Structural characterization and stability analysis in the folding of the second Bromodomain of the BRD2 protein

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Bromodomains (BRDs) are conserved structural motifs involved in a variety of cellular processes such as chromatin remodeling, post-translational modifications or transcriptional control, acting as “reader” of histones acetylation sites. Mutations or chromosomal rearrangements affecting BRDs have been linked to cancer onset. Previous results have shown that BRD domains follow a three-state folding mechanism [1]. In this work, we aim to investigate in detail the structure of the folding transition state of the second bromodomain of BRD2 protein (BRD2d2), by exploiting site-directed mutagenesis and rapid folding kinetics experiments. For this purpose, we generated, expressed and purified, a set of 25 single mutants of BRD2d2, selecting the mutations in the perspective to maintain the native structure of the protein. The structural integrity of the mutants was tested by Circular Dichroism technique and by evaluating the thermal stability of every mutant. We obtained the thermodynamic stability for every mutant, performing urea-induced denaturation experiments at equilibrium condition, and subsequently we analyzed the (un)folding kinetics of BRD2d2 mutants by stopped flow experiments. We then calculated for each variant protein a parameter (phi value) derived from the equilibrium and kinetic data. Since the phi-value is a parameter expressing the destabilization of the folding transition state upon mutation, determining a complete set of phi values allows depicting the structure of the transition state for folding. Overall our analysis sheds light on the kinetic role of different residues that, in some cases, could be important not only during the protein folding pathway of BRD2d2, but also in the interaction with its binding partners.

[1] Petrosino M, Bonetti D, Pasquo A, Lori L, Chiaraluce R, Travaglini-Allocatelli C. (2017) “Unveiling the folding mechanism of the Bromodomains” Biochemistry and Biophysics Reports 11; 99-104.