

ELIDUCATING THE DISTINCTIONS BETWEEN OPEN-STATE MONOMERS AND DIMERS OF HUMAN TISSUE TRANSGLUTAMINASE

S.D. Ivashchenko¹, A.V. Vlasov*^{1,2}

¹Laboratory of Microbiology, BIOTECH University, 125080 Moscow, Russia

²Joint institute for nuclear research, 141980 Dubna, Russia

e-mail: vavplanet@mail.ru

Abstract – Transglutaminase 2 (TG2) is a pivotal enzyme involved in various biological processes such as wound healing, apoptosis, and cell differentiation. Depending on the environmental conditions, TG2 can adopt two distinct conformations: the open and closed states. Notably, the open conformation of TG2 has been associated with the pathogenesis of several diseases, including celiac disease and certain cancers. Recent investigations have demonstrated that within human cells, open-state TG2 can exist both as monomers and as dimers. The monomeric form primarily exhibits transamidation activity, whereas the dimeric form is postulated to exert cytotoxic effects.

While several structures of the monomeric open-state TG2 are available in the Protein Data Bank, structures representing the dimeric form remain elusive. The objective of this study is to elucidate the structural distinctions between TG2 monomers and dimers using small-angle X-ray scattering (SAXS).

INTRODUCTION

Human tissue transglutaminase (tTG, TG2) is a multifunctional enzyme involved in various biological processes, including cell growth, differentiation, and extracellular matrix stabilization [1-2]. Dysregulation of tissue transglutaminase has been implicated in a variety of diseases, including autoimmune disorders and cancer [3-4]. The role of tTG in the pathogenesis of celiac disease (CD), an autoimmune genetic disorder characterized by immune reaction to gluten oligopeptides, has been extensively studied. During the development of the disease, proteolyzed gluten proteins form a complex with tissue transglutaminase in the lamina propria, which leads to conversion of glutamine residues of the bound oligopeptide (Figure 1). This modification enhances the binding affinity of oligopeptides to HLA-DQ2 or HLA-DQ8 receptors of antigen-presenting cells (APCs), which in return triggers two different immune responses: Th1 and Th2 pathways [5].

The celiac disease is widespread: up to 0.6% of the population have CD in Russia, and worldwide the pooled global prevalence of celiac disease was 1.4% [6-7]. Currently there are no registered drugs for the celiac disease, and the only approved treatment is a strict gluten-free diet, which is challenging and in some cases does not eliminate symptoms [8]. Tissue transglutaminase remains as a leading potential drug target in the CD patients; yet the properties of the enzyme are not fully understood.

