

Biochemical characterisation and structure determination of a novel cold-active proline iminopeptidase from the Psychrophilic yeast, *Glaciozyma antarctica* PI12

ABSTRACT

Microbial proteases constitute one of the most important groups of industrially relevant enzymes. Proline iminopeptidases (PIPs) that specifically release amino-terminal proline from peptides are of major interest for applications in food biotechnology. Proline iminopeptidase has been extensively characterised in bacteria and filamentous fungi. However, no similar reports exist for yeasts. In this study, a protease gene from *Glaciozyma antarctica* designated as GaPIP was cloned and overexpressed in *Escherichia coli*. Sequence analyses of the gene revealed a 960 bp open reading frame encoding a 319 amino acid protein (35,406 Da). The purified recombinant GaPIP showed a specific activity of 3561 U mg^{-1} towards L-proline-p-nitroanilide, confirming its identity as a proline iminopeptidase. GaPIP is a cold-active enzyme with an optimum activity of 30° C at pH 7.0. The enzyme is stable between pH 7.0 and 8.0 and able to retain its activity at 10–30° C. Although GaPIP is a serine protease, only 25% inhibition by the serine protease inhibitor, phenylmethanesulfonylfluoride (PMSF) was recorded. This enzyme is strongly inhibited by the presence of EDTA, suggesting that it is a metalloenzyme. The dimeric structure of GaPIP was determined at a resolution of 2.4 Å. To date, GaPIP is the first characterised PIP from yeasts and the structure of GaPIP is the first structure for PIP from eukaryotes.