Introduction

In some cases, production of recombinant proteins for therapeutic development or for in vitro studies is a real challenge and requires a lot of investment in terms of time and cost in order to obtain high yields of pure and active proteins. The aim is to provide a guide on how to easily and rapidly determine in which production and purification conditions these proteins will be the happiest, and how certain innovative solutions can really help in this quest. Our optimization strategies are based on 3 axes (Fig. 1): the optimization of expression systems, purification processes and buffer composition. E. Coli and HEK 293 are the most widely used protein production systems for pharmaceutical target studies. They respectively present considerable advantages.

Indeed, bacterial protein production is cheap and is able to rapidly provide non-recombinant proteins. Generally, HEK 293 cells are able to produce human proteins whereas E. Coli is used for the same genetic expression profiles, well folded and active. First of all, we have focused on how to enhance the transformation efficiency of HEK 293 EBNA cells and the quantity of protein produced in both producing protein and evaluate production systems. In a second time, we have studied how we could stabilize and solubilize unstable proteins during the purification step.

These solutions are not the only ones used within the laboratory. Indeed, an action can also be carried out at the level of the protein conformation, solubilization and activity. Finally, three proteins, one viral and two human candidates which are known to be hard to produce and unstable (Fig. 2). The specific characteristics for the production of toxic or membrane bacterial strains can help. Nevertheless, the production of viral proteins is known to be hard to produce and unstable (Fig. 2). For this reason, the HEK 293 EBNA system will be used.

Material and Methods

The aim of this following optimization strategy is to determine a holistic process for the production and purification of problematic proteins from transcriptional analysis to getting pure proteins in milliseconds by following proven trial based strategies and by performing molecular biology, expression and purification studies (Fig. 3).

Optimization of the protein production process

In bacterial strain system (E. Coli)

To enhance the quantity and the quality of proteins produced in E. Coli, several strategies with innovative approaches have been developed over the last decades. Various tools are available in the market such as a platform of new additives with specific characteristics for the production of toxic or membrane proteins, peptones that are with or without lipids... Moreover, several tags can be used to improve the protein solubility and the purification process or to create a correct folded protein during the cell transfection. Interestingly, the expression, purification and stability of the three proteins produced can be highly enhanced in Superbroth and with the use of innovative additives compared to the amount produced in classical media (LB, 2YT, TB...).

In Human cell system (HEK293 EBNA cells)

As for protein production in E. Coli, the choice of an adapted tag and co-expression of interacting partner peptides can help. Nevertheless, other factors can also be taken into account like the expression vs protein Y intracellular presence. Its expression can be optimized by adding a signal peptide on the DNA sequence (Fig. 6) and the effect of nature and concentration of innovative additives (peptones and/or lipids) added to the media on the quantity of the protein Y produced (Fig. 7).

Conclusion

In conclusion, each protein is a particular case. Nevertheless, taking into account their specific physico-chemistry, performing an efficient Od, implementing new strategies and/or using innovative reagents, and above all taking some time to properly interpret data and screening results make the gene less difficult. This study has permitted to define an efficient, holistic process that can be applied for each protein as a first intention screening. The benefits of our strategy in terms of time and cost are very impressive and of great value, and are accessible to researchers through our protein production platform.