A Novel Protocol for Protein Purification and a Conformational Study of Thermatoga maritima Rad50 Zn-hook via Heteronuclear Single Quantum Coherence Spectrometry

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DNA double strand breaks (DSBs) arise as a result of chromosomal replication errors or exposure to ionizing radiation or genotoxins. If unrepaired, DSBs may cause genomic instability and carcinogenesis. The Mre11-Rad50-Nbs1 (MRN) complex functions in detecting and repairing DSBs. Currently, insight into structure, dynamics, and functionality of MRN and Rad50 is limited. Herein, the Rad50 Zn-hook, a protein motif that tethers broken DNA ends during DSB repair, was investigated with the end goal of developing a model of MRN structure and dynamics in vivo. A novel, efficient protocol for purifying and concentrating residues 387-486 of Thermatoga maritima Rad50 Zn-hook expressed in E. coli was developed and tested successfully. In a short period of time, with relatively few steps, the protocol produces highly concentrated samples that yield high-resolution, highly sensitive NMR images. This protocol can be applied to other proteins with similar chemical properties to those of Rad50 Zn-hook. High resolution 2D 15N, 1H HSQC (heteronuclear single quantum coherence) NMR spectra confirmed that Zn-hook has a stable secondary structure, assumes multiple folded conformations in solution – likely a dimer conformation and a dimer-of-dimers conformation – and vacillates between these conformations rapidly in solution. Spectral evidence of protein aggregation supports the hypothesis that the binding of Zn2+ to the Zn-hook likely acts as a functional switch to facilitate MRN's DNA-repairing activity in vivo. CD (circular dichroism) spectroscopy confirmed that the Zn-hook has a highly alpha-helical structure. This experimentation lays the foundation for a model of Rad50 and MRN structural dynamics. Such a model can be used to drive novel approaches to cancer therapy and genomic repair.