Structural Intermediate of Transglutaminase 2 in Complex with Two Calcium Ions Sheds Light on New Treatments for Celiac Disease

Transglutaminases are a family of calcium-dependent enzymes that form chemical crosslinks between proteins and/or peptides. Transglutaminase 2 (TG2) is one member of this family that has been implicated in many diseases, including the gluten-mediated autoimmune condition celiac disease (CeD)[1,2]. TG2 catalyzes the deamidation of glutamine residues present in gluten peptides in a calcium-dependent manner. This deamidation increases the peptide's affinity for MHCII receptors and therefore their antigenicity. While it is well known that this critical event requires calcium, a high-resolution structure of TG2 bound to this essential metal cofactor has remained elusive. In this work, the authors show, for the first time, the structure of TG2 bound to two calcium ions. This structure, in concert with novel biochemical assays, revealed previously unknown features of the TG2 catalytic cycle and provides greater insight into the catalytically relevant calcium sites.

To become active, TG2 undergoes a large conformational change to adopt an "open" state from its inactive, GDP bound "closed" state[3]. While both states gave valuable insights into TG2's catalytic mechanism, they lacked a key regulatory element. In attempt to capture a more physiological state of the enzyme, extensive screening of crystallographic conditions was performed that gave rise to three high-resolution structures of TG2 in complex with a gluten peptidomimetic inhibitor. One structure adopts the "open" state of the enzyme and is nearly identical to a previously solved conformation of the enzyme without calcium. However, the two other structures, termed the "intermediate" state, revealed a fundamentally new conformation of the enzyme in complex with two calcium ions.

TG2 contains five calcium sites, defined as S1-S5, with varied affinities[4]. The intermediate structure featured a calcium ion bound at the high-affinity calcium site, S1. Additionally, S1 contains a key cysteine redox regulator of TG2, C230[5]. It was known previously that calcium binding at S1 precludes the activity of this cysteine while the absence of calcium allows for its function. The new intermediate conformer shows that Ca^{2+} occupancy at S1 re-orients C230 away from its known redox active partner. In doing so, this new conformer provides a structural basis for this biochemical oxidative 'or' gate **(Fig. 1A)**.

In addition to the deamidation reaction that implicates TG2 in CeD, TG2 can catalyze transamidation reactions when provided with a primary amine co-substrate. It has been hypothesized that the amine co-substrate must be deprotonated by H305 and E363 to serve as a nucleophile[6]. However, in previous TG2 structures H305 and E363 are distant from each other and the active site. This new conformer features a calcium ion at S3 that reorganizes adjacent residues and repositions H305 and E363 near the active site. This structural evidence, in concert with independent LCMS/MS assays, confirmed the role of H305 and E363 in TG2 transamidation **(Fig. 1B)**.

Finally, the intermediate state provides novel insight into protein-peptide interactions that determine TG2 substrate specificity. Two previously unremarkable residues, N333 and K176 make extensive contacts with the gluten peptidomimetic inhibitor in the intermediate state **(Fig. 1C)**. Mutation of N333, specifically, revealed the essentiality of this residue for high affinity substrate recognition and turnover.

In conclusion, the intermediate conformer of TG2 bound to both gluten peptidomimetic inhibitor and calcium structurally rationalizes previously known biochemical requirements for

TG2 activity. These structures also reveal fundamentally new insight into the chemical basis of high-affinity substrate recognition and biochemically functional calcium sites.

The authors conclude that this intermediate conformer is the most physiologically relevant structure of catalytically active TG2 described to date, and as such will be an invaluable tool for further study of the involvement of this enzyme in physiologic and pathophysiologic processes.

