The HHP-NMR unfolding study of the yeast Frataxin Yfh1 at variable temperature gives essential clues on the heat- and cold-induced denaturation of the protein.

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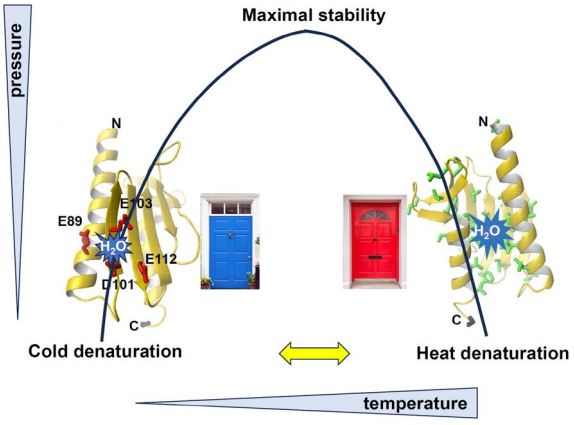
Abstract

Proteins unfold under different environmental insults, among which are heat, cold, high pressure and chaotropic agents. Understanding the mechanisms that determine unfolding under each of these conditions is an important problem that directly relates to the physical forces that determine the three-dimensional structure of a protein. Here, we studied the unfolding transitions of the marginally stable (in the absence of salt) yeast Frataxin Yfh1 using high-hydrostatic pressure nuclear magnetic resonance (HHP-NMR) at variable temperature: this technique is uniquely suited for protein unfolding studies at a residue-level resolution [1-3] , providing local information on the behaviour of different regions of a protein. We compared the cold, heat and pressure unfolded states. We show that the pressure-induced unfolding pathways at low and high temperatures differ [4], suggesting a synergic mechanism between pressure- and temperature-induced denaturation. Our observations help us to reconstruct the structural events determining unfolding and distinguish the mechanisms that rule the different processes of unfolding.



Cartoon showing the forces determining thermal unfolding at low and high temperature under mild pressures. The structure of Yfh1 is plotted to visualize where water starts entering in the two conditions symbolized as two different virtual doors (indicated in blue and red for cold and heat unfolding): at low temperature, where hydrophobic forces are weaker, the electrostatic repulsion between side chains near the N-terminus of the protein will determine opening of the structure, whereas at high temperature the partial exposure of the hydrophobic core due to the shorter C-terminus allows the structure to unfold starting from the C-terminus. The process becomes completely cooperative at high pressure and it is not longer possible to distinguish the two pathways..

References

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[4] Roumestand C et al. (2025) JACS Au. 5 (4):1940-1955. doi: 10.1021/jacsau.5c00185.)