Structure of the Major Single-Stranded DNA-Binding Domain of Replication Protein A Suggests a Dynamic Mechanism for DNA Binding

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Abstract

We report here a 2.5 Å structure of the single-stranded DNA (ssDNA) binding domain of human replication protein A (RPA) (eukaryotic SSB), for which we previously reported a structure in complex with ssDNA. Multiple-wavelength anomalous dispersion data from seleno-methinonine protein crystals were collected at SBC CAT, APS. A comparison of free and bound forms of RPA revealed that ssDNA binding is associated with a major reorientation between, and significant conformational changes within, the structural modules — OB-folds — that comprise the DNA-binding domain. Two OB-folds, whose tandem orientation was stabilized by the presence of DNA, adopted multiple orientations in its absence. Within the OB-folds, extended loops implicated in DNA-binding significantly changed conformation in the absence of DNA. Analysis of intermolecular contacts suggested the possibility that other RPA molecules and/or other proteins could compete with DNA for the same binding site. Using this mechanism, protein-protein interactions can regulate, and/or be regulated by, DNA binding. Combined with available biochemical data, this structure also suggested a dynamic model for the DNA-binding mechanism.

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