Thermostability and Protein Structural Networks: The Role of Hub Residues

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INTRODUCTION: Protein structural network (PSN) analysis has recently been proven to be a significant tool for analysis of protein tertiary structure and detection of important residues to protein function, stability, folding and allostery. In this system, the residues are called nodes, and the interactions between residues are called connections. So, the complete protein tertiary structure is transformed in a network. Despite of proteins have hundreds of residues, the PSN has shown that just a few of them, called hub residues, are important to shorten the pathways linking any two residues over the protein structure.
OBJECTIVE: The aim is to evaluate whether the hubs indeed underlie protein properties, as stability and function.
MATERIALS AND METHODS: The *Spodoptera frugiperda* beta-glucosidase (Sfbglu, 509 residues) was chosen as a model, and its PSN was calculated. There have been founded 11 hubs. To analyze the importance of them, mutant enzymes were produced replacing the hub by alanine. Besides, mutant enzymes were also produced with replacements planned to the environment of the F251 hub. The stability and function of all mutant Sfbglu were assayed.
RESULTS AND DISCUSSION: Circular dichroism and thermofluor assays have shown that Sfbglu mutated in hubs have melting temperature (Tm) 2 to 5 degrees lower than wild type Sfbglu. These results indicate that hubs are important in the thermostability of the enzyme. Besides that, mutations in residues close to F251 hub also affected the Tm. Specifically, the closest ones, V336F and A264F, caused the largest effect, reducing the Tm in approximately 10°C and abolishing the cooperative transition of unfolding. With respect to the function of the enzyme, we have not observed, so far, significant change on the catalytic parameters (k_{cat} and K_m) of the mutant Sfbglu.
Conclusion: The experimental demonstration has shown that hubs indeed underlie protein functional properties, mainly the thermostability.
Keywords: beta-glucosidase; protein structural network; thermostability
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