Structural Analysis of the SBDS Protein Family

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Shwachman-Diamond Syndrome is an autosomal recessive disorder characterized by hematological dysfunction, pancreatic exocrine insufficiency and skeletal abnormalities. Disease-associated mutations were described in a gene designated SBDS that encodes a predicted protein of 250 amino acids. SBDS protein family occurs widely in nature, although its function has not been determined. Aiming at contributing to the understanding of the molecular function of SBDS protein we are performing structural studies on SBDS homologues from *Pyrococcus abyssi*, *Saccharomyces cerevisiae*, *Trypanosoma cruzi* and *Homo sapiens*. The respective genes were cloned and the proteins were overexpressed, purified and submitted to crystallization. SBDS is a three-domain protein and structural flexibility may be responsible for crystallization failure so far. This hypothesis was tested by limited proteolysis and mass spectrometry analyses, which showed that the N-terminal region is more flexible. Based on these results, we have constructed five truncated mutants of human SBDS in order to express the single domains or pairwise domain combinations. Expression assays are in progress. Interestingly, genome sequencing indicated that *T. cruzi* SBDS (TcSBDS) differs from its orthologs by presenting an extended C-terminal. Characterization of TcSBDS by spectroscopic methods (CD and NMR) and limited proteolysis revealed that the C-terminal extension is unfolded. We have shown by immunoblot analysis that endogenous TcSBDS is a ~50 kDa protein consistent with genome sequencing data.

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Key Words

SBDS, Structural Analysis, *Trypanosoma cruzi*