Title:	Folding behavior of a backbone- reversed protein: Reversible polyproline type II to β-sheet thermal transitions in retro-GroES multimers with GroES-like features
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Abstract:	The structural consequences of the reversal of polypeptide backbone direction (retro modification) remain insufficiently explored. Here, we describe the behavior of an engineered, backbone-reversed form of the 97 residues-long GroES co-chaperonin of Escherichia coli. FTIR and far-UV CD spectroscopy suggest that retro-GroES adopts a mixed polyproline type II (PPII)-beta-strand structure with a β II type CD spectrum similar to that of GroES. Gel-filtration chromatography reveals that the protein adopts trimeric and/or pentameric quaternary structures, with solubility retained up to concentrations of 5.0-5.5 mg/ml in aqueous solutions. Mutations inserting a single tryptophan residue as a spectroscopic probe at three different sites cause no perturbation in the protein's CD spectral characteristics, or in its quaternary structural status. The protein is cooperatively dissociated, and non-cooperatively unfolded, by both guanidine hydrochloride and urea. Intriguingly, unlike with GroES, retro-GroES is not unfolded by heat. Instead, there is a reversible structural transition involving conversion of PPII structure to β sheet structure, upon heating, with no attendant aggregation even at 90°C. Retro-GroES does not bind GroEL. In summary, some structure-forming characteristics of GroES appear to be conserved through the backbone reversal process, although the differential conformational behavior upon heating also indicates differences.
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