Engineering of Human Cancer-related Proteins for NMR spectroscopy

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Proteins are involved in both physiological and pathological process, including cancer. Using the SAGE anatomical viewer tool, several cancer-related proteins could be identified by the analysis of their gene expression level in normal and neoplasic human tissues. Once the structural caracterization of these proteins is going to be performed by NMR (Nuclear Magnetic Resonance) spectroscopy, others factors were taken into account for target selection: no significant similarity of amino acid sequence with proteins that had their 3D structure determined, molecular weight less than 25 kDa and no predicted transmembrane segments. Among these targets are C1D and BTF3 whose cDNAs were subcloned into pET43-1.c for *Escherichia coli*. C1D is a protein that participates in apoptosis and BTF3 is a transcriptional factor, both with relevance in various types of cancer. The purification by gel filtration of C1D showed that this protein behaves like a dimmer. Furthermore, the NMR spectra presented several characteristic signals of unfolded regions, which precluded further analysis of its structure. BTF3 had already been solubly produced in *E. coli* but its purification was not possible most likely due to the formation of aggregates. Thereby, we decided to verify for the possibility of truncating these proteins in order to decrease the amount of intrinsically disordered regions (c-termini according to the PONDR algorithm) and consequently avoid protein aggregation due to exposed hidrophobic residues. Truncated C1D and BTF3 were engineered by PCR. Following, we selected the best clone (among 6) and the best expression conditions to give the highest level of soluble recombinant protein production for subsequent purification. The soluble levels of the truncated C1D was highly improved when comparated with wild type.
Support: FAPERJ and CNPq.