Title:	Hyperthermophile protein behavior: Partially-structured conformations of Pyrococcus furiosus rubredoxin monomers generated through forced cold-denaturation and refolding
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Abstract:	Some years ago, we showed that thermo-chemically denatured, partially-unfolded forms of Pyrococcus furiosus triosephosphateisomerase (PfuTIM) display cold-denaturation upon cooling, and heat-renaturation upon reheating, in proportion with the extent of initial partial unfolding achieved. This was the first time that cold-denaturation was demonstrated for a hyperthermophile protein, following unlocking of surface salt bridges. Here, we describe the behavior of another hyperthermophile protein, the small, monomeric, 53 residues-long rubredoxin from Pyrococcus furiosus (PfRd), which is one of the most thermostable proteins known to man. Like PfuTIM, PfRd too displays cold-denaturation after initial thermo-chemical perturbation, however, with two differences: (i) PfRd requires considerably higher temperatures as well as higher concentrations of guanidium hydrochloride (Gdm.HCl) than PfuTIM; (ii) PfRd's cold-denaturation behavior during cooling after thermo-chemical perturbation is incompletely reversible, unlike PfuTIM's, which was clearly reversible (from each different conformation generated). Differential cold-denaturation treatments allow PfRd to access multiple partially-unfolded states, each of which is clearly highly kinetically-stable. We refer to these as 'Trishanku ' unfolding intermediates (or TUIs). Fascinatingly, refolding of TUIs through removal of Gdm.HCl generates multiple partially-refolded, monomeric, kinetically-trapped, non-native 'Trishanku' refolding intermediates (or TRIs), which differ from each other and from native PfRd and TUIs, in structural content and susceptibility to proteolysis. We find that the occurrence of cold denaturation and observations of TUI and TRI states is contingent on the oxidation status of iron, with redox agents managing to modulate the molecule's behavior upon gaining access to PfRd's iron atom. Mass spectrometric examination provides no evidence of the formation of disulfide bonds, but other experiments suggest that the oxidation status of iron (and its extent of burial) together determine whether or not PfRd shows cold denaturation, and also whether redox agents are able to modulate its behavior.
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