclonal antibody for detection of gliadins and glutenins: A step towards reliable gluten quantification

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Celiac disease and food allergy to wheat are the two main pathologies triggered by the consumption of storage proteins from wheat and related cereals. Celiac disease occurs at rates approaching 1% (Green and Cellier, 2007), and prevalence of food allergy to wheat is in the range of 0.2–0.5% of the population (Zuidmeer et al., 2008). Until now, a life-long gluten-free diet has been the only causal therapy for people suffering from these pathologies. Gluten-free products must respect a threshold established at 20 mg/kg by the Codex Alimentarius (ALINORM 08/31/26, Appendix III, 2008).

Storage proteins of wheat, rye, barley and possibly oats are termed gluten in the field of celiac disease. Gluten proteins are gliadins and glutenins in wheat, secalins in rye, hordeins in barley, and avenins in oats. These storage proteins have also been termed prolamins in the literature (Shewry and Tatham, 1999). Based on their solvent solubility and electrophoretic mobility, gliadins are divided into α-, β-, γ-, and ω-gliadins. Glutenins are composed of low-molecular-weight and high-molecular-weight glutenin subunits (LMW-GS and HMW-GS) (Tatham and Shewry, 2008). In some food products, gluten is also added after partial hydrolysis or deamidation (conversion of some glutamine residues to glutamic acid) to enhance their use as functional ingredients (Bollecker et al., 1990; Popineau et al., 2002). All of these proteins, both in their native and modified forms, can be detrimental and should thus be avoided by celiac patients or people suffering from food allergy to wheat. It is therefore necessary to detect or quantify them by analytical methods.

Fortunately, prolamins of the *Triticeae* tribe share repeated homologous peptides (Shewry and Tatham, 1999), constituting a target of choice for their detection by a unique ELISA assay (Denery-Papini et al., 1999). Moreover, among the numerous antibodies described to detect gluten, the two monoclonal antibodies (mAbs), R5 (Valdes et al., 2003) and 401/21 (Skerritt and Hill, 1990), recommended by the AOAC or by the Codex Alimentarius are directed against these repeated sequences. The R5 mAb recognises the epitope, QQ(Q/P)FP, that is present in all gliadins (Osman et al., 2001). The epitope bound by the 401/21 mAb has not yet been fully identified. Andrews and Skerritt (1994) showed that the 401/21 mAb recognises at least one epitope, Q(Q/S/P)G(Y/H)YP, in the repeated domain of HMW GS. However, these mAbs differentially bind the individual gluten proteins (van Eckert et al., 2010) and do not recognise hordeins and secalins with the same intensity as wheat prolamins (Kanerva et al., 2006; Thompson and Mendez, 2008). Moreover, deamidation dramatically affects their binding abilities (Kanerva et al., 2011). As a consequence, gluten quantification with these mAbs depends on the gluten composition, its chemical modification as well as on the composition of the standard chosen in the assay (Allred and Ritter, 2010; Seilmeier and

Abbreviations: LMW, low molecular weight; HMW, high molecular weight; GS, glutenin subunits; ELISA, enzyme-linked immunosorbent assay; mAbs, monoclonal antibodies; IgG, immunoglobulin G; IgM, immunoglobulin M.

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Wieser, 2003). For example, because R5 mAb mainly detects gliadins, gluten concentration is extrapolated from the gliadin concentration, assuming a constant gliadin/glutenin ratio equal to 1 within all samples. This, however, is not always the case. Gluten composition depends on multiple parameters such as the species, the cultivars, the agronomical conditions, as well as on the process the products were subjected to (Wieser and Koehler, 2009).

Wieser and Koehler (2009) and van Eckert et al. (2010) suggested that an antibody or a mixture of antibodies that recognises gliadins and GS to similar degrees might be the next step toward a reliable method. However, the development of an assay combining two or more antibodies is complex and can lead to a complicated method development and an expensive assay.

In this study, our aim was to find a unique antibody able to react with the different gliadin and GS classes with equivalent affinity, as well as with prolamins from related cereals and from modified gluten. We therefore decided to explore those antibodies directed to repeated sequences from our mAbs library, which contains almost 100 prolamin-specific antibodies (Denery-Papini et al., 1995; Kuktaite et al., 2011; Loussert et al., 2008).

Gliadins were extracted from wheat flour (C.V.Hardi) using a sequential procedure. The different gliadin classes (α, β, γ, and ω), LMW-GS and HW-GS were further purified using several chromatography steps (Battais et al., 2003; Popineau et al., 1986; Popineau and Pineau, 1985).

Grains of rye (cv. Petkus), spelt (genotype épeautre rose), barley (cv. Sonja) and triticale (cv. Calao) were ground in liquid nitrogen. Total prolamin fractions were extracted from these ground grains and from wheat flour (cv. Hardi) with the so-called "Mendez cocktail", described in Garcia et al. (2005).

Deamidation was initiated by dispersing 20 mg of gluten in 1 mL of 0.1 N hydrochloric acid, followed by heating at 90 °C for 1 h. The reaction was stopped by neutralising with 0.1 N NaOH, and the resulting mixture was dialysed against deionized water and then centrifuged in order to recover the supernatant, which was finally lyophilised.

