PROGRESS REPORT

Review of methods for measuring gliadins in food

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Patients with coeliac disease are advised to take a strict gluten free diet, and usually avoid wheat, rye, barley, and oats. Although there is no doubt that sensitivity to gluten varies between patients¹⁻³ and some can tolerate small amounts, strict adherence to a gluten free diet is usually strongly recommended, particularly in view of the possibility of protection from malignancy.⁴

A reliable and specific means of assessing the gluten content of food would be of great benefit to patients, dietitians, food manufacturers, and gastroenterologists. In this regard there have been several recent reports of enzyme linked immunoassays to detect gluten in foods. These assays are generally sensitive, specific, and relatively easy to perform and their role in testing gluten toxicity will be reviewed here.

Windemann *et al*⁵ described an ELISA assay for wheat α gliadin and whole gliadin using polyclonal rabbit or mouse enzyme labelled antisera. This assay was very sensitive and could detect 0.4 ng α gliadin or 4 ng whole gliadin. They went on to use the assay to detect α gliadin and whole gliadin in unheated gluten containing and gluten free foods.⁶ There were some problems, however, since different extraction solvents had to be used for foods containing cocoa and the assays were too specific since α , β , and γ gliadins only could be detected, not ω gliadin. Nor did the assay react with constituents of other toxic cereals such as rye. The test was not used on cooked food. These problems make this assay unsuitable for routine use.

McKillop *et al*⁷ described a similar ELISA using a polyclonal rabbit antiserum. The detection limit was 3.3 ng gliadin and the assay detected other coeliac toxic cereals. There were no details of which foods were tested, however, and whether they were cooked.

Troncone *et al*⁸ developed a similar assay for gliadin, also using a rabbit polyclonal antiserum. This assay was sensitive, could detect 0.5 ng gliadin, and also reacted with avenins from oats. There were, however, positive results with rice and maize prolamins, which are not toxic to people with coeliac disease. The authors state that these crossreactions make the assay unsuitable for testing foods for coeliac patients. In a later paper, the same authors⁹ showed that both human and rabbit antisera crossreacted with non-toxic proteins such as wheat albumins and globulins and zeins from maize. They suggest that there is a dissociation between the immunogenic properties of cereal proteins and toxicity in coeliac disease.

Skerritt and coworkers have developed a

sensitive monoclonal antibody based test for gluten detection which can be used on a variety of cooked and uncooked foods.¹⁰⁻¹² They used a mouse IgM monoclonal antibody to ω gliadin as the basis of their detection system. ω Gliadin specificity was chosen since ω gliadin is heat stable whereas the other wheat gliadins are denatured during cooking. This assay gave negative results for a variety of non-cereal food proteins but positive reactions for all known cereals toxic to patients with coeliac disease, including weakly positive reactions for some wheat starches and 'gluten free' bread mixes. The limit of detection for wheat gliadin was 2 μ g. This assay was sensitive and specific enough to detect the 'gliadin-like immunoreactivity' in cooked and processed foods.

Freedman *et al*^{13 14} have also used a mouse IgM monoclonal antibody to detect wheat gliadin, principally in gluten free foods. This assay had a lower limit of detection for unfractionated gliadin of 0.75 ng. The assay detected all gliadin fractions as well as prolamins from rye, barley, and oats. Positive reactions were also obtained for wheat glutenins and for gluten free flours based upon wheat starch rather than maize. Negative reactions were obtained for a variety of food proteins not toxic to coeliac patients. No cooked or processed foods were tested. This assay seems to be sensitive and specific enough to be used for the monitoring of gluten free products.

Most recently Friis¹⁵ has described an ELISA assay using a purified rabbit polyclonal IgG antibody to detect gliadin and gliadin-like polypeptides. This assay had a lower detection limit of 1 ng gliadin. There were negative reactions with most cereals that are known not to be toxic to people with coeliac disease but weak reactivity with buckwheat. Positive reactions were also obtained with gluten free flours based upon wheat starch. The method was recommended by the author to be used only with flours and other unprocessed foods since he was concerned about the accuracy of the assay for detection of proteins that may have been potentially denatured by heating and cooking.

There are, therefore, several reported assays available for the immunological detection of gliadin in a variety of flours and foods. But several questions have to be raised about the assays before they can be recommended for routine use. One has to be satisfied about their sensitivity and specificity but perhaps most importantly about the relevance of what they are

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measuring to the exacerbation of coeliac disease – namely the toxicity as opposed to the immunogenicity of gliadin.

Of the assays described above, that used by Windemann, *et al*⁵ and Fritschy *et al*⁶ were very sensitive but too specific. That of McKillop *et al*⁷ has not been tested extensively and cannot therefore be recommended at present. Troncone *et al*⁸ also described a very sensitive assay but it had such a wide specificity they themselves could not recommend it for use in testing the gliadin content of food.

Of the remaining three assays, those of Freedman *et al*^{13,14} and Friis¹⁵ are more or less equally sensitive; they are able to detect 0.75 ng and 1 ng gliadin respectively. In addition both assays are suitably specific in that they gave positive reactions with all coeliac toxic cereals and were negative for non-toxic cereals. Interestingly, Freedman *et al*¹³ found a positive reaction with wheat glutenin and Friis¹⁵ with buckwheat. Although the toxicity of both these materials has yet to be definitely established in coeliac disease, the presumption is that both are non-toxic. Both authors detected low gliadin-like reactivity in so called gluten free flours based on wheat starch. They did not use their assays to test cooked food; Friis¹⁵ thought his assay would be unreliable in these circumstances.

The remaining assay is that described by Skerritt.¹⁰⁻¹² This assay seems to be ideally specific in that correct positive and negative results with a variety of cereals were obtained. Skerritt's antibody was directed against epitopes in ω gliadin, which is not destroyed in cooking, and the assay certainly retained its specificity when used with cooked and processed foods. He therefore feels that this assay is very suitable for routine use in assessing the gliadin content of food, and indeed it is now commercially available in Australia (Qlone Ltd, Darra, Queensland, Australia). This assay is not as sensitive as those of Freedman *et al*^{13,14} or Friis¹⁵ as its lower limit of detection is 2 μ g. Nevertheless, this level of detection is still more than adequate for current recommendations on the gluten content of food for coeliac patients. This area is a matter of some debate but the *Codex Alimentarius* recommends that a gluten free product should contain less than 0.3% protein from wheat, rye, barley, or oats. With regard to wheat in particular, 40% of the protein is gliadin,¹⁶ or 0.12%, representing 1.2 mg/g flour. All the gluten free flours tested and found positive in the above assays were said to contain much less than this amount of gliadin; nevertheless bread baked with these flours may lead to symptomatic relapse in treated coeliac patients.¹⁷ The availability of such sensitive immunological assays may lead therefore to a redefinition of the amount of gluten, if any,

which can be allowed in a so called gluten free product.

If one of the above assays is to be recommended for routine use we conclude that the one described by Skerritt and coworkers seems to be suitable.

As already suggested, the main problem still remaining revolves around the question of gliadin toxicity as opposed to immunogenicity. This was addressed by Troncone *et al* to some extent in 1987.⁹ All the originators of the assays described allude to this problem and base their claims for specificity on the known toxicity or not of the various cereals and cereal fractions. We maintain, however, that the onus must be on those who report these immunological methods for detecting gliadin-like material to show, preferably by in vivo but at least by in vitro methods, that the material they are detecting is both necessary and sufficient for toxicity in patients with coeliac disease.

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