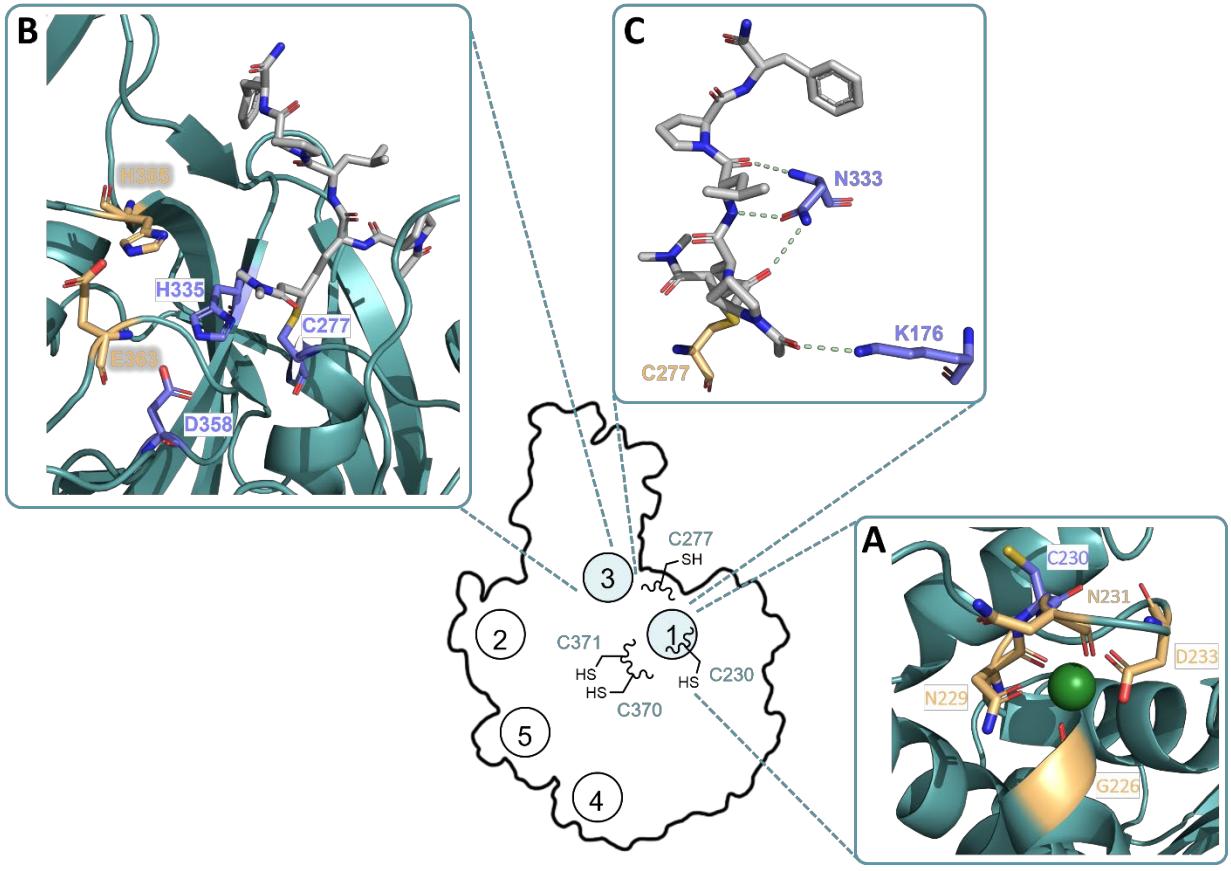

Figure 1: Regulation of Transglutaminase 2 using a Ca^{2+}-bound Intermediate State. TG2 core domain depicted as a cartoon with five defined calcium sites and four key regulatory cysteines (C230, C277, C370, and C371). Calcium sites that are occupied in the intermediate state are shown blue. **(A)** Intermediate state of TG2 with calcium bound at S1 and S3 provides structural evidence of the oxidative 'or' gate. Intermediate structure in teal, Ca^{2+} at S1 in green, coordinating residues (N229, N231, D233, and G226) in yellow, C230 in blue. **(B)** Calcium occupancy at S3 allows repositioning of key transamidation residues H305 and E363, shown in yellow, in proximity to TG2's catalytic triad C277, H335, and D358, shown in blue. Active site specific peptidomimetic inhibitor of TG2 shown in grey. **(C)** Peptidomimetic inhibitor, shown in grey, bound to active site cysteine C277, shown in yellow, is well coordinated by specificity residues N333 and K176, shown in blue.

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