Investigating the Interaction of the Inhibitor Novobiocin with Hsp90, a Molecular Target Against Cancer

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The 90 kDa heat shock protein (Hsp90) family is integrally involved in cell signaling, proliferation, and survival, and is ubiquitously expressed in cells. Many proteins in tumor cells are dependent upon the Hsp90 protein folding machinery for their stability, refolding, and maturation. Inhibition of Hsp90 uniquely targets client proteins associated with all hallmarks of cancer. Thus, Hsp90 has emerged as a promising target for the treatment of cancer. Hsp90 exists as a homodimer, which contains three domains. The N-terminal domain contains an ATP-binding site that binds the natural products geldanamycin and radicicol. The middle domain is highly charged and has high affinity for co-chaperones and client proteins. Several studies suggested a second ATP-binding site in the C-terminus of Hsp90, a domain that can also bind other small molecules, like novobiocin. Here, we present the purification of the recombinant C-terminal domain of Hsp90α and the initial tests of its binding with novobiocin. The folded state of the recombinant protein was investigated by circular dichroism and intrinsic emitted fluorescence showing that the protein is stable. However, the presence of novobiocin seemed to affect both the stability, as investigated by thermal-induced unfolding experiments, and the conformation of the domain. Pull-down experiments and calorimetric assays are underway to investigate the thermodynamic parameters of novobiocin-CHsp90 interaction.