

Biochemistry of coeliac disease

The significance of an enzyme deficiency in people with coeliac disease is that it is likely to be the basic cause of the disease. Thus enzyme therapy can relieve the difficulties of a strict gluten-free diet, says Hugh Cornell.

What is coeliac disease?

Coeliac disease is a permanent intolerance to the gluten proteins found in cereals such as wheat, rye and barley. The 70% ethanol-soluble proteins of these cereals, known as prolamins, have been shown to include the main proteins responsible for the symptoms of the disease.¹ These symptoms are the result of damage to the small intestine, causing malabsorption of vital nutrients, such as iron, calcium and folic acid. The damage can be a protracted process, but other symptoms such as diarrhoea, bloating, stomach pains and lassitude may develop to varying degrees, making the disease difficult to diagnose in many individuals. People who suspect they have coeliac disease should undertake a blood test, which measures the antibodies to tissue transglutaminase (tTG). If this is positive, a biopsy of the duodenum/jejunum, where most of the damage to the mucosal surface occurs, should be taken to confirm the diagnosis. Further biopsies can be performed, if necessary, after several months on a gluten-free diet and after a specific gluten challenge.² The standard treatment for the disease is then to remain on a gluten-free diet. The incidence of the disease in Caucasian races is about 1 in 200 individuals.

Toxic action of the prolamins

The prolamins of wheat, called gliadins, have been studied the most and have become the best characterised of the toxic cereals. It has been shown that the toxicity is still present after digestion of the gliadins with pepsin, trypsin and pancreatin to oligopeptides.³ Fractionation of this digest on SP Sephadex (Pharmacia, Sweden) showed that one of these fractions prevented recovery of the mucosa from patients with active coeliac disease, much more than the other fractions of the same digest, as shown by electron microscopy.⁴ These findings were based on important studies that showed the intestinal mucosa from patients with active coeliac disease recovered histologically after 24 hours of culture in the absence of gliadin, but remained damaged in the presence of gliadin.

Further digestion of these fractions *in vitro* using mucosa from patients with coeliac disease in remission, where the mucosa had recovered after a gluten-free diet, in comparison

with digestion with mucosa from normal individuals, showed that fraction 9 was incompletely digested by coeliac mucosa but completely digested by normal mucosa. All the other fractions were well digested by coeliac and normal mucosa.⁵ These studies provided further support for the enzymopathic hypothesis, put forward many years before, based on experiments showing that digests of gluten made using pepsin and trypsin were harmful to patients with coeliac disease in remission, but after digestion with hog intestinal mucosa they were not.⁶ This hypothesis suggested that the basic cause or aetiology of coeliac disease was a missing or defective enzyme in individuals with this disease, causing high concentrations of damaging peptides in the small intestine.

Composition of the toxic peptides

The elucidation of the amino acid sequence of A-gliadin afforded a very important break-through in these studies.⁷ Based on this structure, two key sequences, each of four amino acids (called motifs) were found to be associated with toxicity and found in this toxic fraction of the gliadin.⁸ These motifs were QQQP and PSQQ. When found in larger peptides from A-gliadin, they were shown to be toxic to cultured mucosa from patients with coeliac disease.

Pursuing the toxic peptides present in the relevant fraction, it was found that a sub-fraction from reversed-phase HPLC contained both the QQQP and PSQQ motifs and corresponded to the sequence residues 9-19 of A-gliadin.⁹ This peptide was subsequently synthesised and its toxicity verified.

The other sub-fraction was one that corresponded to residues 75-86 of A-gliadin, and although it did not have as high an activity in a foetal chick assay, it was capable of causing immunological reactions that can result in damage to tissue.¹⁰ The structure of this peptide was RPQQPYQPQPQ, one without the two motifs previously associated with toxicity.

An insight into the importance of motifs containing phenylalanine (F), tyrosine (Y), proline (P) and glutamine (Q) came with the aid of proteomics. A search of protein databases looking for matches with coeliac-toxic cereals has provided