NMR Solution Structure Determination of a Psychrophilic Thiol-Disulfide Oxidoreductase

CollinsT , Santos H., Matzapetakis M

ITQB - Inst. Tecn. Química e Biológica, Univ. Nova de Lisboa, Oeiras, Portugal

Psychrophilic enzymes produced by cold-adapted micro-organisms have successfully overcome the low temperature challenge and have adapted to maintain high catalytic rates in their permanently cold environments [1]. The current consensus is that this high activity at low temperatures is mainly achieved through an increase in the flexibility of the protein structure, thereby allowing for the molecular motions necessary for activity in the low thermal energy environment. Nevertheless, while the demonstrated decreased stability and high activity at low temperatures of psychrophilic enzymes does support this hypothesis, there is, as yet, no direct experimental evidence of an increase in flexibility. In effect, the flexibility of a protein is a difficult parameter to assess since it may be related to the frequency or the amplitude of the fluctuations and limited to only a specific part of the protein, in particular at or near the active site. Previous attempts to demonstrate this proposed increased flexibility of psychrophilic enzymes used approaches such as measurement of hydrogen/deuterium exchange rates, fluorescence quenching, neutron scattering and even molecular dynamics simulation studies, with variable and conflicting results being obtained. We are using NMR to probe the flexibility of a thiol-disulfide oxidoreductase (DsbA) from an Antarctic bacterium as this technique offers the advantage of allowing analysis of both local motions and global movements over various time scales. The gene encoding the psychrophilic enzyme (187 amino acids) was isolated, cloned and overexpressed in *E. coli* and the $^{15}N^{13}C$-labeled protein was purified from the periplasmic extracts. Here, the preliminary results for the determination of the solution structure of the reduced form of this cold adapted oxidoreductase at pH 7.2 and 25 ºC will be presented as well as the future strategies to investigate the flexibility of its structural edifice.

[1] D'Amico, S., Collins, T., Marx, J.C., Feller, G. and Gerday, C. (2006) Psychrophilic microorganisms: challenges for life. *EMBO Rep.*, **7**, 385-389.