Study of the Correlation between Structural Network and Structural and Catalytic Properties of Beta-glucosidases

SOUZA, V. P.; ARANTES, G. M., MARANA, S. R.
Departamento de Bioquímica, IQ, USP, SP, Brazil

Introduction and Aims: The protein structure can be represented as a network in which residues of amino acids are the nodes and their interactions are edges or connections. The central nodes contribute to shorter paths (sets of connections) between different nodes on the network. Thus, it has been suggested that central nodes made important contributions in various enzymatic properties, as stability, allostery and catalytic activity. Additionally, it has been suggested that the structural protein network is "robust", that is, random mutations tend to reach the non-central nodes and therefore cause only local effects that do not propagate damaging the overall structure of the protein. On the other hand, mutations directed to central nodes or their proximity may easily diffuse and significantly modify the protein structure. The aim of this project is to test this hypothesis in experiments designed to determine catalytic and stability parameters for the β-glucosidase Sfbglu.

Results and Conclusion: The mutant Sfbglu were constructed via site-directed mutagenesis kit (Stratagene) and sequenced. WT and mutant Sfbglu were produced as recombinant protein in E. coli BL21 gold DE3 using vector pET46 and purify using affinity chromatography in Ni-NTA resin. The homogeneity of these samples was verified by SDS-PAGE. Furthermore, the structural properties of the Sfbglu WT were initially characterized. For this, we collected circular dichroism and tryptophan intrinsic fluorescence spectra. Moreover, the enzyme stability was evaluated using guanidinium chloride as denaturant agent. The thermal stability of WT Sfbglu was studied by determining the remaining activity after incubation at 45°C and by differential scanning fluorimetry experiments. The kinetics of Sfbglu refolding after dilution of the denaturation agent (guanidinium chloride) was also evaluated. Therefore, these experiments set up an overall picture of the structural stability for WT Sfbglu, which will be a reference for comparison to mutant Sfbglu.

Keywords: beta-glucosidase, network, structural studies

Supported: CNPq and FAPESP