Role of Disulfide Bonds in Proteins Examined by Molecular Dynamics Simulations

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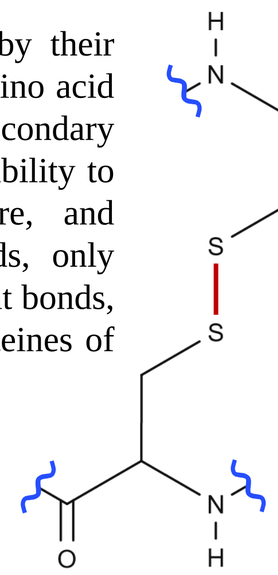
Disulfide Bonds

Role of proteins in nature is mainly determined by their structure, which is influenced by several factors: amino acid sequence (primary structure), presence of secondary structure elements, such as α-helices and β-sheets, ability to interact with each other to form 3D structure, and environment. Out of 20(21) standard amino acids, only cysteine can form disulfide bonds, which are covalent bonds, formed by oxidation of the thiol groups of two cysteines of inter- or intramolecular origin.



Why Study Them?

Disulfide bonds are present in more than 20% of known proteins, yet their function is still not fully understood. Disulfide bonds were believed to be responsible mostly for structural stability of proteins, but recent studies showed that it is not always true[1] and introduction of an additional disulfide bond may not increase stability,[2] or lack of one of native disulfides bonds may not reduce it.[3] On the other hand, presence of disulfide bonds can prevent enzymatic proteolysis, influence amyloidosis, be used as a method to control biochemical processes or impact viral efficiency.

Studied Proteins

In our studies we examined 3 different proteins: (i) the bovine pancreatic ribonuclease A (RNase A) (PDB code: 1KF5); (ii) the Lipid Transfer Protein (LTP) From Pea Pisum Sativum (PDB code: 2N81); (iii) second domain of Tumor necrosis factor receptor superfamily member 14 (HVEM) (from LIGHT-HVEM complex, PDB code: 4RSU). These systems were selected based on their unique properties: RNase is one of the most extensively experimentally researched proteins and it does not bind any ligand; LTP can be found in plants, and it can bind lipids, while HVEM is an immunologically important protein that can form complex with LIGHT protein.

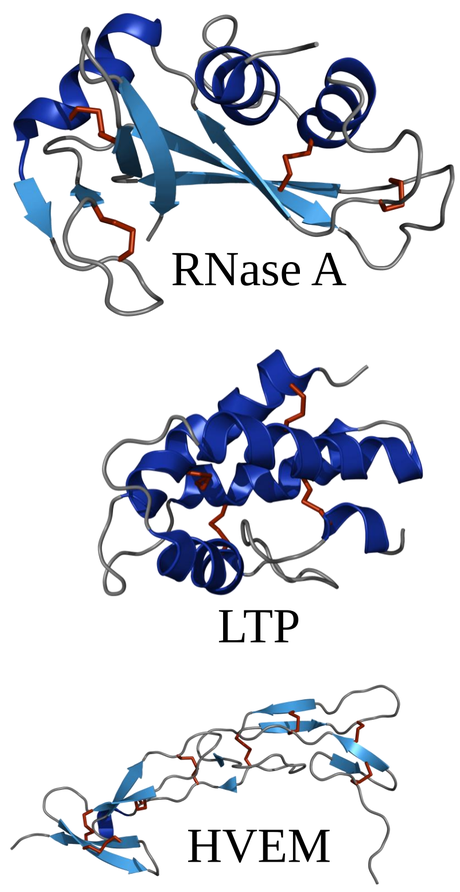


Figure 1. Cartoon representations of RNase A, LTP and HVEM. Disulfide bonds marked as red sticks, α-helices as navy and β-sheets as blue.

Methodology

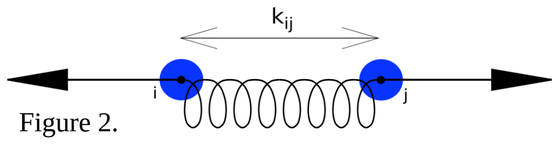


Figure 2.

Selected system were studied at two levels of resolution, all-atom and coarse-grained, to fully capture observed phenomena in various timescales. Our primary method of investigation is Molecular Dynamics (MD) and its variation - Steered MD (SMD), in which external force is applied (Fig 2.). All-atom simulations were performed with the use of all-atom AMBER package and coarse-grained simulations with UNRES force fields.

Results

Below we present representative results describing the role of disulfide bonds in RNase A [4] (Fig.3), CRD2 of HVEM [5] (Fig. 4 top) and LTP [6] (Fig. 4 bottom).

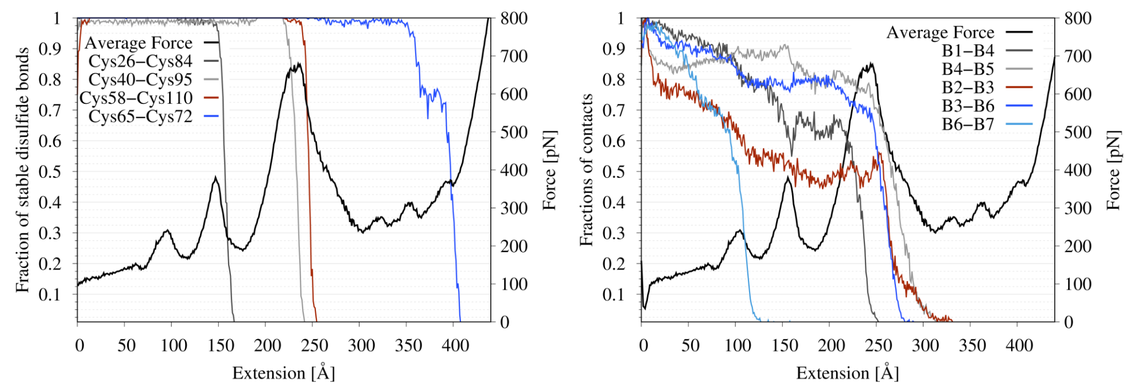


Figure 3. Fraction of stable: disulfide bonds (left panel) and β-sheets (right panel) during RNase A SMD simulations.

