



# Linker length affects expression and bioactivity of the onconase fusion protein in *Pichia pastoris*

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**ABSTRACT.** The aim of this study was to analyze the effect of linker length on the expression and biological activity of recombinant protein onconase (ONC) in fusion with human serum albumin (HSA) in *Pichia pastoris*. Four flexible linkers with different lengths namely Linker L0, L1: (GGGGS)<sub>1</sub>, L2: (GGGGS)<sub>2</sub>, and L3:(GGGGS)<sub>3</sub> were inserted into the fusion gene and referred to as HSA-n-ONC, where N = 0, 5, 10, or 15. The sequence of the fusion gene HSA-ONC was designed based on the GC content and codon bias in *P. pastoris*; the signal peptide of albumin was used as the secretion signal. Gene sequences coding for the fusion protein with different linkers were inserted into pPICZ $\alpha$ -A to form recombinant plasmids pPICZ $\alpha$ -A/HSA-n-ONC, which were then transformed into *P. pastoris* X-33 for protein expression. Ideal conditions for expression of the fusion proteins were optimized at a small scale, using shake flasks before proceeding to mass production in 10-L fermenters. The recombinant fusion proteins

were purified by aqueous two-phase extraction coupled with DEAE anion exchange chromatography, and their cytotoxic effect on the tumor cell was evaluated by the sulforhodamine B assay. The results showed that the expressed amount of fusion proteins had no significant relationship with the length of different linkers and rHSA-0-ONC had no cytotoxic effect on the tumor cells. While rHSA-5-ONC and rHSA-10-ONC had a weak cytotoxic effect, rHSA-15-ONC could kill various tumor cells *in vitro*. In summary, the biological activity of the fusion protein gradually improved with increasing length of the linker.

**Key words:** Linker; Onconase; Human serum albumin; Fusion protein; Activity detection