Physical and functional characterization of distinct single-stranded DNA-binding subdomains in purine-rich element binding protein B (Purβ)

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Purβ is a ~34 kDa single-stranded DNA (ssDNA)-binding protein that represses transcription of the smooth muscle α-actin gene (*ACTA2*) in vascular and stromal cell types. Mechanistically, Purβ has been reported to interact with multiple sites in the purine-rich strand of a 5′ *ACTA2* enhancer sequence to form a high affinity 2:1 protein:ssDNA complex. In this study, the structural basis for site-specific ssDNA-binding and *ACTA2* repression was explored by evaluating the physical and functional properties of specific subdomains formed by putative intra- and intermolecular associations between homologous repeats I, II, and III of Purβ. Cell-based assays indicated that elaboration of full *ACTA2* repressor activity required all three repeats (Purβ I-II-III). Calibrated size exclusion chromatography validated that the intramolecular domain (Purβ I-II) exists as a monomer in solution, while Purβ I-II-III and the intermolecular domain (Purβ III) self-associate to form dimers. Comparative ligand binding assays revealed that the inter- and intramolecular subdomains exhibit quantitatively distinct ssDNA and transcription factor interaction properties relative to the full-length protein and Purβ I-II-III. Collectively, our findings suggest that the tripartite modular organization adopted by the Purβ dimer is uniquely suited for cooperative ssDNA interaction and consequent repression of *ACTA2* expression.