CHARACTERIZATION OF WILD-TYPE P53 AND MUTANT R248Q INTERMEDIATE STATES

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p53 is a transcription factor that maintains the genome integrity. Its function is lost in 50% of human cancers; the majority of the mutations is clustered in the core domain, where mutations in the R248 residue are the most frequent. p53 has a flexible structure that allows it to acquire distinct conformations. We investigated if wild-type (WT) p53 and R248Q acquire a "molten-globule" state (MG) by analyses at different pHs. At low pHs, the fluorescence data demonstrate exposure of aromatic residues and circular dichroism measurements show that these proteins maintain a substantial amount of secondary structure. Moreover, the binding of WT p53 and R248Q to the compound 8-anilino-2-naphthyl sulfonic acid indicate exposure of hydrophobic regions. We have worked at pH 5.0 and compared with the results at pH 7.2. Our data show that the high-pressure effect on WT and mutant p53 MG state is reversible and does not lead to protein aggregation, in contrast with the results at pH 7.2. In addition, we observed that upon chemical denaturation, these proteins presented different unfolding profiles at both pHs. Curiously, incubation at high temperature (up to 55^{o}C) maintained the secondary structure content and lead to formation of a WT p53 aggregate. Further studies about the flexibility of p53 may help to explain its folding.
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