

Characterization of the interaction between the Amphipathic Cell Penetrating Peptide p28 and the Wild Type and Mutated p53 by Raman and Atomic Force Spectroscopies combined with Surface Plasmon Resonance

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Keywords: Raman Spectroscopy, Atomic Force Spectroscopy, Docking, p53, Mutations, Cell penetrating peptide

Tumor suppressor p53 belongs to the class of intrinsically disordered proteins characterized by large unstructured regions. These proteins can adopt an ensemble of diverse, dynamically interchanging, and thermodynamic stable, secondary structure conformations [1]. This highly dynamic nature allows p53 to recognize a wide number of biological targets, without sacrificing specificity, acting as a central hub in a plethora of signaling pathways and playing a key role in the prevention of carcinogenesis [2]. Notably, p53 is often mutated in human cancers, chiefly in the DNA-Binding Domain (DBD), with drastic effects on its functionality; this making p53 an attractive target for anticancer strategies developing drugs addressed to restore its functions. p28, an amphipathic, nontoxic, cell-penetrating peptide, derived from the bacterial copper protein Azurin, can bind the DBD of wild type (WT) as well as of some mutants p53 through hydrophobic interaction inducing an increase of their intracellular levels and functionality, without altering the overall conformation [3][4]. In this context, the investigation of the structural properties of WT [5] and mutants p53 [6] could be useful to explore whether and how DBD punctual mutations can alter p53 conformations and, importantly, if the DBD alterations, in term of conformation and surface hydrophobicity, can correlate with the p28 binding affinity and capability; this aiming to refine the p28 peptide structure and to improve its ability to restore mutant to wild type p53 performance. Accordingly, we have combined Molecular Modeling, Raman spectroscopy coupled with a solvent perturbation strategy, Atomic Force Spectroscopy (AFS) and Surface Plasmon Resonance (SPR) studies to comprehensively characterize site-directed (L114D, A119D, C124D, and C229D) and naturally occurring (K164E, R273H and P223L/V274F) mutant DBDs, also in connection with their interaction with p28. We showed that mutations altering hydrophobicity within non-mutable and mutable regions of the p53-DBD can affect DBD secondary structure potentially reducing p28 binding. The affinity of p28 was positively correlated with the β -sheet content in naturally occurring mutants of DBD, being reduced by an increase of unstructured or random coil regions that resulted from a loss in hydrophobicity and redistribution of surface charge. The combination of the aforementioned techniques allowed us to conclusively demonstrate that p28 does bind to the DBD of p53 and confirm predictions of those binding motifs, providing an alternative approach to define how local alterations in secondary structure affect the binding of p28 to the p53-DBD. These results can help to refine our knowledge of the conformational properties of wild type and mutants p53 and their involvement in p28 binding, therefore getting insight on how potential structural modification in p28 can raise its anticancer activity [6].

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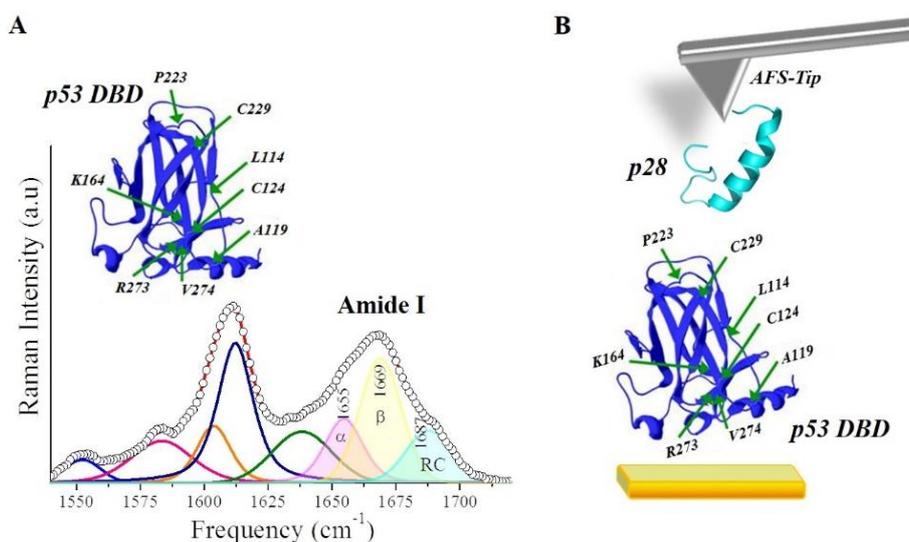


Figure 1. (A) Amide I Raman band (black circles) of wild type DBD fitted using three-component Voigt model (red line) to obtain the structural content of the main conformations present in the protein: α -helix (magenta band), β -sheet (yellow band) and random coil (blue band). The same fitting analysis was applied on both the site-directed and naturally occurring DBD mutants. (B) Schematic sketch of the AFS approach employed to study the interaction of p28 with both the site-directed and naturally occurring DBD mutants.