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| Title: | Design of a soluble mini-protein through tandem duplication of the minimally engineered beta hairpin 'tongue' motif of alpha-hemolysin |
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| Abstract: | In an attempt to fashion a globular protein out of two conjoined beta hairpin structural motif(s), we created a gene encoding, in tandem, two copies of the 40 residues-long transmembrane beta hairpin tongue (BHT) motif of the pore-forming toxin, alpha-hemolysin, of Staphylococcus aureus. Seven selected hydrophobic residues on each copy of the BHT motif's lipid-facing surface were mutated to hydrophilic residues, to prevent or reduce any non-specific aggregation based on hydrophobic interactions. Tandem BHT turned out to be expressed as a soluble polypeptide which could be raised to concentrations of ~2 mg/ml. It displayed several characteristics of a folded mini-protein, although not the characteristics of a typical well-folded globular protein. These characteristics include (i) far-UV CD and FTIR spectra indicative of the presence of sheet structure mixed with polyproline type II secondary structure, (ii) a near-UV CD spectrum, indicating some formation of tertiary structure, (iii) evidence of unfolding and dissociation transitions in the presence of denaturants, accompanied by increase in random coil content, and (iv) the ability to transform from sheet to helical structure through a biphasic structural transition in the presence of the cosolvent, trifluorethanol. Importantly, however, tandem BHT displayed no cooperativity during unfolding; taken together with the poor structural content revealed in the far-UV CD spectrum and some non-canonical gel filtration behavior seen in the presence of denaturants, this suggests a partially unsuccessful instance of protein design. |
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