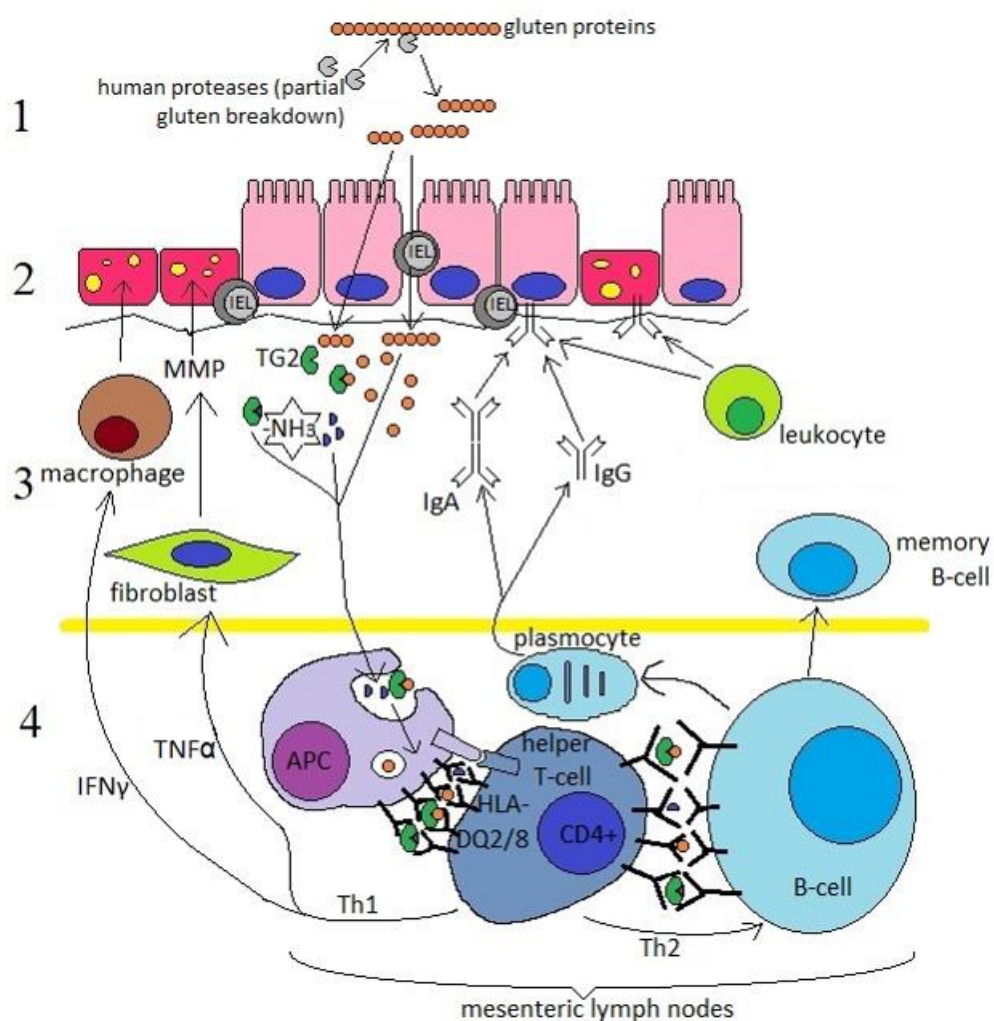


Figure 1. Schematic representation of the molecular mechanism behind celiac disease. TG2 – tissue transglutaminase; APC – antigen-presenting cell; MMP – matrix metalloproteinase; IEL – intraepithelial lymphocyte; IFN γ – interferon gamma; TNF α – tumor necrosis factor alpha.

Tissue transglutaminase in the human body exists in two distinct stable conformations - open and closed, - depending on the extrinsic conditions and presence of ligands with the affinity to specific conformation of tTG [9]. The conformations of tTG differ not only in their structural organization, but also in their cellular localization and functions. While closed conformation of the protein is identical to G α h subunit of the dimeric G-protein Gh, and is not involved in the CD development, the open conformation plays a crucial role in the modification of gluten oligopeptides during disease development [10]. The cysteine amino acid residue of open form transglutaminase (C277) attacks glutamine residue of gluten peptide, forming a complex. In the tTG-peptide complex the reaction can go two ways: either transamidation, when the oligopeptide connects to acyl acceptor, or deamidation [11]. Interestingly, during the modification process, ammonia is released in both possible reactions with the peptide, which means that the enzymatic activity of tTG can be detected using Nessler's reagent [12].

Several studies have demonstrated that the open conformation of transglutaminase is capable of dimerization resulting in the formation of dimers which engender inter-molecular crosslinking activity and are probably cytotoxic [9]. There are four resolved structures of monomeric open form of tissue transglutaminase in the ProteinDataBank (2q3z, 3s3j, 3s3p, 3s3s); however, there is none for the dimeric form of tissue transglutaminase. The binding domain for tTG dimerization was established (residues 593-600), and it was shown that the information about oligomeric state can be obtained using small-angle X-ray scattering (SAXS) analysis [13]. There is no data on whether the ligand binding of the open-form tTG affects the oligomeric state of the protein, which in return can lead to inhibitor dysfunction and should be taken into consideration during the CD drug development.

In this study, we applied CRY SOL modeling [14] to understand whether we can distinguish between monomeric and dimeric states of the human tissue transglutaminase using SAXS as well as between open and closed forms of the protein and between different dimeric organizations.

RESULTS

The high-resolution structure of the open conformation of human tissue transglutaminase was obtained from 2q3z PDB file. We used HDOCK modeling [15] to explore possible dimer organization of open form tTG knowing the localization of the protein dimerization domain (residues 593-600). The most representative diverse structural organizations of modelled dimers are shown in Figure 2; since it is known that the dimeric state enhances crosslinking activity [13], the most probable dimeric state is on the Figure 2B, where both of the binding sites are accessible.

