Abstract

Xylanases are proteins that degrade xylan, which is a component of plant cell walls. Thermostable xylanases are useful for the paper and pulp industry because they can be used as substitutes for chlorine in certain methods. The prerequisite of a useful industrial xylanase is that it should be thermostable, but also active at lower temperatures (40ºC-60ºC) than most thermostable xylanases (which tend to be thermo-active, as well as thermostable). Further, in cold climes, a xylanase needs to be able to act at even lower temperatures, to degrade plant waste. In order to try and create a xylanase with unusual characteristics normally absent in naturally-occurring xylanases, we attempted to make a 'chimeric' xylanase by transplanting catalytic and other loops from a beta -alpha barrel xylanase derived from Cryptococcus adeliae, an Antarctic yeast adapted to cold environments, onto the structural scaffold of a mesophile xylanase of known high kinetic thermostability (the NG-27 Xylanase, also known as BSX, or Bacillus sp. xylanase). The chimeric xylanase was constructedon the basis of principles of protein engineering. NG -27 Xylanase shows optimal activity at 70 ºC while C. adeliaexylanase is expected to show optimal activity in the range of 0-10 ºC. So, a encoding the novel protein-engineered xylanase was constructed by one of my seniors, by transplanting the latter’s loops onto the former’s scaffold. However, there was a frame shift mutation in the gene which introduced a stop codon. The first step of this project was, therefore, to correct this mutation. After correction of the mutation, the idea was to produce the xylanase, and study various characteristics including whether it folds and whether it is active. Since it is a novel protein, there was a need of optimizing the conditions under which it can be expressed and purified. This was achieved by exploring various different conditions at each and every step during purification and expression. After optimizing the conditions for expression and purification, we proceeded to check whether the chimeraformed was active or not. Standard xylanase degradation assay (Miller 1962) was made use of for checking the activity in which the reducing sugars react with DNSA and gives colour which is monitored at 540nm. Also, plate-based

assays were tried, to check for activity through visual evidence of degradation of substrate (xylan) in the form of zones of clearance of suspended (turbid) xylan around cavities in which enzyme was placed. To characterize the protein and have a better understanding of the system, we also used different techniques like CD (Circular Dichroism) and gel filtration chromatography. While performing these experiments, the main findings were: a) the protein was expressed and present in inclusion bodies, so it was extracted out with the help of denaturants, b) it can be refolded by removing denaturant using dialysis, or through on column refolding, or rapid removal of denaturant using a desalting column, c) the refolded protein displays signature features of alpha helix and beta sheet in the CD spectrum, suggesting that the protein folds into mixed beta alpha structures, d) refolding through dialysis leads to formation of soluble aggregates containing polypeptides in structured beta-alpha form, but refolding through desalting-based removal of denaturant leads to formation of some dimer population too, and e) the soluble aggregate does not show activity under any of the conditions or temperatures tested, indicating that such refolding and activity assays need to be optimized, while the dimers obtained through desalting have not yet been fully tested for activity (although initial tests at one pair of chosen high, and low,temperatures suggests that there is no activity even in the dimer). Further studies are needed.