CALORIMETRIC ANALYSIS OF THERMODYNAMIC STABILITY OF THE ISOLATED BIR3-XIAP DOMAIN AND ITS COMPLEX WITH AVPI-SMAC PEPTIDE

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The X-linked inhibitor of apoptosis protein (XIAP) is a potent inhibitor of apoptosis, which has an essential role in the homeostasis of all multicellular organisms. The development of new drugs that block BIR3 domain of XIAP is a promising strategy for inhibiting the antiapoptotic activity of XIAP and for overcoming apoptosis resistance of cancer cells. The N-terminal tetrapeptide AVPI from Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP-binding protein with low pI) can bind and block BIR3 domain action. Herein, calorimetric techniques, fluorescence spectroscopy and circular dichroism were used to investigate changes in thermodynamic stability of BIR3 domain and BIR3-AVPI complex. Our results showed that BIR3 is very stable to high temperature and chemical denaturing agents. DSC data indicate that BIR3 domain unfolds via two-state and that AVPI stabilizes BIR3 domain increasing the melting temperature of the second state by around 9°C. BIR3-AVPI binding parameters were obtained by ITC which reveals an exothermic reaction with prevalence of hydrophobic interactions. All these thermodynamic data provide essential information about Smac/DIABLO-XIAP interaction. The understanding of this process is very important for the design of anticancer drugs.

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