A Peptide Inhibitor Reveals an Extended Conformation of Transglutaminase 2

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Abstract

Transglutaminase 2 (TG2) is a ubiquitous enzyme which catalyzes the crosslinking of proteins via formation of epsilon-(gamma-glutamyl) lysine bonds as well as deamidation of glutamine residues to glutamate in suitable substrates. The activity of Transglutaminase 2 has been implicated in the pathology of several diseases including Celiac Disease and diseases involving protein aggregation such as Huntington's and Parkinson's Disease [X]. We have synthesized a potent peptide inhibitor containing the unnatural amino acid 6-diazo-5-oxo-norleucine (DON) and have obtained a 2Å crystal structure of the enzyme-inhibitor complex. The peptide inhibitor stabilizes an extended conformation of Transglutaminase 2 in which the active site is exposed. The structure gives insights into enzyme-substrate interactions, has immunological implications for Celiac Sprue, and acts as a basis for the rational design of small molecule inhibitors.

Peptides derived from gluten which have the repeat motif PQPQLPY have been shown to be resistant to hyrdolysis by gastric and pancreatic proteases. On the brush border of the intestinal lumen, the peptides containing this motif are regiospecifically deamidated to PQPELPY by TG2. In patients who are genetically predisposed to CS, the deamidated peptides are potent activators of a T cell autoimmune response, which is responsible for villous atrophy in the upper GI and consequently a variety of intestinal and non-intestinal maladies.

Recognizing the high affinity of this gluten motif for TG2, the peptide Ac-PELPF-NH_2 was synthesized by solid phase peptide synthesis and derivatized to Ac-P(DON)LPF-NH_2. DON (6-diazo-5-oxo-norleucine) is glutamine isostere, which is known to react with cysteine-histidine-aspartate active site catalytic triads, such as that of TG2, to form non-hydrolyzable thioether bonds. The potent irreversible inhibitor was reacted with TG2 and crystallized to reveal an extended conformation, which differs dramatically from the prolate ellipsoid conformation seen in previously crystallized transglutaminases. The overall conformation of the enzyme-inhibitor complex along with recent immunological studies [X,Y] suggests a mechanism for the formation of anti-TG2 autoantibodies, which are a hallmark of Celiac Sprue.