# Inhibitors of transglutaminase 2: A therapeutic option in celiac disease

Ina Lindemann*, Jark Böttcher*, Kai Oertel**, Johannes Weber**, Martin Hils**, Ralf Pasternack**, Uwe Linne***, Andreas Heine*, Gerhard Klebe*

*Department of Pharmaceutical Chemistry, Philipps University Marburg, Pilgrimstein 2, 35032 Marburg, Germany
**ZEDIRA GmbH, Rösslerstr. 83, 64293 Darmstadt, Germany
***Department of Chemistry, Hans-Meerwein-Str., 35032 Marburg, Germany

Email: ina.lindemann@staff.uni-marburg.de

## Introduction

Human transglutaminase 2 (TG2), an enzyme with a variety of physiological and pathological functions, catalyzes the transamidation and deamidation of glutamine residues in peptides. Amongst others, the enzyme is involved in the pathogenesis of celiac disease, a T-cell mediated inflammatory disorder of the small intestine. Here, gluten peptides are the environmental factors required for disease activation in genetically predisposed individuals. TG2 deamidates glutamine residues from these peptides and converts them into glutamic acids, increasing the binding affinity to HLA-DQ2/DQ8-receptors. As TG2 thus increases the pathological effect of the gluten peptides, TG2 inhibitors are potential therapeutic agents to treat celiac disease and to improve the patient`s quality of life: Up to now, no treatment whatsoever has been developed, patients have to follow a strict glutenfree diet.[1]

The aim of our work was to determine the binding mode of a TG2 inhibitor by crystallization and X-ray analysis of a TG2-inhibitor complex. The information obtained about the interactions between TG2 and the inhibitor forms the basis for further drug design.

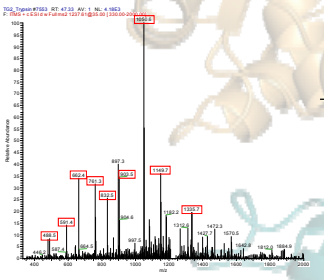
## Rational design of transglutaminase inhibitors

Based on a peptide substrate of TG2 a new class of inhibitors was designed. The lead substance consists of a modified tetrapeptide. The substrate glutamine amino acid was replaced by a head group addressing the active site of the enzyme. Since TG2 is a cysteine dependent enzyme a selective pharmacophore had to be synthesized. Combining a side chain localized Michael-acceptor derivative with a tight binding peptide backbone a highly potent lead substance was generated. Based on the determined structure the molecule is currently optimized towards a druggable peptidomimetic.

## Production, purification and inhibition of TG2

Escherichia coli cells producing recombinant TG2 were centrifuged at 3,700 rpm for 20 min after an induction period of 20 hours at 20°C. The pellet was resuspended in buffer and lysed by high pressure homogenization. After centrifugation, the supernatant was applied to a column containing Ni-NTA resin. The column was rinsed with buffer until baseline was reached. TG2 was then eluted in a buffer containing 300 mM imidazole. Fractions containing TG2 were pooled and further purified by anion exchange and gel permeation chromatography. Preparation of inhibited TG2 for crystallization was performed by incubating freshly prepared TG2 with inhibitor at a ratio of 1 to 50 at room temperature for 30 min and then at 4°C overnight. Excess inhibitor was removed by anion exchange chromatography. TG2-inhibitor conjugate was concentrated to 8 mg/mL. Glycerol was added to a final concentration of 10%. Storage was performed at -80°C.

## Confirmation of the covalent binding mode by mass spectrometry



**Fragment Ion Table, monoisotopic masses**

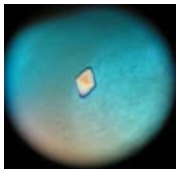
| Seq | # | B | Y | # (+1) |
|---|---|---|---|---|
| Y | 1 | 164.07120 | 2474.27733 | 16 |
| G | 2 | 221.09266 | 2311.21401 | 15 |
| Q | 3 | 349.15124 | 2254.19254 | 14 |
| C | 4 | 1139.56042 | 2126.13397 | 13 |
| W | 5 | 1325.63974 | 1335.72478 | 12 |
| V | 6 | 1424.70815 | 1149.64547 | 11 |
| F | 7 | 1571.77656 | 1050.57705 | 10 |
| A | 8 | 1642.81368 | 903.50864 | 9 |
| A | 9 | 1713.85079 | 832.47153 | 8 |
| V | 10 | 1812.91921 | 761.43441 | 7 |
| A | 11 | 1883.95632 | 662.36600 | 6 |
| C | 12 | 1986.96550 | 591.32889 | 5 |
| T | 13 | 2088.01318 | 488.31970 | 4 |
| V | 14 | 2187.08160 | 387.27262 | 3 |
| L | 15 | 2300.16566 | 288.20361 | 2 |
| R | 16 | 2456.26677 | 175.11955 | 1 |

