Enhancement in the production, structure-activity relationship, and chaperone assisted folding of recombinant biotherapeutic protein - human paraoxonase-1

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ABSTRACT

In the present work, I attempted to shed light on the improvement of protein production levels of an enzyme, human paraoxonase 1 (hPON1). The protein has a molecular weight of 43kDa and has a beta-barrel structure. The enzyme catalyzes the degradation of organophosphates and their derivatives, such as nerve agents. Along with this, several literatures suggest its extensive role in various diseases. Inside humans, it is produced in the liver tissue and found to be associated with high-density lipoproteins in the blood.

Despite their importance in tackling organophosphates and other diseases, available literatures suggest the yield of the protein from various host systems is meagre. The primary source of hPON1 is human serum; however, its availability is limited and poses a threat when used for purification if it is contaminated. Therefore, various hosts widely used for protein production have been utilized till now in this direction. However, they are either difficult to overexpress or form inclusion bodies. These inclusion bodies (IBs) are distinct structures inside the cells and are formed on protein overexpression. These IBs usually contain proteins in non-functional and aggregated form. Here, *Escherichia coli* (*E. coli)* is considered one of the highly advantageous host organisms for protein production. It has contributed significantly and accounts for producing 30% FDA (Food and Drug Administration) approved drugs. Several other benefits include simpler and cheaper substrates, and it is easy to grow, reach high cell density in a short period, and can be scaled to a fermentation scale with less cost. However, expression of hPON1 in *E. coli* suffers with very low expression levels and the formation of inclusion bodies that makes it a difficult task to produce it inside the *E. coli* despite numerous benefits.

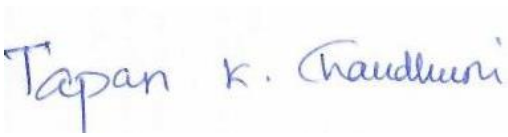
In the present work, hPON1 has been chosen to enhance its functional yield for industrial applications, and also to understand the basic principles of its folding. Firstly, we have

optimized the conditions for overexpression of recombinant hPON1 in *E. coli* system. During the optimization, several *E. coli* strains were taken, which were selected based on their ability to support the translation of eukaryotic genes in *E. coli*. Other factors considered are reducing environment, ability to survive the toxic protein expression inside the cells, ability to survive at lower temperatures, and bypassing the codon bias.

After choosing the expression host, the expression level was further enhanced by taking into consideration various cellular environmental parameters that affect cell growth and recombinant protein expression levels. Parameters like induction type, induction time, post-induction duration, growth media, and different inducer concentrations were considered.

Even though protein has overexpressed sufficiently in the *E. coli* cells, as discussed above, they usually form inclusion bodies and are non-functional. Therefore, we attempted to fold the protein both *in vivo and in vitro*.

Firstly, the recombinant hPON1 was produced in soluble form *in vivo* using a combination of different factors that affect protein folding. The most crucial factor is chaperones known for their role in protein folding and providing stability. Molecular chaperones are generally present inside the cells and assist in maintaining the protein quality inside the cells. During protein folding, hPON1 protein may form different intermediates, some of which get kinetically trapped and form irreversible aggregates. Chaperones play an important role here; they prevent the conversion of misfolded protein to non-functional aggregates while, on the other hand, accelerating the attainment of native structure from it. Different molecular chaperones used here are GroEL/GroES (GroEL/ES), Trigger factor (TF), and DnaK–DnaJ–GrpE (DnaKJE). Along with this, we also used chemical chaperones, also termed as osmolytes. These osmolytes are small organic molecules that help stabilize macromolecules inside the cells under various



environmental stresses and protein folding. Its example includes trehalose, glycerol, sorbitol,

TMAO (trimethylamine N-oxide), etc. Another factor that was utilized was temperature, which aids in facilitating the proper refolding of proteins. By employing the lower temperature and harnessing the combinatorial effect of molecular and chemical chaperones, we achieved significant improvement in the protein yield within the *E. coli* cells *in vivo*.

Secondly, we focussed on refolding the protein which we obtained as inclusion bodies from *E. coli*. This involved the isolation of inclusion bodies from *E. coli*, ensuring minimal loss and the presence of the lowest possible amounts of contaminants. Protein was solubilized using the denaturant and subsequently refolded using on-column refolding with the incorporation of various additives in the refolding buffer. Through careful optimization of the different buffer additives and on-column refolding conditions, efficient and functional refolding of hPON1 was achieved.

Further, with the help of various biophysical techniques, we were able to establish partial bio-similarity of recombinant hPON1 with its serum counterpart. Finally, the role of chaperones in the soluble production of hPON1 was investigated. Here, we have used GroEL/ES chaperone system, as it is extensively studied. The results showed that it actively participates in hPON1 folding and contributes to our understanding of its role in facilitating hPON1 folding.