Hop (Hsp 70-Hsp90 Organizing Protein) Co-chaperone is Monomer in Solution

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Molecular chaperones are important proteins involved in the quality control of cellular processes. The most important molecular chaperone is Hsp70 that helps nascent proteins to reach native conformation and forms a pivot system that delivers partially folded proteins to other molecular chaperones. A relevant function of Hsp70 is to form a foldase system with Hsp90 that activates several proteins involved in apoptosis, signal-transduction pathways and cell-cycle regulation, which are usually deregulated in cancers. As a consequence, the functional understanding of this system has been considered of remarkable importance because it is an appealing target for cancer therapeutics. The interaction between Hsp70 and Hsp90 is mediated by the co-chaperone Hop (Hsp70-organizing protein) which is also involved in substrate binding and here we present the cloning, purification and biophysical characterization of the human protein. The recombinant protein was purified by affinity chromatography, ion-exchange and gel filtration. The protein was purified folded as shown by intrinsic emitted fluorescence (single Triptophan: $?_{max}$= 339 ± 1 nm and $\langle l \rangle$= 345 ± 1 nm) and circular dichroism (alpha-helical spectrum, 64% according to calculations) spectroscopies. Thermal-induced unfolding measurements showed that Hop unfolded through a sigmoidal transition curve with Tm = 54.0 ± 0.1 ºC and reversible refolded to its native conformation if heated to 60 ºC. Hydrodynamic experiments (Dynamic Ligth Scattering, Analytical Gel Filtration, Analytical Ultracentrifugation and Blue Native Gel) suggested that Hop is a monomer in solution ($D = 5.4x10^{-7} cm^2/s$, Stokes radius = 31.9 Å ± 2.0, Molecular mass = 66.400 kDa).

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