The Role of Disulfide Bonds in Structural Stability and Dynamics of Human TFF1

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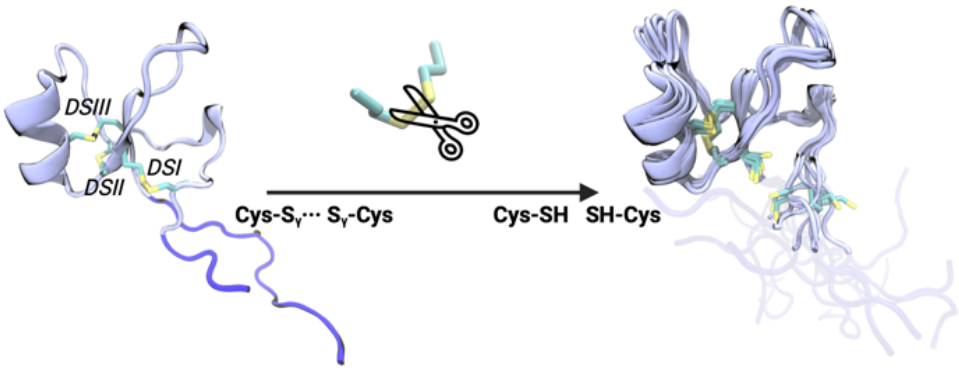
Mucosal epithelial cells secrete a variety of disulfide-rich small proteins, such as trefoil factors (TFFs), for the protection of the epithelial barrier and the maintenance of mucosal viscoelasticity. Human TFF1 is a small polypeptide consisting of sixty amino acids, primarily expressed in gastric surface mucous cells. TFF1 contains a highly conserved three-looped TFF domain stabilized by three intramolecular disulfide bonds between residues Cys7-Cys33, Cys17-Cys32, and Cys27-Cys44.[1] The distinctive spatial conformation of the TFF domain is associated with the adaptation of TFF proteins to a highly acidic environment and their resistance to thermal and proteolytic degradations.[2] However, the structural stability of TFF1 towards reduction and their relevance to its biological functions remained elusive. With this motivation, we performed microsecond-long atomistic molecular dynamics simulations to elucidate the role of the three disulfide bonds on the structural integrity and dynamics of the TFF domain. In human TFF1 these disulfide bonds were sequentially removed in all possible combinations (i.e. fully oxidized, mono-, di-, and fully reduced states). Our results show that, even though the removal of disulfide bonds induces some local alterations in the structure, the overall structural integrity and compactness of the domain remain almost unaffected. In particular, the inter-residue distance between Cys17 and Cys32 is preserved even when the disulfide bond is removed. This remarkable integrity of the TFF domain structure is attributed to the preservation of an extensive non-bonded interaction network within the domain. Despite the reduction of the disulfide bonds, the corresponding Cys residues are involved in additional interactions with nearby residues, further contributing to the domain stability.



[1] W. Hoffmann, *Int. J. Mol. Sci.*, **2020**, *21(12)*, 4535.
[2] S. Kjellev, *Cell Mol. Life Sci.*, **2009**, *66(8)*, 1350-1369.