Abstract

The aim of this project was to construct a psychro-active thermostable chimeric protein by transplanting the postulated catalytic and substrate contacting residues from a thermolabile, cold adapted (β/α)8 barrel xylanase onto the core scaffold of a homologous, thermostable xylanase. The question driving this study was to understand whether individual secondary structures are capable of folding autonomous of the rest of the protein. Thus the idea was to provide certain loops that assemble to form the catalytic site in one enzyme (the cold adapted C. adeliae xylanase), the opportunity to undergo folding and assembly in a different (but structurally homologous) thermostable enzyme (the NG-27 xylanase), to examine whether the catalytic site has folded correctly. Moreover, the combination of a psychro-active thermostable xylanase which does not exist in nature however, might find use in paper and pulp industry, owing to its dual quality of being thermostable as well as active at room temperature conditions. The catalytic sites in most β/α barrel proteins are harboured in the β- loop-α units. Since the crystal structure of the psychrophilic xylanase was not known, these random loops were demarcated employing a rational approach which used the pair wise sequence alignment of the two proteins and the crystal structure of the NG-27 xylanase. These β-loop-α units were then exchanged with their known counterparts in NG-27 xylanase and incorporated into the gene sequence of this protein. This was done through a series of splicing by overlapping extension (SOE) polymerase chain reactions. Even though the recombinant gene containing the foreign sequences was obtained, there was a single point mutation causing a frame shift in the amino acid sequence and therefore the desired protein was not obtained. Currently the gene sequence is being corrected and the work needs to be continued.