The aim of the experiment was to show that the inhibitor binds to the active site Cys277. The TG2-inhibitor complex was fragmented by trypsinolysis. The fragments were separated by HPLC, ionized by ESI and afterwards sorted in a FT-ICR mass analyzer. The desired fragment, whose molecular mass was calculated previously, could be identified. To find out whether the inhibitor is bound not only to the right fragment but also to the active site Cys277, the respective fragment was fragmented again and another mass spectrum was generated (shown above).
The calculated masses of the possible resulting fragments are shown in the table on the right. Depending on the position of the charge, the fragments are classified in the Y-series and the B-series. Fragments from the Y-series are charged at the N-terminus, fragments from the B-series are charged at the C-terminus.
The fragments 4 to 12 from the Y-series are all found in the mass spectrum, indicating the inhibitor's binding to one of the first four amino acids of the fragment. The active site cysteine is amino acid number 4 in this table.

## Crystallization and X-ray analysis

The crystal structure of TG2 in complex with a covalently bound inhibitor was determined at 2.5 Å resolution. The refinement of the structure is currently in progress.

**Crystallization conditions:**
1.75 – 2.25M $(NH_4)_2SO_4$
100mM HEPES pH 6.75 – 7.5
Sitting-drop vapor diffusion method
18°C
Rhombic-shaped crystals usually appeared in 5-7 days.



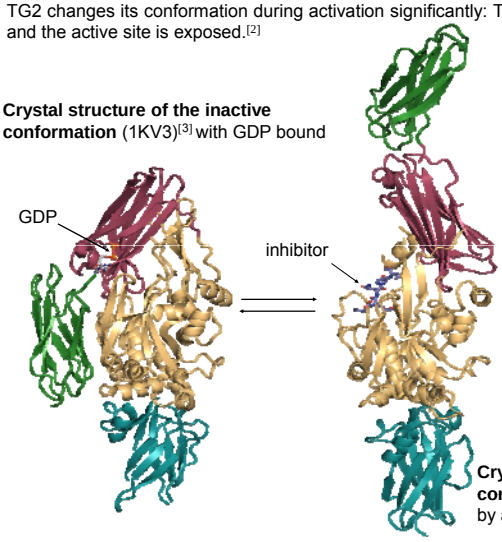
**Space group:** $P4_12_12$
**Unit cell constants:**
a, b = 71.0 Å, c = 310.4 Å

The data set was collected with synchrotron radiation at BESSY II in Berlin.

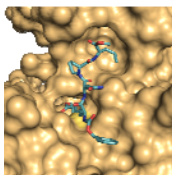
TG2 changes its conformation during activation significantly: Two domains are shifted away and the active site is exposed.[2]

**Crystal structure of the inactive conformation** (1KV3)[3] with GDP bound

Description of the domains:
**blue**: β-sandwich domain
**gold**: catalytic core domain
**red** and **green**: β-barrel domains[3]

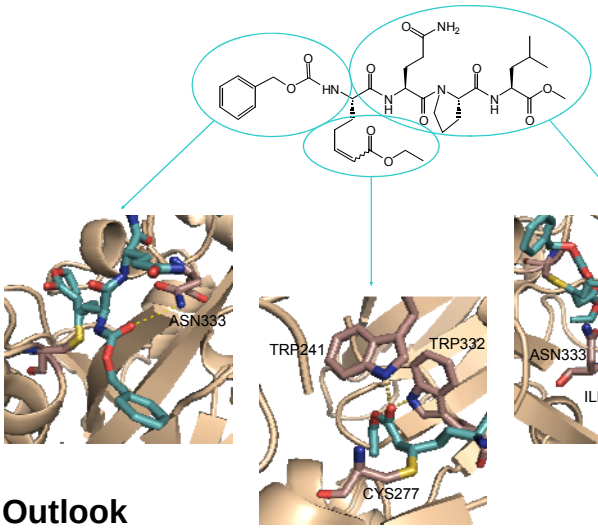



**Crystal structure of the active conformation** which is stabilized by a covalently bound inhibitor



**Position of the inhibitor in the binding site:**

The inhibitor (shown in cyan) is covalently bound by a Michael addition reaction to the active site Cys 277, which is located in the catalytic tunnel. It mimics the gluten peptide substrates. The main part of the inhibitor fits onto the protein surface, forming several hydrogen bonds and stabilizing the open conformation.




## Outlook

The crystallization of further inhibitor complexes with new TG2 inhibitors will provide information for the subsequent drug design cycles. Final goal is to develop TG2 inhibitors as potential therapeutics of celiac disease.

**References**
[1] Siegel, M., Khosla, C. (2007) *Pharmacology and Therapeutics* **115**, 232-245
[2] Pinkas, D.M. et al. (2007) *PLoS Biology* **5**: e327
[3] Liu, S. et al. (2002) *PNAS* **5**, 2743 - 2747

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