Increased production of human granulocyte-macrophage colony stimulating factor (hGM-CSF) by the addition of stabilizing polymer in plant suspension cultures

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Abstract

The effect of stabilizing polymer on hGM-CSF production was investigated in suspension cell cultures of transgenic tobacco. Secreted human GM-CSF from cell suspension cultures was detected in the medium at a maximum concentration of 180 μg/L by ELISA. However, the secreted hGM-CSF was unstable in the medium, and rapidly degraded after day 5. In order to stabilize the secreted hGM-CSF, three stabilizing polymers were tested, polyethylene glycol, polyvinylpyrrolidone and gelatin. Gelatin was the most effective in stabilizing the secreted GM-CSF. Following the addition of 5% (w/v) gelatin, the maximum GM-CSF concentration reached 783 μg/L, a 4.6-fold increase over control. *Keywords:* *h*GM-CSF, gelatin, protein stabilizer

Introduction

Plant cell cultures have several advantages compared to microbial or animal cell cultures. They have a reduced risk of mammalian viral contaminants, oncogenes and bacterial toxins, and they can also produce correctly folded proteins and assemble multimeric protein such as antibodies[1]. As a result, reports on the development of transgenic plants for foreign protein expression are now accumulating[2, 3]. Plant cell culture for foreign protein production is not yet well-developed technologically. Foreign proteins produced in plant cell cultures are harvested from plant biomass, from culture supernatant, or from a mixture of the two. Despite the dilution of secreted protein to low concentrations in a relatively large liquid volume, plant cell culture of foreign protein can be advantageous. Since plant cell culture medium contains no native proteins, recovery and purification of secreted proteins is relatively inexpensive. However, accumulation of the secreted protein depends largely on its stability in the extracellular environment, and this is of particular importance for proteins with

complicated structures. The use of protein-stabilizing agents such as polyvinylpyrrolidone (PVP) has been shown to improve antibody production in suspension cultures, suggesting that the medium is the site of antibody degradation[4,5]. We demonstrated similar results in studies of bioactive hGM-CSF production in tobacco suspension culture, where the accumulation of hGM-CSF was drastically reduced during the exponential phase of cell growth. In this study, we attempted to improve the yield of hGM-CSF in tobacco suspension culture by testing three different polymer stabilizing agents.

Materials and methods

Flask culture : Suspension culture of transgenic cells were cultured in 50 ml (in 300 ml flask) of MS medium containing 1 mg/l of 2,4-D, 0.05 mg/l of kinetin, 3% sucrose, and 100 mg/L kanamycin at 25℃ in a shaking incubator with a rotation speed of 100 rpm. The pH of the medium was adjusted to 5.8 with 0.5 M KOH. The suspension cell culture was kept alive by transferring a fifth of a proceeding culture into fresh medium every 7 days.

Stabilizing polymer treatment : To investigate the effect of polymer on stabilize of protein, transgenic cells were cultivated in a medium containing with various concentration of polyvinylpyrrolidone (PVP 360,000: 0.05, 0.1, 0.2%), polyethylene glycol (PEG 3,350: 0.5, 1, 2, 3, 5%), Gelatin(type B, from bovine serum albumin: 0.5, 1, 2, 3, 5%).

Quantitative analysis of hGM-CSF : The cultured broth was centrifuged at 10,000 rpm for 5 min, and the supernatant was collected for ELISA. For the detection and quantification of hGM-CSF, an ELISA kit was used according to the manufacturers instructions (PharMingen, Inc., USA). Sample concentrations were determined by comparison to a standard curve of recombinant hGM-CSF. MS medium, PVP, PEG, gelatin did not interfere with the ELISA analysis.

