Characterisation and molecular dynamic simulations of J15 asparaginase from Photobacterium sp. strain J15

Abstract

Genome mining revealed a 1011 nucleotide-long fragment encoding a type I l-asparaginase (J15 asparaginase) from the halo-tolerant Photobacterium sp. strain J15. The gene was overexpressed in pET-32b (+) vector in E. coli strain Rosetta-gami B (DE3) pLysS and purified using two-step chromatographic methods: Ni2+-Sepharose affinity chromatography and Q-Sepharose anion exchange chromatography. The final specific activity and yield of the enzyme achieved from these steps were 20 U/mg and 49.2%, respectively. The functional dimeric form of J15-asparaginase was characterised with a molecular weight of ~70 kDa. The optimum temperature and pH were 25°C and pH 7.0, respectively. This protein was stable in the presence of 1 mM Ni2+ and Mg2+, but it was inhibited by Mn2+, Fe3+ and Zn2+ at the same concentration. J15 asparaginase actively hydrolysed its native substrate, l-asparagine, but had low activity towards l-glutamine. The melting temperature of J15 asparaginase was ~51°C, which was determined using denatured protein analysis of CD spectra. The Km, Kcat, Kcat/Km of J15 asparaginase were 0.76 mM, 3.2 s-1, and 4.21 s-1 mM-1, respectively. Conformational changes of the J15 asparaginase 3D structure at different temperatures (25°C, 45°C, and 65°C) were analysed using Molecular Dynamic simulations. From the analysis, residues Tyr24, His22, Gly23, Val25 and Pro26 may be directly involved in the 'open' and 'closed' lid-loop conformation, facilitating the conversion of substrates during enzymatic reactions. The properties of J15 asparaginase, which can work at physiological pH and has low glutaminase activity, suggest that this could be a good candidate for reducing toxic effects during cancer treatment.

Keyword: J15 asparaginase; Photobacterium sp.; Expression; Purification; Molecular Dynamic (MD) simulations