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Research Article

Monoclonal Antibody Characterization of the Coeliac Toxic Gluten Content of Wheat Starch Standards for a Gluten Test Kit - a

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ABSTRACT

Coeliac Disease (CD) is treated with a strict Gluten-Free Diet (GFD). Gluten is comprised of gliadin, Low (LMWG) and High (HMWG) Molecular Weight Glutenins. Many commercially available, nominally gluten-free foods are based on purified wheat starch. The only Food and Agriculture Organization of The United Nations (FAO) certified assay for gluten quantitation is based on the R5 monoclonal antibody (MAB) that recognizes gliadin but not glutenin. The R5 estimated gluten content of gluten-free foods involves multiplying the R5 gliadin value by two to yield a gluten value. We have raised a panel of monoclonal antibodies to CD toxic motifs. We assessed the gluten content of three wheat starches A, B, & C that are supplied as standards for the Transia gluten quantitation kit, which is based on a MAB to omega-gliadin. We employed separate ELISAs to quantify gliadin, Low (LMWG) and High Molecular Weight (HMWG) glutenins. Gliadin content in all three starches, as measured by one of the antibodies, was always higher that that measured with the other, but the ratio between measurements made by the 2 MABs varied from 3.1 to 7.0 fold. We also report significant variation in glutenin: gliadin ratios for different wheat starches. We suggest quantitation of the gluten content of nominally gluten-free foods, particularly those based on purified wheat starch, should be determined by measuring gliadin and glutenin, the values for which should be summated, since measurement of gliadin alone, followed by multiplication by two to yield a gluten content, appears to be invalid for processed foods.

INTRODUCTION

The only generally accepted treatment for coeliac disease (gluten-sensitive enteropathy, CD) is a lifelong strict Gluten-Free Diet (GFD) that involves avoidance of wheat, rye and barley. Wheat gluten contains gliadin, Low (LMWG) and High (HMWG) Molecular Weight Glutenin proteins, all three of which have been shown to be CD toxic [1-3]. Many gluten-free foods are available. This includes those that are commercially marketed, many of which are based on purified wheat starch. Foods that are supplied as gluten-free are required to contain <20 mg/ kg gluten and those labelled "very low gluten" 21-100 mg/ kg gluten. The only Food and Agriculture Organization of the United Nations (FAO) certified assay to quantify the gluten content of foods for individuals with CD is based on the R5 Monoclonal Antibody (MAB) that recognizes gliadin but not glutenin [4]. The value for the gluten content of a given food for this assay is determined by quantifying the gliadin content, the value for which is multiplied by two to yield the gluten content of a given food. This extrapolation, based on the gliadin content may be invalid due to the differing solubility of gluten proteins that is gliadin and glutenins, when food is processed.

AIM

We wished to improve the extent and accuracy of quantification of Coeliac Disease (CD) triggering peptides in purified wheat starch that is a common ingredient of many commercially available processed gluten-free foods for individuals with CD.

MATERIALS AND METHODS

We have generated three MABs to wheat gluten proteins. This includes PN3 to wheat gliadin, which is raised against and detects coeliac toxic A-gliadin AA31-49 [5,6]. CDC5 to the CD toxic immuno-dominant epitope in wheat gliadin, raised against and detects α_2-gliadin AA57-75 [7] and CDC7 to wheat glutenin generated against the protein 1Dy10 HMWG subunits [8]. We developed three separate competitive ELISAs employing the three separate MABs, PN3, CDC5 and CDC7. We assessed the gluten content of three wheat starches termed A, B & C that are supplied as standards for the Transia kit that is marketed to quantify the gluten content of foods, based on the use of a MAB raised against ω-gliadin [9].

RESULTS

The gliadin content of wheat starches A, B and C were 34.2, 52.9 and 234.6 mg/ kg as determined by the PN3 MAB. The gliadin content of the wheat starches measured using the CDC5 MAb followed the same trend of increasing gliadin content from starch A to C, but the values were higher. The gliadin contents were 106.9, 370.8 and 1033.1 mg/ kg respectively. The wheat starches A, B and C contained 114.1, 431.1 and 1481.4 mg/ kg glutenin as assessed with CDC7 MAB (Table 1).

The gluten content was then calculated based on the values obtained employing our three MABs. Two approaches were undertaken:

1. Following the standard method of extrapolating the gliadin content to the total gluten by multiplying the gliadin value by two;
2. Summating the gliadin and glutenin values to obtain the gluten content. Two different results were obtained, depending on whether the gliadin content was measured with either the PN3 or CDC5 MAB (Table 2).

When PN3 MAB measurement was used to extrapolate the gliadin content of starch A to total gluten, the obtained value was 68.4 mg/kg gluten which is within the limit for "very low gluten" labelling of foodstuffs. When another anti-gliadin MAB (CDC5) was used for the same starch, the gluten content was more than three times higher (213.8 mg/ kg), exceeding the 100 mg/ kg cut off value for "very low gluten".

