STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF TRANSGLUTAMINASE 2 IN RELATION TO SIGNAL TRANSDUCTION AND COELIAC DISEASE

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Coeliac disease (GSE) is the most frequent, chronic small intestinal autoimmune disorder with broad spectrum of manifestation in genetically predisposed persons. The main autoantigen of GSE is the transglutaminase 2 (TG2).

We identified that the specific TG2 autoantibodies also exist in small-bowel antibody deposits in seronegative patients. Our study confirms the hypothesis that the anti-TG2 autoantibodies are specific markers of GSE and are present in every coeliac patient.

Our finding raises the possibilities that TG2 and anti-TG2 autoantibodies can play important role in pathogenesis of GSE. We found that immunoglobulins from patients with severe malabsorption enhanced the transglutaminase activity of TG2. This activating effect was dose-dependent, most pronounced with immobilised glutamine-acceptor substrates, and correlated inversely with the basal specific activity of the enzyme and with dietary treatment. A similar activation could be demonstrated also with the TG2-specific fraction of autoantibodies and in transamidation activity assays which use fibronectin-bound TG2 and thereby mimic *in vivo* conditions. These results suggest that coeliac antibodies may stabilise the enzyme in a catalytically advantageous conformation.

GTPase activity of TG2 decreased in the presence of antibodies raising the possibility that inhibition of GTPase activity may affect cellular signalling.

Since the TG2 could be a key player in GSE and the Ca^{2+}-dependent function and structure relations were not completely characterised we examined the Ca^{2+}-binding properties of TG2. We identified 5 non-canonical Ca^{2+}-binding sites, out of which 3 by homology with known Ca^{2+}-binding sites of TG3 and Factor XIIIa and the other 2 with negative surface potentials using site directed mutagenesis. CD spectroscopy, antibody binding assay and GTPase activity measurements indicated that the amino acid substitutions did not cause major structural alterations. ^{45}Ca equilibrium dialysis and isothermal calorimetric titration showed that the wild type and active site deleted enzymes bind 6 Ca^{2+}. Each mutant binds less Ca^{2+} than these and mutation of a site resulted in the loss of more than one Ca^{2+} ions. All mutants were deficient in transglutaminase activity and similarly to the wild type enzyme GTP inhibited remnant activities. Similarly to the wild type form GTPase activities of the mutants were sensitive to Ca^{2+}-concentration except in case of S4 and S5 which exhibited increased GTPase activity. Testing reactivity of Ca^{2+} mutants with coeliac autoantibodies revealed that the S4 site strongly influenced antigenicity and the interaction of autoantibodies with TG2.

Keywords: transglutaminase 2, transglutaminase activity, calcium-binding, coeliac disease, dermatitis herpetiformis, antibody enhancing, GTPase activity, antigenicity, coeliac epitope

Kulcsszavak: transzglutamináz 2, transzglutaináz aktivitás, kalcium-kötés, coeliakia, dermatitis herpetiformis, antitest erősítés, GTPáz aktivitás, antigenitás, coeliakia epitóp