The 4th Iranian Conference on
Systems Biology

In Silico Selection of an Optimal Signal Peptide for Improve Expression and Secretion of Human Interferon Alpha 2b (IFN-2b) in *E. coli*

Mousa Naderi[1]*, Mohammad Rezaei[2], Roghayeh Ghaderi[1], fatemeh velayatipor[1], Bijan Bambai[1]

1. Department of System Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

2. Malek Ashtar University of Technology, Tehran, Iran

musanaderi2@gmail.com

Abstract

Production of recombinant proteins in the periplasm of the bacterium Escherichia coli has a number of advantages, including the ability to form disulphide bonds, aiding correct folding, and the relative ease of release and subsequent capture and purification. Signal peptides are one of the most important factors for prosperous secretion of the recombinant proteins. The nucleotide sequence encoding the signal peptide also influence 5'mRNA secondary structure, which is an important factor in translation efficiency. In this study, we evaluated different signal peptides and theoretically determined appropriate signal sequences for improved expression and secretion of interferon alpha 2b (IFN-2b) in the E. coli. In silico analysis of the influencing factors identified MalE signal peptide as a theoretically suitable signal peptide for both improved expression and higher secretion of IFN-2b in E. coli.

Keywords: Signal Peptide, interferon alpha 2b, *in silico,* Secretory Expression, *E. coli*

Introduction

Escherichia coli is the most commonly used microbial host for the expression of recombinant proteins. However, cytoplasmic expression is the main challenges in the production of recombinant proteins in E. coli, due to formation of insoluble aggregates known as inclusion bodies. To prevent the formation of inclusion bodies, several strategies such as the use of E. coli strains with oxidative cytoplasmic environment, co-expression of chaperones and secretion of proteins into the periplasm or culture medium have been developed. Periplasmic expression offers several advantages over intracellular production such as a more oxidative environment for effective protein folding and strait forward downstream purification process without cell lysis. The N-terminal signal peptide is highly essential for secretion of recombinant proteins into the periplasm. Therefore, one of the strategies to improve the secretion efficiency is the optimization of signal peptide. The nucleotide sequence encoding the signal peptide also influence 5'mRNA secondary structure, which is an important factor in translation efficiency. Therefore, by harmonizing gene expression levels with secretion capacity, protein production in the periplasm can be optimized. Selection of an optimal signal sequence is the first important step in constructing an extracellular recombinant protein secretion system. However, there is no universal rule for selecting an appropriate signal peptide for the secretion efficiency. the available bioinformatics tools, employed in different biological fields, assist researchers to bypass some of these costly and time-consuming steps, and enable them to start the experimental processes with a more clear view or at least some preliminary forecasts. So in this study, an in silico approach is adopted to evaluate several SPs for finding the best candidate for improve expression and secretion of interferon alpha 2b (IFN-2b) in the E. coli

Methods

Probability and cleavability of different signal peptides connected to IFN-2b were predicted using SignalP 4.0 server. Different physicochemical characteristics, which are important for selecting an appropriate signal peptide, were evaluated using ProtParam server. Modeller software and ZDOCK server applied for the signal peptide modelling and molecular docking (signal peptide – protein docking), respectively. The secondary structure and (ΔG) of the 5′ mRNA TIR with different signal peptides were predicted using mfold server.