Summation of the values of gliadin and glutenin measurements for starch A to obtain a total gluten content led to two different

Table 1: Gliadin and glutenin content (in mg/kg) of wheat starches A, B and C as determined with PN3, CDC5 and CDC7 MAb.

Antigen measurement	Starch A	Starch B	Starch C
Gliadin (mg/kg) with PN3	34.2	52.9	234.6
Gliadin (mg/kg) with CDC5	106.9	370.8	1033.1
Glutenin (mg/kg) with CDC7	114.1	431.1	1481.4

Table 2: Gluten content (in mg/kg) of wheat starches A, B and C as determined by multiplying gliadin content by two versus summation of the values for gliadin and glutenin content.

Gluten content (in mg/kg)	Starch A	Starch B	Starch C
a) gluten = 2x gliadin (CDC5)	68.4	105.8	469.2
Gliadin (mg/kg) with CDC5	213.8	741.6	2066.2
b) gluten = gliadin (PN3) + glutenin (CDC7)	148.3	484	1716
b) gluten = gliadin (CDC5) + glutenin (CDC7)	221	801.9	2514.5

results: 148.3 and 221 mg/ kg depending on whether the results of PN3 or CDC5 measurements were summated with the CDC7 value (Table 2). Interestingly, the calculation for total gluten based on the approach gluten = 2x gliadin (PN3) was more than 2-fold lower than when gluten was calculated by summation of gliadin (PN3) plus glutenin (CDC7) which equaled 148.3 mg/ kg, for wheat starch A. On the contrary for the CDC5 MAB these two approaches resulted in very similar final gluten contents (213.8 and 221 mg/ kg respectively) (Table 2).

Similarly, when the gliadin content of wheat starch B was extrapolated to total gluten (by multiplying the gliadin content by two), the obtained value was 105.8 mg/ kg for PN3 measurement and a 7-fold higher value for gluten content (741.6 mg/ kg) for CDC5 measurement. When the total gluten of starch B was obtained by the other approach, involving summation of the values of gliadin and glutenin (CDC7) measurements, they resulted in 484 and 801.9 mg/ kg gluten for PN3 and CDC5 measurements respectively. The gluten content calculated by gluten = 2x gliadin as opposed to gluten = gliadin + glutenins (CDC7) differed 4.6-fold for PN3 MAb measurements and 1.1-fold for CDC5, the higher values obtained by gluten = gliadin + glutenin approach (Table 2).

The results for wheat starch C had a similar trend. The gluten content obtained by multiplying gliadin measurements by two resulted in 4.4-fold higher total gluten content for CDC5 measurement (2066.2 mg/ kg) than PN3 measurements (469.2 mg/ kg). The alternative approach whereby glutenin content (obtained with CDC7 measurement) was summated with the gliadin content resulted in a 3.6-fold increase of gluten content with PN3 MAB measurements (from 469.2 to 1716 mg/ kg) and a 1.2-fold increase of gluten content, employing CDC5 MAB measurements (from 2066.2 to 2514.5 mg/ kg) (Table 2).

Further ratios of glutenin to gliadin content of the wheat starches were determined by dividing the glutenin values obtained with CDC7 MAB by gliadin values assessed either by the PN3 or CDC5 MABs (Table 3).

The gliadin content of starches A, B and C depended on whether PN3 or CDC5 was chosen for the measurement (Table 1). The ratios of gliadin contents in the three wheat starches were determined by dividing the gliadin content obtained with CDC5 by the gliadin content obtained with PN3. The scale of difference in gliadin content as measured by CDC5 or PN3 varied amongst the starches (3.1- to 7.0-fold) (Table 4).

Table 3: Ratios of glutenin to gliadin contents in wheat starches A, B and C depending on which gliadin monoclonal antibody (PN3 or CDC5) is used for comparison with CDC7 MAB measurements.

Glutenin: gliadin ratio	Starch A	Starch B	Starch C
CDC7 : PN3	3.3	3.3	3.3
CDC7 : CDC5	1.1	1.2	1.4

Table 4: Ratios of gliadin contents in wheat starches A, B and C as obtained with the two gliadin antibodies (CDC5 and PN3).

Gliadin: gliadin ratio	Starch A	Starch B	Starch C
CDC5:PN3	3.1	7	4.4

DISCUSSION

We have demonstrated that a broadened repertoire of MABs specific for CD triggering peptides enabled improved measurement of CD-toxic gluten in foods, by allowing a more realistic measurement of the CD triggering epitopes within the glutenins. This applied particularly to the measurements where the PN3 MAB was used to measure the gliadin content and then obtaining the total gluten content either by the "R5 standard method" involving multiplying the gliadin value by two or by summation of the gliadin and glutenin measurements. This observation was less applicable to CDC5 measurements as multiplying the gliadin content obtained by the CDC5 MAB by two differed very little in comparison to obtaining the gluten content by summation of the food's gliadin and glutenin and content. The total gluten based on CDC5 and CDC7 MAB measurements indicate that the epitopes that these two MABs detect were more equally distributed amongst the three wheat starches, as opposed to those detected by PN3 and CDC7 MABs in the three starches.

