

SL301

Application of Yeast Secretion System for Large Production and Secretion Analysis of Human α_1 -Antitrypsin

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The similarities between yeast and animal cell secretion pathway have made yeast not only a promising host system for large production but also a valuable model system for analysis of secretion of human proteins. Using the signal sequence derived from inulinase (INU1A) of *Kluyveromyces marxianus*, a yeast expression vector was constructed to direct secretion of a human glycoprotein, α_1 -antitrypsin (α_1 -AT), from *Saccharomyces cerevisiae*. The yeast transformants harboring the expression vector secreted about 70% of the total α_1 -AT synthesized into the culture media. The fed-batch cultivation of the recombinant yeast led to the secretion of biologically active and glycosylated α_1 -AT up to 75 mg l⁻¹. The N-terminal sequencing of the purified α_1 -AT protein showed that the secreted α_1 -AT was processed correctly at the Kex2 cleavage site as expected from the sequence of INU1A signal peptide. The results demonstrate that the yeast secretion system using the inulinase signal sequence is usefully applicable for the high-level secretion of human α_1 -AT from *S. cerevisiae*.

The yeast secretion system was also exploited to investigate the relationship between folding and secretion of the Z variant α_1 -AT. While the normal human α_1 -AT protein was efficiently secreted from the yeast cells, the Z variant protein failed to be secreted and underwent degradation rapidly inside the yeast cells, as observed in human liver cells. The thermostable mutations suppressing the folding defect of Z variant *in vitro* relieved the intracellular degradation as well as the secretion defect of Z variant in the yeast secretion system in proportion to the extent of suppression in the folding. The results obtained using the yeast secretion system provide a direct supporting evidence that the *in vivo* defect associated with the Z variant is derived from the folding defect. The presented studies show that the yeast secretion system is a useful tool in the expression of human α_1 -AT for industrial use and for basic research as well.