Characterization of the External Domain of Human TOM70, an Outer Membrane Translocase in Mitochondrial Protein Import

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Mitochondria accomplish a great variety of metabolic processes in eukaryotic cells. These organelles are involved in processes related to cell life and death, and therefore also in tumoral transformation. At molecular level these processes have to be executed by a large number of different proteins, of which the majority being nuclear encoded, synthesized as proteins precursors on cytosol, and imported into the organelle via translocation complexes in the outer and inner mitochondrial membranes. Mitochondrial precursor proteins contain targeting signals that are recognized by receptors of the *t*ranslocase of the *o*uter mitochondrial *m*embrane (*TOM* complex). TOM70 is the major surface receptor in TOM complex, it is a protein composed of multiple tetratricopeptide repeat (TPR) motifs related to those found in a range of other co-chaperones of Hsp90 and Hsp70 which provide a specific binding site for the Hsp90 and Hsp70 chaperones. Interaction with cytosolic chaperones is considered an essential first step in TOM70-dependent protein precursor targeting followed by contact between the precursor and TOM70 itself. Here we present the purification and biophysical characterization of the human cytosolic region of TOM70 (residues 111 to 680, recombinant). Circular dichroism and emitted fluorescence spectroscopy indicated that the protein is well folded and stable, CD showed a protein with approximately 65% of α-helices and thermal-induced unfolding with a Tm of 51°C. The quaternary structure was investigated by analytical ultracentrifugation, dynamic light scattering, analytical gel filtration and blue native gel indicating that the domain is a monomer with an elongated shape. Pull-down assays validate the interaction between Hsp90 and TOM70 and isothermal titration calorimetry studies are underway to characterize this interaction.