None of the three wheat starches were "gluten-free", as they all contained more than 20 mg/ kg gluten. When the anti-gliadin MAB PN3 was used to measure the gluten contamination of starch A this resulted in values that would be classified as a "very low gluten" foodstuff as it contained less than 100 mg/ kg gluten. This was applicable for the "standard" method of determining the total gluten by multiplying the gliadin value by a factor of two. However, when total gluten content was assessed by summation of the values obtained with the PN3 MAB, and anti-glutenin MAB, CDC7, the gluten values were well above the cut of value for "very low gluten". This data clearly demonstrates the importance of measuring both groups of proteins in gluten responsible for CD toxicity (gliadins and glutenins). This is particularly important for processed foodstuffs such as purified wheat starches where we and others have shown the gliadin: glutenins ratios vary significantly [10,11].

The glutenin

Gliadin ratios of the purified wheat starches varied between 1.1-8.1. Our results demonstrate that multiplying gliadin content by two to estimate the glutenin and in turn the gluten content may be invalid for processed foodstuffs, which is in agreement with Wieser and Koehler's [10] observations. We suggest measurement of gliadin alone therefore cannot predict the total gluten content of foods. The standard method of multiplying the gliadin amount by two would lead to gross underestimation of gluten content in the wheat starches if the antibody used for detection of gliadin was PN3.

Lower glutenin

Gliadin ratios were obtained when comparing the glutenin contents with gliadin determined with the CDC5 MAB. The higher ratio of glutenin: gliadin obtained with PN3 as an anti-gliadin antibody can be explained by the lower amounts of the gliadin peptide that PN3 detected in the wheat starches. This was further confirmed by calculating the gliadin: gliadin ratios determined by PN3 and CDC5 antibodies which showed that the amount of detected gliadin in a foodstuff depends significantly on which antibody is used for quantification. This concept was demonstrated previously in a study of van Eckert, et al. [12] who showed that two different anti-gliadin antibodies (PN3 and R5) reacted with different individual proteins in different protein sub-fractions of the reference gliadin separated by two dimensional electrophoresis with Western immuno-blotting.

Further, our results differed from the reference values for gluten contamination of the three wheat starches A, B and C. The reference values were provided in the manufacturer's information sheet and had

been obtained using a monoclonal antibody which detects ω-gliadins and were as follows: <100 mg/ kg gluten for starch A, <300-600 mg/ kg gluten for starch B and <1000-2500 mg/ kg for starch C [9]. Our results of gluten contamination followed the same trend of increasing gluten contamination from starch A to C, but the values were not the same. Gluten contamination assessment with antibodies of different specificity can therefore result in different gluten amounts, which is consistent with Allred and Ritter's observations [13]. It should be noted the manufacturer of the three starches A, B and C did not provide information as to which wheat cultivars the starches had been obtained. In this respect we do not know whether the flour from the same cultivar was used and the resultant starches subjected further to three different washing processes or whether flour from either three different or a variety of cultivars was used.

There is dilemma in the field of CD-toxic gluten measurement as to what should be quantified in order to access the overall toxicity of foods for CD sufferers [14]. There are several CD-triggering epitopes [15]. It is probably unrealistic to detect all of them. The gliadin fraction of wheat gluten has long been established as CD-triggering. However, the glutenins have only relatively recently been shown to exacerbate the affection. Our monoclonal antibodies detect CD triggering epitopes distributed amongst both groups of proteins, for which there is substantial clinical data confirming their role in CD pathogenesis. It is interesting, although not surprising, that contamination of wheat starches with glutenins were notably higher than with the gliadins. This is likely to be due to the different solubility characteristics of the gluten protein fractions as a result of food processing [13,16,17]. The glutenins are less water soluble and therefore more likely to stay adsorbed to starch granules after washing [16]. It is therefore crucial to detect glutenin contamination of processed foodstuffs as well as gliadin and thereby improve the extent of measured gluten components. Our findings are consistent with Allred [13] who demonstrated that all processed foodstuffs (n = 40) tested in their study contained 4 to 10-fold higher gluten values when assessed with a MAB that has high affinity to glutenins as opposed to the R5 MAB with high affinity for gliadins but none to glutenins.

CONCLUSION

We suggest that a broadened repertoire of MABs specific for CD triggering peptides enables improved measurement of gluten in foods for individuals with coeliac disease, by allowing a more realistic measurement of the CD triggering epitopes within the glutenins. The total gluten content depended on the specificity of the MAB(s) used for quantitation. In addition, the gluten to gliadin ratios varied greatly between wheat starches. We therefore suggest that multiplying the gliadin content by a factor of two to estimate the total gluten content of a given nominally gluten-free food, particularly those that are based on purified wheat starch may be invalid for processed foodstuffs.

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