Title:	Introduction of a thermophile-sourced ion pair network in the fourth beta/alpha unit of a psychophile-derived triosephosphate isomerase from Methanococcoides burtonii significantly increases its kinetic thermal stability
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Abstract:	Hyperthermophile proteins commonly have higher numbers of surface ionic interactions than homologous proteins from other domains of life. PfuTIM, a triosephosphate isomerase (TIM) from the hyperthermophile archaeon, Pyrococcus furiosus, contains an intricate network of 4 ion pairs in its 4th beta/alpha unit, (β/α)4, whereas MbuTIM, a triosephosphate isomerase from a psychrophile archaeon, Methanococcoides burtonii, lacks this network. Notably, (β/α)4 is the first element of the structure formed during folding of certain TIM-type (beta/alpha)8 barrel proteins. Previously, we have shown that elimination of PfuTIM's ion pair network in PfuTIM significantly decreases its kinetic structural stability. Here, we describe the reciprocal experiment in which this ion pair network is introduced into MbuTIM, to produce MutMbuTIM. Recombinant MbuTIM displays multi-state unfolding with apparent Tm values of autonomous structural elements approaching, or above, 70 C, when a temperature scanning rate of 90 C/h is used. The protein displays significant intrinsic kinetic stability, i.e., there is a marked temperature scan rate-dependence of the Tm values associated with unfolding transitions. The Tm values drop by as much as ~ 10 C when the temperature scanning rate is lowered to 5 C/h. MutMbuTIM, incorporating PfuTIM's ion pair network, shows significantly higher apparent Tm values (raised by 4-6 C over those displayed by MbuTIM). MutMbuTIM also displays significantly higher kinetic thermal stability. Thus, it appears that the thermal stability of triosephosphate isomerase can be increased, or decreased, by either enhancing, or reducing, the strength of ion pair interactions stabilizing (β/α)4, presumably through reduced cooperativity (and increased autonomy) in unfolding transitions.
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