Monoclonal antibodies directed to repeated sequences were produced in our laboratory according to standard procedures (Denery-Papini et al., 1995). They were raised against synthetic peptides deduced from the sequence of ω5-gliadins (series ORE) or γ-gliadins (series PQQ, S1 and S3 (Denery-Papini et al., 2001), or against a purified polypeptide corresponding to the repetitive domain of a γ-gliadins obtained after hydrolysis by pepsin (series H) or against deamidated gluten (series GLU). All results in this study were obtained with cell culture supernatants. MAbs401/21 and R5 were taken from commercial kits (Diffchamb S.A., France).

The reactivity of the antibodies towards the different gliadins and GS fractions was assessed by indirect ELISA as described in Battais et al. (2003) for sample preparation and coating. mAbs were detected with peroxidase conjugated goat anti-mouse IgG (H + L) (ref 170–6516, Bio-Rad, France) and ortho-phenylenediamine dihydrochloride (SIGMA-Aldrich, France) as substrate. Titration curves were built, and a working dilution corresponding to the top of the slope for the best-recognised fraction was established for each mAb. At this working dilution, reactivity towards each prolamin fraction was expressed as a percentage of the maximal signal obtained with the best-recognised fraction (set to 100%). In the case of R5 and 401/21 mAbs, their initial low concentration prevents the building of complete titration curves, and percentages of reactivity were defined with reagent diluted according to manufacturer's instructions.

Cross reactivity profiles towards some species of the *Triticeae* tribe were obtained with an ELISA performed on a macro-array. Briefly, prolamin extracts were spotted onto a 96-well microtiter plate with a printer loaded with solid pins (BioOdyssey Calligrapher MiniArrayer, Bio-Rad, France) so that each well contained two spots of extracts from wheat, rye, barley and triticale. A classical indirect ELISA as described above was then carried out with chemiluminescent substrate (SuperSignal West Dura Extended Duration Substrate; Pierce, Rockford, IL, USA). Luminescence was measured by a CCD camera (Luminescent Image Analyzer LAS 3000; Fujifilm, Japan). Percentages of cross reactivity were determined as described above with reactivity on wheat set to 100%.

We focused on 17 antibodies from our mAb library obtained after immunisation with either polypeptides or peptides from the repetitive domains of γ- or ω5-gliadins or with deamidated gluten. These antibodies were characterised for their reactivity towards purified gliadins and GS and deamidated gluten (Table 1). Marketed versions of mAbs R5 and 401/21 were used for comparison.

The profiles reported for the two commercial antibodies are in accordance with previous descriptions: 401/21 mAb mainly bound to GS and ω-gliadins (Skerritt and Hill, 1990; van Eckert et al., 2010), while R5mAb mainly reacted with gliadins (Valdes et al., 2003).

Within our collection, some mAbs displayed reactivity to few proteins but, as expected, most of them had a broad reactivity. On the whole, these results underlined the versatility of antibody production and the absolute necessity of a fine characterisation. It is noteworthy that immunisation with a unique short immunogenic peptide generates antibodies with different reactivity profiles, as illustrated by the S1 series profiles in Table 1.

Independently of the immunogenic material, three arbitrary groups of mAbs could be considered according to the extent of their reactivity profile.

The first group consists of four mAbs that display a reactivity of more than 50% to all gliadins and GS: GLU 12F3B1, GLU 19H6E6, PQQ 3B4 and PQQ 7F12 (in bold in Table 1). In contrast, a second group is formed with four mAbs that display a reactivity restricted to two or three fractions: GLU19H5E2, S1DG3 5, S3B5 and ORE15F10. Finally, a third group includes the mAbs with an intermediate extent of reactivity.

From this first screening, the mAbs of the first group were retained for further characterisation since they were able to bind all types of gluten proteins. Among these candidates, mAbs GLU 12F3B1 and PQQ 3B4 appeared to be of particular interest. Their titration curves on purified prolamins are grouped over the whole range of dilutions (see mAb PQQ 3B4 on Fig. 1). They clearly illustrated the effective and equivalent recognition of all gluten protein types.

A valuable mAb must also be able to recognise deamidated gluten. The decrease of reactivity of mAbs R5 and 401/21 on deamidated gluten was recently reported by Kanerva et al. (2011): reactivity of mAb R5 decreased by two orders of magnitude and that of mAb 401/21 was completely eliminated. Two mAbs from the broad reactivity group, GLU 19H6E6 and PQQ 3B4, fulfilled this objective since they bound deamidated gluten and purified prolamins to similar degrees (i.e., with reactivity of over 75%, Table 1).

Analytical methods for gluten determination also require the detection of gluten from other cereals. This was achieved with R5 mAb (Valdés et al., 2003) but not with 401/21 mAb, which barely recognised hordeins (Skerritt and Hill, 1990). The reactivity of mAbs GLU 12F3B1, GLU 19H6E6 and PQQ 3B4 was then assessed on prolamin extracts from rye, barley, triticale and spelt, and compared with reactivity to wheat extract (Table 2). They all recognised the extracts to a similar extent of reactivity.

With the initial aim of finding an antibody suitable for accurate gluten quantification, we found four candidates able to detect all gliadins and GS. They are all of the IgM isotype. This isotype is considered unsuitable for assay development because IgM often display lower affinity than IgG. However, in the case of repeated epitopes, the pentameric structure of IgM may lead to high-avidity antibodies and become valuable (Devey and Steward, 1986).