

The effect of site-directed mutagenesis on cold adaptation of VPR; a subtilisin-like serine proteinase from a psychrophilic *Vibrio*-species.

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Psychrophilic enzymes have very similar 3D structures as their homologous enzymes from mesophilic and thermophilic organisms. Main characteristics of enzymes from psychrophiles are their high catalytic efficiency (k_{cat}/K_m values) and thermolability. Both of these characteristics have been related to increased structural flexibility. A subtilisin-like serine proteinase from a psychrophilic *Vibrio*-species (VPR) shows these characteristics when compared to homologous enzymes from mesophilic and thermophilic organisms (Kristjansson et al., 1999). The VPR gene was cloned, sequenced and expressed in *E.coli* (Arnorsdottir et al., 2002). Recently the X-ray crystal structure has been determined to a 1.84 Å resolution (Arnorsdottir et al., 2005). Structural comparisons have been carried out and have led to hypotheses about some of the structural factors that may determine the cold adaptation of VPR. Some of these hypotheses have been examined with site-directed mutagenesis. The specific residue exchanges were selected with the objective to incorporate stabilizing interactions into the cold adapted enzyme which were deemed to be present in related thermostable homologues. These include incorporation of prolines into loops, introduction of a new potential salt-bridge, as well as substitutions aimed at improving packing in the hydrophobic core (Ala to Val) and decreasing apolar exposed surface. In addition, we have introduced Ser to Ala substitutions at three different locations in the cold-adapted enzyme, but Ser to Ala exchanges were the most frequently observed amino acid exchange observed when the sequence of the enzyme was compared to those of more thermostable homologues. Here we report on the catalytic and stability characteristics of the selected mutants.

References:

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