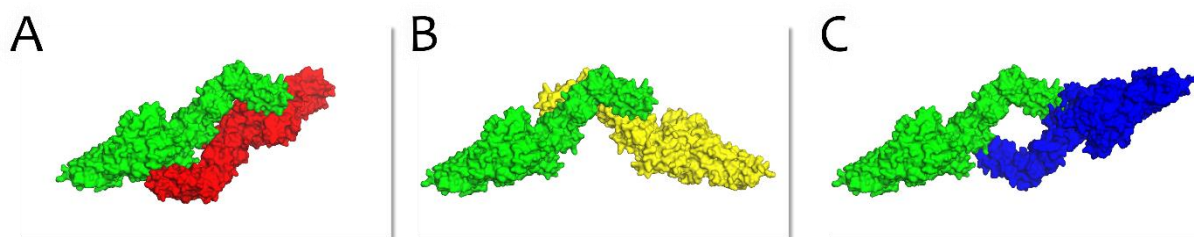


Figure 2. Possible dimer organization of open form tTG.

To understand whether we can distinguish between tissue transglutaminase conformations and oligomeric states we applied CRY SOL modeling. Using PDB structures of monomeric open tTG form and closed tTG form as well as HDOCK models of dimeric open tTG form we got theoretical results that are shown in Figure 3. According to the obtained data, we will be able to distinguish between monomeric and dimeric state of the open conformation using only SAXS data. Moreover, we can distinguish open and closed conformations using only this method, and we will be able to confirm the correctness of one particular HDOCK dimer model, because the difference between scattering intensities is higher than 10% and therefore it is detectable via SAXS method.

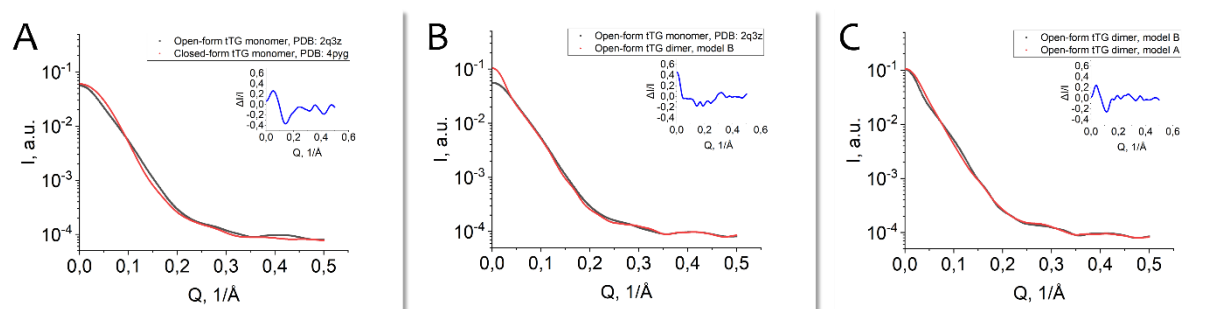


Figure 3. Plots using theoretical SAXS data from CRYSOLE modeling. On the horizontal axis the modulus of vector of momentum transfer in reciprocal space is displayed (scattering vector Q). Vertical axis shows the scattering intensity in arbitrary units. Additional plot shows the ratio of difference between scattering intensities to the scattering intensity. **A.** Comparison of the open-form monomer with the open-form dimer. **B.** Comparison of the open-form monomer with the closed-form monomer. **C.** Comparison of two open-form HDock dimer models.

CONCLUSION

According to the obtained data, open-form monomers and dimers of tissue transglutaminase are easily distinguishable, and the difference between open and closed forms of the protein is likely to be detected using SAXS method. Thus, in further experiments one can check by SAXS whether the ligand binding affects oligomeric state and conformation of the protein. The difference between HDock models of tissue transglutaminase dimers is also detectable by SAXS, so it is easier to understand structural organization of the dimeric state of the protein. Future structural studies of tTG can also include a wide-angle X-ray scattering (WAXS) for more accurate assessment of the protein conformation.

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