

Cytoglobin, a recently discovered hemeprotein, is part of a globin protein family including hemoglobin, myoglobin, and neuroglobin. Numerous studies have reported the role of cytoglobin in tissue protection as well as a tumor suppression. However, the molecular mechanism of how cytoglobin carries out its protective functions under physiological and pathological conditions remains unclear. The cytoglobin structure includes a globin core that is homologous to myoglobin and a 20 amino acid residue extension at the N terminus and the C terminus that is unique among vertebrate globins. This study aims to provide a molecular insight into the cytoglobin folding mechanism with a focus on the role of N- and C- terminal extensions in modulating protein stability and its folding mechanism. The protein stability will be monitored by exposure of wild type (WT) cytoglobin and cytoglobin construct missing the C-terminal extension ( $\Delta$ C cytoglobin) to an increasing concentration of guanidine hydrochloride (GuHCl). The changes in the secondary and tertiary protein structure will be monitored by circular dichroism and steady-state fluorescence of intrinsic tryptophan residues, respectively. CD spectra experiments indicate that WT cytoglobin folding proceeds as a two state mechanism ( $F \leftrightarrow U$ ) with midpoint GuHCl concentration of 3.5 M. Analogous results were obtained by steady-state fluorescence experiments. This study will provide new insights into the structure - function relationship of newly discovered cytoglobin and the importance of its C- and N- terminals in protein stability and its folding mechanism. The information provided in this study adds to the growing amount of data on cytoglobin and provides structural data that can be of future use in discovering the overall molecular function of cytoglobin.