Results and discussion

Effect of stabilizing polymers on hGM-CSF production: We examined the effect of three polymers known to have protein stabilizing effects : PEG, PVP, and gelatin. Gelatin had a significant stabilizing effect on hGM-CSF (Fig. 1), while the addition of PVP and PEG did not significantly increase the extracellular production of hGM-CSF (data not shown). Addition of gelatin did not affect cell growth until the mid-exponential growth phase (day 5). After day 5, gelatin had

a negative effect on cell growth (Fig. 2A), and at the same time hGM-CSF production started to decrease (Fig. 2B).

In orther to confirm the positive stabilizing effect of gelatin, we examined the effect of the timing of gelatin addition (2%, w/v) on hGM-CSF stabilization. As shown in Fig. 3, the hGM-CSF concentration started to increase on the day gelatin was added.

Degradation of hGM-CSF in the presence of a stabilizing polymer: To confirm the stabilizing effect of gelatin, we determined the residual amount of pre-produced hGM-CSF under various conditions in cell broth that did not produce hGM-CSF. Addition of PVP (0.05%) and PEG (2%) could not stabilize hGM-CSF (Fig. 4), and all hGM-CSF was degraded in one day.

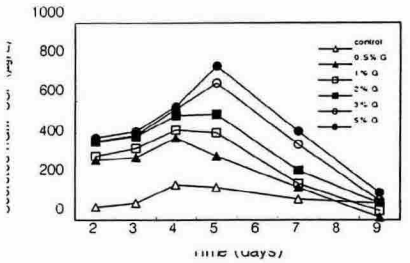

Figure 1. Effect of various concentration of gelatin on the extracellular hGM-CSF production during batch suspension cultured.

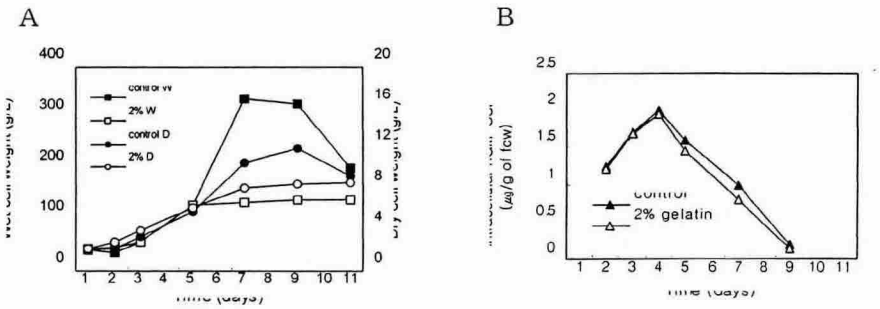

Figure 2. Effect of 2% gelatin on cell growth and intracellular concentration of hGM-CSF during batch suspension culture. The concentration of intracellular hGM-CSF was calculated by measuring hGM-CSF extracted from 1g fresh cell.

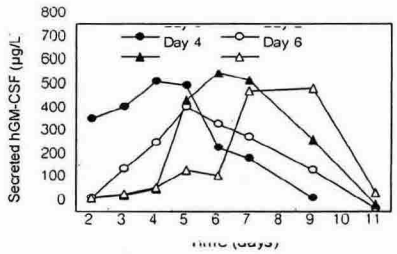

Figure 3. Effect of the time of gelatin addition (2%) on the extracellular concentration of hGM-CSF during batch suspension culture

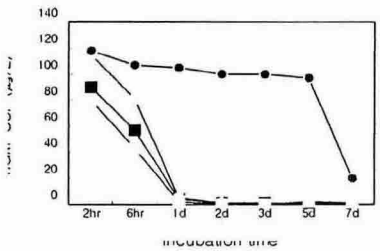

Figure 4. Effect of polymers on survival of hGM-CSF degradation of hGM-CSF was reduced by the addition of 2% gelatin(●), 0.05% PVP (○), 2% PEG (■), compared to control (□).

Acknowledgements

This work was supported by a grant from the National R&D Project (National Research Laboratory Program) of the Korean Ministry of Science and Technology.

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