Soluble expression and catalytic properties of codon-optimized recombinant bromelain from MD2 pineapple in Escherichia coli

ABSTRACT

Bromelain, a member of cysteine proteases, is found abundantly in pineapple (Ananas comosus), and it has a myriad of versatile applications. However, attempts to produce recombinant bromelain for commercialization purposes are challenging due to its expressibility and solubility. This study aims to express recombinant fruit bromelain from MD2 pineapple (MD2Bro; accession no: OAY85858.1) in soluble and active forms using Escherichia coli host cell. The gene encoding MD2Bro was codon-optimized, synthesized, and subsequently ligated into pET-32b(+) for further transformation into Escherichia coli BL21-CodonPlus(DE3). Under this strategy, the expressed MD2Bro was in a fusion form with thioredoxin (Trx) tag at its N-terminal (Trx-MD2Bro). The result showed that Trx-MD2Bro was successfully expressed in fully soluble form. The protein was successfully purified using single-step Ni2+-NTA chromatography and confirmed to be in proper folds based on the circular dichroism spectroscopy analysis. The purified Trx-MD2Bro was confirmed to be catalytically active against N-carbobenzoxyglycine p-nitrophenyl ester (N-CBZ-Gly-pNP) with a specific activity of 6.13 ± 0.01 U mg−1 and inhibited by a cysteine protease inhibitor, E-64 (IC50 of 74.38 ± 1.65 nM). Furthermore, the catalytic efficiency (kcat/KM) Trx-MD2Bro was calculated to be at 5.64 ± 0.02 × 10–2 μM−1 s−1 while the optimum temperature and pH were at 50 °C and pH 6.0, respectively. Furthermore, the catalytic activity of Trx-MD2Bro was also affected by ethylenediaminetetraacetic acid (EDTA) or metal ions. Altogether it is proposed that the combination of codon optimization and the use of an appropriate vector are important in the production of a soluble and actively stable recombinant bromelain.