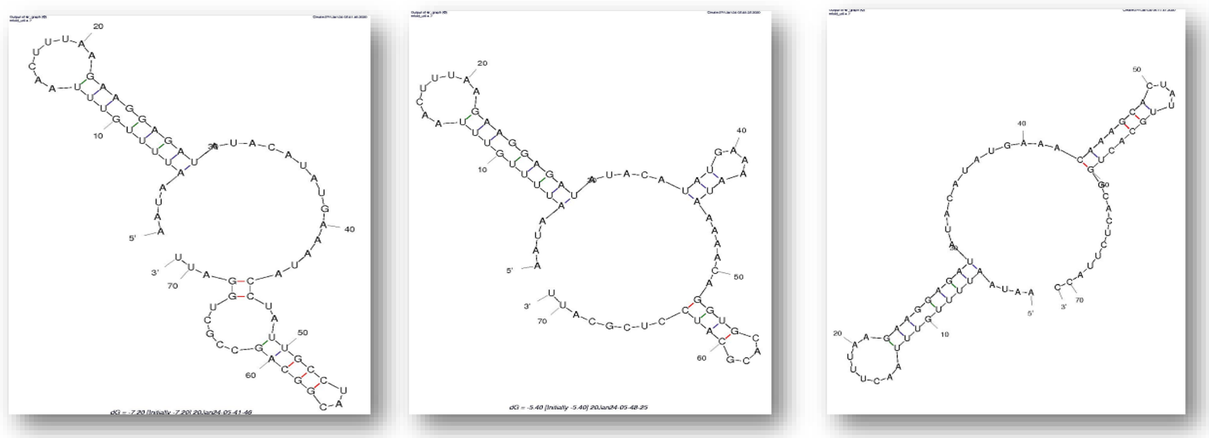
Results

*Probability and cleavability of different signal peptides connected to IFN-2b were predicted by SignalP 4.0 server, as shown in Table **1**.*

***Table 1.** In silico analysis of the signal peptides sequences by SignalP 4.0 server*

Signal peptide	Amino acid sequence	Cleavage site	D-score of SP+ IFN-2b
PelB	MKYLLPTAAAGLLLLAAQPAMA	AMA	.893
MalE	MKIKTGARILALSALTTMMFSASALA	ALA	.876
DsbA	MKKIWLALAGLVLAFSASA	ASA	.872
OmpA	MKKTAIAIAVALAGFATVAQA	AQA	.858
LamB	MMITLRKLPLAVAVAAGVMSAQAMA	AMA	.851
PhoE	MKKSTLALVVMGIVASASVQA	VQA	.815
AnsB	MEFFKKTALAALVMGFSGAALA	ALA	.810
PhoA	MKQSTIALALLPLLFTPVTKA	TKA	.679
LPP	MKATKLVLGAVILGSTLLAG	Non-cleavable	.565
TorA	MNNNDLFQASRRRFLAQLGGLTVAGMLGPSLLTPRRATA	Non-cleavable	.234

The secondary structures of the nascent 5'mRNA TIR for the IFN-2b were predicted theoretically with essential architectures shown in Fig. 1.



PelB
ΔG= - 7.2 Kal/mol

MalE
ΔG= - 5/4Kal/mol

PhoA
ΔG= - 4.4Kal/mol

***Figure 1.** Predicted secondary structure of TIR and minimum free energy of the 5'mRNA TIR with different signal peptides, including 36 bases upstream of the start codon and 35 bases downstream of the start codon.*

Conclusion

In silico Study of the critical parameters identified that the pelB signal peptide was a good choice for improving the secretion of the IFN-2b and the PhoA signal peptide facilitated IFN-2b overexpression. However, our results demonstrate that the MalE signal peptide is potentially a suitable candidate for both improve expression and higher secretion of IFN-2b in E. coli. Moreover, a system biology approach is needed for designing a high productivity secretory system, taking into account the balance between different important factors, mainly gene expression, the protein translocation process, host characteristics, and the culture medium composition. As considering all these factors together is not easy and still demands more knowledge about the determinants and their importance, the in silico predictions are the required initial step for the practical experiments.

References

Bai C, Wang X, Zhang J, Sun A, Wei D, Yang S (2014) Optimization of the mRNA secondary structure to improve the expression of interleukin-24 (IL-24) in Escherichia coli. Biotechnol Lett 36(8): 1711–1716

Freudl, R. (2018) Signal peptides for recombinant protein secretion in bacterial expression systems. Microb. Cell Fact. 17, 52.