Title:	Partial destabilization of native structure by a combination of heat and denaturant facilitates cold denaturation in a hyperthermophile protein
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Keywords:	Guanidine Recombinant enzyme Triosephosphate isomerase,
Issue Date:	2008
Publisher:	Wiley-Liss, Inc.
Citation:	Proteins: Structure, Function and Genetics, 72 (2), pp. 539-546.
Abstract:	Cold denaturation is a phenomenon seen in many different proteins. However, there have been no reports so far of its occurrence in hyperthermophile proteins. Here, using a recombinant triosephosphate isomerase (PfuTIM) from the hyperthermophile archaeon, Pyrococcus furiosus, we show that the heating of this protein through the low temperature side of its thermal unfolding transition in the presence of guanidinium hydrochloride (GdmCl) results in the formation of partially-disordered conformational ensembles that retain considerable native-like secondary and tertiary structure. Unlike PfuTIM itself, these thermochemically obtained partially-disordered PfuTIM ensembles display cold denaturation as they are cooled to room temperature. The protein thus shows hysteresis, adopting different structural states in a manner dependent upon the nature of the heating and cooling treatment, rather than upon the initial and final conditions of temperature and GdmCl concentration, indicating that some sort of a kinetic effect influences structure adoption and retention. The structure lost through cooling of partially-disordered PfuTIM is found to be regained through heating. The ability of GdmCl to thus apparently destabilize the highly thermodynamically and kinetically stable structure of PfuTIM (sufficiently, to cause it to display observable cold-denaturation and heat-renaturation transitions, in real-time, with cooling and heating) offers support to current ideas concerning the how hyperthermophile proteins achieve their high kinetic stabilities, and suggests that desolvation-solvation barriers may be responsible for high kinetic stability.
Description:	Only IISERM authors are available in the record.
URI:	http://onlinelibrary.wiley.com/doi/10.1002/prot.22077/pdf (http://onlinelibrary.wiley.com/doi/10.1002/prot.22077/pdf)
Appears in Collections:	Research Articles (/jspui/handle/123456789/9)

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