Understanding the thermostabilization of citrate synthase using Constraint Network Analysis

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Citrate synthase (CS) is a homodimeric enzyme which catalyzes the first step of the TCA cycle – the conversion of oxaloacetate to citrate employing acetyl-CoA. Being a central enzyme in carbohydrate metabolism, CS is found in nearly all life forms. CS is one of the rare proteins for which crystal structures are available in the PDB from organisms living at temperatures from as extreme as 0°C to 100°C. To understand structural implications that lead to such a range of thermostability of CS, we apply Constraint Network Analysis (CNA) on five citrate synthase (CS) structures over a temperature range from 37°C to 100°C. CNA is a front-and-backend for the FIRST software [1] for characterizing mechanical rigidity (and flexibility) of proteins modeled as networks of atoms (sites) connected by covalent and non-covalent interactions (edges). [2-4] In the present study, for the first time, we introduce an ensemble-based variant of CNA in order to circumvent the sensitivity of the method on the input structure. Furthermore, we model the temperature dependence of hydrophobic interactions in the constraint network as hydrophobic interactions strengthen at higher temperatures.

From a macroscopic point of view, a very good correlation between the predicted thermostabilities of CS and optimal growth temperatures of their source organisms ($R^2 = 0.88$, $p = 0.017$) is obtained, which validates that CNA is able to quantitatively discriminate between less and more thermostable proteins even within a series of orthologs. From a microscopic point of view, the top 5% structural weak spots predicted by CNA on a less thermostable CS show a higher mutation ratio in the corresponding more thermostable CS than other sequence positions. Furthermore, highly ranked weak spots that are otherwise highly conserved in a multiple sequence alignment of CSs are nevertheless found to be mutated in the next more stable CS in the series of structures analyzed. Finally, while deducing the mechanisms at an atomic level that lead to reinforcement of weak spots, we observe that the thermophilic CSs incorporate a better hydrogen bonding in order to achieve a higher thermostability whereas hyperthermophilic CSs employ more hydrophobic contacts for the same. All in all, these findings suggest that CNA can be applied as a pre-filter in data-driven protein engineering for suggesting residues that, when mutated will more likely improve thermostability. [5]

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