ABSTRACT

Recombinant DNA technology has rapidly become a prime choice for the pathogen-free protein-based therapeutics preparation in large quantities with batch consistency which is an impossibility with the natural source. However, various challenges are involved with the protein preparation in the heterologous hosts which limits the recombinant production of the desired protein. Human Serum Albumin (HSA) is one of such most demanded therapeutic protein of immense potential which is still obtained primarily from the natural source i.e. human blood plasma.

In the present work, we have developed a strategy to enhance the functional production of HSA in *Escherichia coli*. After its successful functional preparation in *E. coli* cytosol, *E. coli*-derived HSA has been isolated and its physiochemical properties were discerned and compared with the plasma-derived form followed by its stability studies.

HSA- one of the most demanded therapeutic protein with immense biotechnological applications- is 66.5kDa multidomain protein containing 17 disulfide bonds. The current prime source of HSA is human blood plasma. Blood is a limited and an unsafe source. Thus, there exists an indispensable need to promote non-animal derived rHSA production.

*E. coli* is one of the most convenient hosts which has contributed to the production of more than 30% of the FDA-approved recombinant pharmaceuticals. It grows rapidly and reaches high cell density using inexpensive and simple substrates. *E. coli*-derived recombinant products have more economic potential as fermentation processes are cheaper compared to the other expression hosts. The major bottleneck in exploiting *E. coli* as a host for - a disulfide-rich multidomain protein-rHSA production was aggregation. Majority of the expressed recombinant protein was forming inclusion bodies (more than 90% of the total expressed rHSA) in the *E. coli* cytosol. Recovery of functional rHSA form inclusion body is not preferred because it is tedious, time-consuming, laborious and expensive. Because of such limitations, *E. coli* host system was neglected for rHSA production for past few decades.

In the present work, we have exploited the capabilities of *E. coli* as a host for the enhanced functional production of rHSA (~60% of the total expressed rHSA in the soluble fraction). Parameters like intracellular environment, temperature, induction type, duration of induction, protein extraction conditions etc. which play an important role in enhancing the level of production of the desired protein in its native form has been optimized. We have studied the effect of assistance of different types of exogenously employed chaperone systems in the functional expression of rHSA in *E. coli* host system. Different aspects of cell growth parameters during the production of rHSA in *E. coli* has also been studied.

Upon overcoming the difficulties to produce functional rHSA in *E. coli*, it has been possible to produce significant levels of functional protein through engineering the biological system of protein folding in the cell, the *E. coli*-derived rHSA has been purified to homogeneity. Its detailed physicochemical characterization has been performed by monitoring its conformational properties,
secondary and tertiary structure elements, surface properties, ligand binding properties, stability issues etc. These parameters of the recombinant protein have been compared with the naturally occurring protein from the human source. The outcome of the comparison reveals that the recombinant protein resembles exactly the same as the natural one.

Hence, we propose that the *E. coli*-derived rHSA is an ideal biosimilar for human blood plasma-derived serum albumin. Therefore, in the present study, we have introduced and promoted the *E. coli*- derived rHSA as an alternative to the preparation from a human source, pHSA.