Enhanced protein stability through disulfide engineering

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The CSK-homologous kinase (CHK) is an endogenous inhibitor of Src-family protein tyrosine kinases (SFKs). Excessive SFK activity contributes to cancer formation and progression, thus endogenous SFK-inhibitors play a vital tumour-suppressor function in normal cells (reviewed in Chong *et al.,* 2005). The cellular function of CHK is modulated by its subcellular localisation, which is controlled by the ligand-binding properties of its Src homology-2 (SH2) and Src homology-3 (SH3) domains. For some years we have been studying the structure and function of CHK, including NMR studies of the SH2 and the SH3 domains (Mulhern *et al.,* 2002; Chong *et al.,* 2004; Chong *et al.,* 2006). Our initial attempt at NMR analysis of the CHK SH3 domain was hampered by the intrinsic instability of the recombinant protein, which resulted in slow irreversible unfolding at ambient temperatures. We adopted a protein engineering approach to generate a CHK SH3 construct more suitable for structural and functional analysis. We have successfully enhanced the stability of the CHK SH3 domain through an engineered disulfide bond. The mutant protein (DS-SH3) has a melting temperature (T_m) more than 20°C higher than that of the wild type SH3 domain. This increase in T_m is considerably greater than that reported for other comparable examples of disulfide engineering. The structural integrity of the domain was confirmed by circular dichroism (CD) spectropolarimetry and nuclear magnetic resonance (NMR) spectroscopy. A full-length version of CHK carrying the DS-SH3 mutations was expressed in insect cell culture and purified for functional analysis.

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