# Celiac Disease

Lamaso Sada



Celiac disease is an autoimmune disorder of the small intestine. Some symptoms of celiac disease include: Pain and discomfort in the digestive tract, diarrhea, constipation, and fatigue. Celiac disease is caused by the body becoming intolerant to gliadin. Gliadin is a prolamin protein found in wheat, barley, rye, and other grains. Prolamin proteins are plant storage proteins that causes celiac disease to a genetically susceptible individual. When gliadin is modified by transglutaminase 2, the modified protein and the immune system cross react with the small bowel tissue, causing an inflammatory reaction.

Transglutaminase 2 is a calcium dependent enzyme that crosslinks proteins between a ε-amino group of a lysine residue and a γ-carboxamide group of a glutamine residue *(1)*. A thiol group is involved in the catalytic mechanism for crosslinking in human transglutaminase 2. The thiol group from the cysteine residue in the active site of transglutaminase 2 attacks the carboxamide on the surface of protein releasing ammonia and producing a thioester intermediate *(5)*. The thioester intermediate can crosslink with a lysine residue or get hydrolyzed. The hydrolyzation of the thioester intermediate results in the conversion of the glutamine residue to glutamic acid by deamidation *(5)*. Deamidation is a reaction that removes an amide group from an organic molecule. The catalyzed deamidation of glutamine by transglutaminase 2 is suggested to be linked to the immune response to gluten in celiac disease *(2)*.

Interferon gamma (IFN-γ) plays an important role in celiac disease pathogenesis because it causes thioredoxin to be released. *(3)*. IFN-γ is a strong inflammatory cytokine. Cytokines are a class of small proteins important in cell signaling. A gluten reactive T cell that releases IFN-γ

is located in the small intestinal mucosa of celiac patients. IFN-γ can raise the trans-epithelial flux of antigen-sized peptides that forms an autocrine signaling process when gluten peptides are deamidated to transform into strong T cell antigens *(3)*. The autocrine signaling process causes gluten-induced toxicity.

The experiment done in the article *Activation of Extracellular Transglutaminase 2 by Thioredoxin* required the co-culture of Tamm-Horsfall protein (THP)-1 and Winstar Institute (WI)-38 fibroblasts. THP-1are white blood cells derived from a leukemia patient. WI-38 fibroblasts are human cell culture lines derived from lung tissue of a three-month-old white female fetus. THP-1 and WI-38 were cultured as suspension cells in Roswell Park Memorial medium (RPMI 1640). RPMI 1640 is used for the growth of human lymphoid cells. The co-cultured THP-1 and WI-38 are known to have increased levels of thioredoxin when exposed to IFN-γ. Thioredoxin is a small redox protein that enables the reduction of other proteins by cysteine thiol-disulfide exchange. THP-1 cells were incubated with 1000 units/ml IFN-γ for forty-eight hours. Western blotting was used to measure thioredoxin levels in THP-1. Western blotting is a technique used to identify specific proteins from a complex mixture of proteins. Three techniques are used to identify proteins: Separation by size, transfer to a solid support, and marking the specific protein with an antibody to distinguish it.

The samples used in Western blotting were diluted in a one-to-one ratio concentration with a buffer containing β-mercaptoethanol, which reduced disulfide bonds and searched for hydroxyl radicals. The samples were added to sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). SDS disrupts the tertiary structure of a protein, bringing the protein to its linear form. PAGE is the gel matrix that the linear proteins move through at different rates depending on their molecular weight, charge and length. The proteins from the SDS-PAGE were transferred to a

polyvinyldiene fluoride (PVDF) membrane at eighty volts (V) for two hours in a transfer buffer containing tris, water, glycine, and methanol. The membranes were then blocked using five percent nonfat dry milk, then washed three times with Tris-Buffered Saline (TBS-T). The membranes were exposed to thioredoxin after washing. The blots were quantified using Typhoon fluorescence imager, fluorescing thioredoxin. Typhoon fluorescence imager has the capability to detect gels and blots with fluorescence, storage phosphor, and chemiluminescence. It transmits sensitive fluorescent recognition of nucleic acids and proteins in agarose and polycrylamide gel formats.

The treated THP-1 cells were co-cultured with WI-38 monolayers in an eight well chamber for six hours. THP-1 cells were washed off using phosphate-buffered saline (PBS). To the eight well chamber, Tris-hydrochloride (Tris-HCl) buffer containing five mM calcium chloride ($CaCl_2$) was added. The cells were incubated at 37º C for an hour, then washed three times with PBS. One percent bovine serum albumin (BSA) in PBS was used to block the contents of each cell. The cells were washed two more times with PBS and stained with Alexa Fluor 555-conjugated streptavidin. Alexa Fluor 555-conjugated streptavidin is used to detect a variety of proteins, protein motifs, nucleic acids, and other molecules. Alexa Fluor 555-conjugated streptavidin is a family of dyes that are synthesized through sulfonation of coumarin, rhodamine, xanthene, and cyanine. Sulfonation attaches a sulfonic acid group to a carbon in an organic compound that makes the dyes negatively charged, hydrophilic, more stable, brighter, and less pH-sensitive. The cells were detected using fluorescence microscopy. Using fluorescence microscopy, transglutaminase 2 activity was detected around many fibroblasts. The experiment showed that IFN-γ causes the extracellular release of thioredoxin, which activates extracellular transglutaminase 2. It is yet to be determined how TG2 activity is triggered in

response to inflammatory signals. However, previous studies have shown that IFN-γ is a potent pro-inflammatory cytokine that is secreted by gluten-reactive, disease-specific T cells that reside in the small intestinal mucosa of celiac patients.

