DEPARTMENT OF BIOLOGICAL SCIENCES

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Molecular insights into the structural stability and biological activity of T4 bacteriophage DNA polymerase processivity factor

The well-coordinated efforts of DNA polymerase (DNAP) and the accessory proteins result in the replication of DNA of an organism with high fidelity (errorless synthesis) and processivity (number of nucleotide added before 'falling-off'). Although DNAPs inherently have high fidelity, they require sliding clamp protein to enhance their processivity [1]. Sliding clamps are ring-shaped proteins that encircle DNA and function as DNA polymerase processivity factor. They are ubiquitously present in all living organisms. Notwithstanding their high significance, the molecular details of clamps pertaining to the factors contributing to their stability are presently lacking. The T4 bacteriophage gp45 sliding clamp is a homotrimeric molecule that not only assists in DNA replication, but also moonlights as a transcription factor. In this study, we have carried out a detailed characterization of gp45 to understand the role of structural hallmarks and molecular interactions involved in stability and functioning of the protein. Several biochemical and biophysical tools have helped us discover that the two domains present in gp45 display asymmetric characteristics. While the C-terminal domain shows stability and rigidity, we find that the N-terminal domain is unstable and flexible, which probably confers easy loading and stability on DNA [2]. Our findings explain how gp45 acts as a highly dynamic clamp. Tryptophan scanning mutagenesis experiments with gp45 allow us to conclude that the C-terminal domain of the protein undergoes a global conformational change at intermediate urea concentrations and adopts a molten globule state before complete denaturation. We demonstrate for the first time that protein's molten globule state could be visualized on Blue-Native PAGE and could also be trapped in vitro under native conditions, thus opening avenues for studying on-pathway folding/unfolding intermediates.

Additionally, we carried out a detailed analysis of the subunit-subunit interface of gp45 and identified one specific mutation, S88P that leads not only to monomerization, but also results in an unstable molecule. Very interestingly, two domains display an intermolecular cross-talk that governs structural stability and biological activity in gp45 [3]. Furthermore, we were able to uncouple the two biological activities of gp45 – replication and transcription – by means of site-directed mutagenesis; two of the mutations render gp45 inactive for T4 phage late promoter transcription, whereas strand-displacement DNA replication ability remains unaltered. We believe that our data will help in understanding the clamps behavior at molecular level to use them as drug target to combat bacterial infections and cancer by directly targeting the DNA replication.

References:

1. Kong XP, Onrust R, O'Donnell M, & Kuriyan J (1992) *Cell* 69:425-437.
2. Singh MI & Jain V (2016) *Biochemistry* 55:588-596.
3. Singh, MI, Ganesh, B, & Jain, V (2017) *Biochimica et Biophysica Acta* 1861:3300-3310.