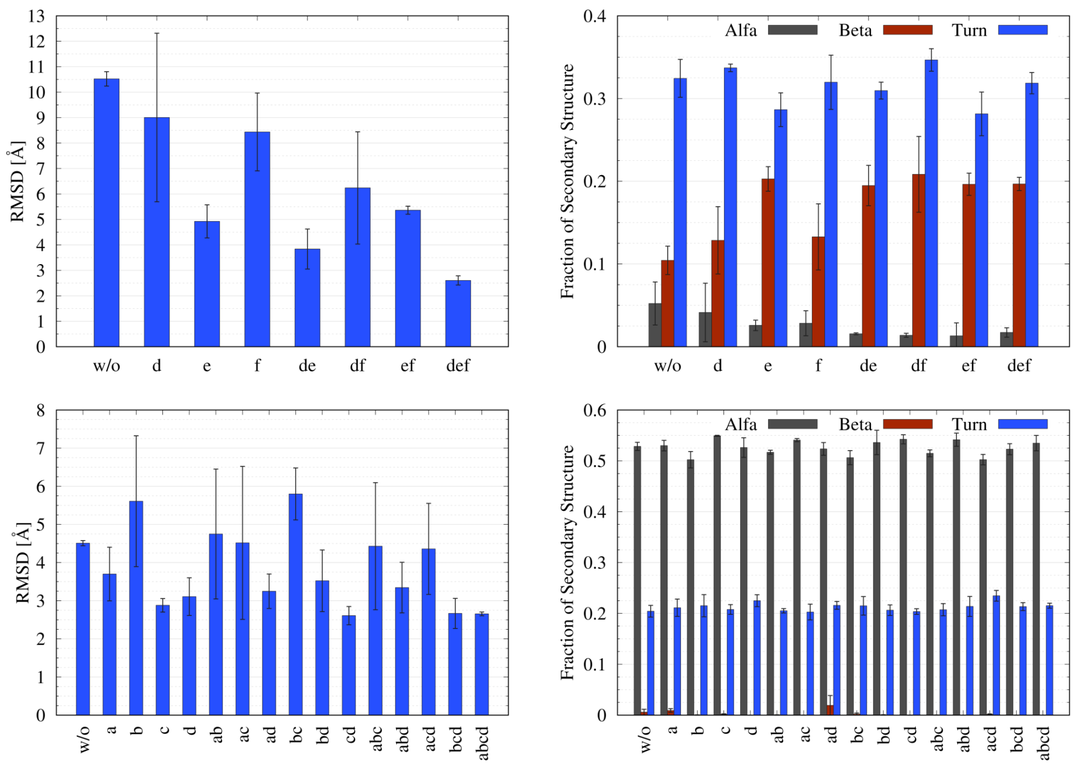


Figure 4. RMSD (left top panel) and fraction of secondary structures (right top panel) of CRD2 of HVEM. Letters d-f stand for different disulfide bonds present in native structure d: Cys40-Cys55, e: Cys58-Cys73, f: Cys61-Cys81. RMSD (left bottom panel) and fraction of secondary structures (right bottom panel) of LTP. Letters a-d stand for different disulfide bonds present in native structure a: Cys4-Cys53 b: Cys14-Cys30 c: Cys31-Cys76 d: Cys51-Cys90.

Disscusion & Conclusions

- Each disulfide bond in the protein can have different function and environment can impact it in a different manner.
- In the proteins and peptides of low structural stability, such as HVEM_CRD2, disulfide bonds can stabilize secondary structure (β-sheets).
- Some, but not all, of the disulfide bonds are usually important for proteins to maintain proper tertiary structure.
- Even for very stable proteins such as LTP, presence of at least one disulfide bond can further increase stability of the protein.
- Some of the disulfide bonds can destabilize the proteins by imposition of internal tension of non-local protein fragments.

What's next?

We plan to study how influence of disulfide bonds:

- impacts LTP stability in ligands binding,
- impacts LTP stability in presence of environmental factors: temperature, ions concentration, pH and lipid bilayer,
- impacts inhibitory properties of HVEM-LIGHT complex.

[1] Castellanos, M. M.; Colina, C. M. Molecular Dynamics Simulations of Human Serum Albumin and Role of Disulfide Bonds. *J. Phys. Chem.* B 2013, 117, 11895– 11905
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[3] Liu, H.; Schittny, V.; Nash, M. A. Removal of a conserved disulfide bond does not compromise mechanical stability of a VHH antibody complex. *Nano Lett.* 2019, 19, 5524– 5529
[4] Pamela Smardz, Adam K. Sieradzan, and Paweł Krupa, Mechanical Stability of Ribonuclease A Heavily Depends on the Redox Environment *J. Phys. Chem. B* 2022, 126, 33, 6240–6249
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