ABSTRACT

Human-Granulocyte Macrophage Colony Stimulating Factor (h-GMCSF) is an acidic glycoprotein produced by mitogen activated helper T lymphocytes and other cell types. It stimulates the growth and differentiation of haemopoietic precursor cells from various lineages, including granulocytes, macrophages, eosinophils, and erythrocytes. Clinically, h-GMCSF has achieved immense significance by virtue of its role in elevating neutrophil levels in primates. It is used to treat neutropenia induced by a variety of causes from cytotoxic chemotherapy to bone marrow transplantation and AIDS. Recently, recombinant h-GMCSF has been used for autologous and allogeneic bone marrow transplantation followed by immunosuppressive therapy for aplastic anemia. The wide spectrum in which GM-CSF can be used makes it a very important pharmaceutical product.

The concentration of h-GMCSF produced by the different mammalian cell lines is low and hence other methods for producing higher levels of such molecules are essential. The use of recombinant DNA technology for the large-scale production of molecules that are otherwise difficult to extract from natural sources has been a boon to medical treatment. The objective of this study has been to compare the efficacy of h-GMCSF produced by E. coli and insect cells. In E. coli, attempts were made to produce the bioactive protein in different compartments like cytoplasm and periplasm (secretory).

In the current work, recombinant h-GMCSF was expressed in E.coli cytoplasm as a fusion protein with polyhistidine tag (pVM1) as well as a non-fusion mature form (pVM4/NS) using T7 expression system. The over-expression of h-GMCSF resulted in the formation of inclusion bodies (IB) in the cytoplasm of E. coli under the induced conditions (IPTG). The purification procedure required a prior denaturation and renaturation process before checking the biological activity of the target protein. The results showed that the activity of recombinant h-GMCSF generated from the recombinant clones pVM1 and pVM4/NS were significantly lower when compared to the commercially
available standards. The possible reason for the lesser biological activity may be due to the uncertainty about the percentage of properly refolded target protein after denaturation. In order to overcome the formation of IB while expressing the protein in the cytoplasm, different cloning strategies were used to obtain the soluble protein in the periplasmic space of E. coli. To achieve this, hybrid clones were developed by the fusion of prokaryotic signal sequences from E.coli β-lactamase and Shigella apyrase with the N-terminus of the mature h-GMCSF sequence.

Comparative analysis of expression and secretion of h-GMCSF by different recombinants (pVM2/bla, pVM3/apy and pVM4/NS) was carried out under identical culture conditions. The results showed that the recombinant h-GMCSF generated from hybrid gene [bla signal sequence with mature h-GMCSF sequence (pVM2/bla)] was in two different forms (secretory product and unprocessed precursor molecules). This heterogeneity in the product formation may be due to competition for processing of bla signal sequence between β-lactamase and recombinant protein. On the other hand, recombinant h-GMCSF produced from the hybrid gene [apy signal sequence with mature h-GMCSF sequence (pVM3/apy)] was only in the secretory form. However, constructs containing the prokaryotic signal sequences (pVM2/bla and pVM3/apy) expressed relatively lower levels and soluble form of recombinant h-GMCSF compared to pVM4/NS that exhibited over-expression of the target protein resulting in the formation of IB in the cytoplasm.

In the current study, recombinant h-GMCSF was also expressed in insect cells using baculovirus expression system. The target protein was expressed as a secretory form into the culture medium clearly suggesting that the insect cells recognized the mammalian (native) signal sequence of h-GMCSF cells. The biological activity was found to be significantly less in cytoplasmic fractions (pVM1 and pVM4/NS) of E.coli when compared to periplasmic fractions (pVM2/b/a, pVM3/apy) and supernatants from insect cells.