An essential step in the pathogenesis of celiac disease is the modification of gluten peptides by transglutaminase 2. The redox-sensitive cysteine triad causes oxidative inactivation of transglutaminase 2. The cysteine triad is $Cys^{230}$, $Cys^{370}$, and $Cys^{371}$. $Cys^{230}$ is the entrance for disulfide bond formation. The vulnerability of $Cys^{230}$ to oxidation results in the inactivity of transglutaminase 2. In the article *Redox Regulation of Transglutaminase 2 Activity* the cysteine residue vulnerability to oxidation was identified.

In order to identify the cysteine residues that were vulnerable to oxidation, transglutaminase 2 was treated with glutathione (GSH) over glutathione disulfide (GSSG) *(4)*. GSH can be stimulate GSSG by glutathione reductase (GSR). GSR produces a temporary FADH anion when nicotinamide adenine dinucleotide phosphate (NADPH) reduces flavin adenine dinucleotide (FAD) to FADH. The FADH anion breaks disulfide bonds. Following the incubation of transglutaminase 2, the free cysteine residues underwent alkylation with 2-iodoacetamide (IAM). Separation was done using sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). The protein band was eliminated and the disulfide bonds were reduced using dithioreitol (DTT) and then alkylated with indole-3-acetic acid (IAA). DTT is a reducing agent that forms a stable six-membered ring with internal disulfide bonds. Using mass spectrometry the redox-sensitive cysteine triad was identified.

The cysteine triad identified included: $Cys^{230}$, $Cys^{370}$, and $Cys^{371}$ were found to be vulnerable to oxidation. $Cys^{370}$ was found to have two disulfide bonds, one with $Cys^{371}$ or one with $Cys^{230}$. Following the identification of the cysteine triad, a GSH/GSSG titration was done.

The results showed that oxidation of the cysteine triad increased when the relative amounts of GSH/GSSG was decreased. When the conditions were not oxidizing $Cys^{230}$ and $Cys^{370}$ formed more readily than $Cys^{230}$ and $Cys^{371}$.

Celiac disease is an abnormal immune response to gluten in the small intestine. The thiol group from the cysteine residue in the active site of transglutaminase 2 attacks the carboxamide on the surface of protein releasing ammonia and producing a thioester intermediate. The thioester intermediate can crosslink with a lysine residue or get hydrolyzed. The hydrolyzation of the thioester intermediate results in the conversion of the glutamine residue to glutamic acid by deamidation. The consumption of food containing gluten results in a catalytic activity of transglutaminase 2. When the food containing gluten reaches the small intestine the immune system attacks gliadin. This results in antibodies attacking the lining of the small intestine causing damage. The damage leads to the symptoms of celiac disease. This autoimmune disorder that affects the digestive system is a result of the mechanism of transglutaminase 2 and its catalytic activity in developing gluten peptides that act as antigens.

Reference

1. Ghanta, K. S.; Pakala, S. b.; Reddy, S.; Li, D.; Nair, S.; Kumar, R. MTA1 Coregulation of Transglutaminase 2 Expression and Function During Inflammatory Response. *The Journal of Biological Chemistry*. 2011, 286, 7132-7138. From the Department of Biochmeistry and Molecular Biology and Institute of Coregulator Biology, The George Washington University Medical Center, Washington, D.C. 20037

2. Iwai, K.; Shibukawa, Y.; Yamazaki, N.; Wada, Y. Transglutaminase 2-Dependent Deamidation of Glyceraldehyde-3-Phosphate Dehydrogenase Promotes Trophoblastic Cell Fusion. *The Journal of Biochemistry*. 2014, 289, 4989-4999. From the Department of Molecular Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan.

3. Jin, X.; Stamnaes, J.; Klock, C.; DiRaimpondo, T. R.; Sollid, L. M.; Khosla, C. Activation of Extracellular Transglutaminase 2 by Thioredoxin. *The Journal of Biochemistry*. 2011, 286, 37866-37873. From the Department of Chemistry and Chemical Engineering, Stanford University, Stanford California 94305.

4. Stamnaes, J.; Pinkas, D. M.; Fleckenstein, B.; Khosla, C.; Sollid, L. M. Redox Regulation of Transglutaminase 2 Activity. *The Journal of Biochemistry*. 2010, 285, 25402-25409. From the Centre for Immune Regulation, Institute of Immunology, University of Oslo and Oslo Hospital, N-0027 Oslo.

5. Wang, Z.; Collighan, R. J.; Pytel, K.; Rathbone, D. L.; Li, X.; Grffom, M. Characterization of Heparin-binding Site of Tissue Transglutaminase. *The Journal of Biochemistry*. 2012, 287, 13063-13083. From the School of Life and Health Science, Aston University, Aston Triangle, Birmingham, B4 7ET, United Kingdom.