World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:11, No:06, 2017

Production of Insulin Analogue SCI-57 by Transient Expression in Nicotiana benthamiana

Authors : Adriana Muñoz-Talavera, Ana Rosa Rincón-Sánchez, Abraham Escobedo-Moratilla, María Cristina Islas-Carbajal, Miguel Ángel Gómez-Lim

Abstract : The highest rates of diabetes incidence and prevalence worldwide will increase the number of diabetic patients requiring insulin or insulin analogues. Then, current production systems would not be sufficient to meet the future market demands. Therefore, developing efficient expression systems for insulin and insulin analogues are needed. In addition, insulin analogues with better pharmacokinetics and pharmacodynamics properties and without mitogenic potential will be required. SCI-57 (single chain insulin-57) is an insulin analogue having 10 times greater affinity to the insulin receptor, higher resistance to thermal degradation than insulin, native mitogenicity and biological effect. Plants as expression platforms have been used to produce recombinant proteins because of their advantages such as cost-effectiveness, posttranslational modifications, absence of human pathogens and high quality. Immunoglobulin production with a yield of 50% has been achieved by transient expression in Nicotiana benthamiana (Nb). The aim of this study is to produce SCI-57 by transient expression in Nb. Methodology: DNA sequence encoding SCI-57 was cloned in pICH31070. This construction was introduced into Agrobacterium tumefaciens by electroporation. The resulting strain was used to infiltrate leaves of Nb. In order to isolate SCI-57, leaves from transformed plants were incubated 3 hours with the extraction buffer therefore filtrated to remove solid material. The resultant protein solution was subjected to anion exchange chromatography on an FPLC system and ultrafiltration to purify SCI-57. Detection of SCI-57 was made by electrophoresis pattern (SDS-PAGE). Protein band was digested with trypsin and the peptides were analyzed by Liquid chromatography tandem-mass spectrometry (LC-MS/MS). A purified protein sample (20µM) was analyzed by ESI-Q-TOF-MS to obtain the ionization pattern and the exact molecular weight determination. Chromatography pattern and impurities detection were performed using RP-HPLC using recombinant insulin as standard. The identity of the SCI-57 was confirmed by anti-insulin ELISA. The total soluble protein concentration was quantified by Bradford assay. Results: The expression cassette was verified by restriction mapping (5393 bp fragment). The SDS-PAGE of crude leaf extract (CLE) of transformed plants, revealed a protein of about 6.4 kDa, non-present in CLE of untransformed plants. The LC-MS/MS results displayed one peptide with a high score that matches SCI-57 amino acid sequence in the sample, confirming the identity of SCI-57. From the purified SCI-57 sample (PSCI-57) the most intense charge state was 1069 m/z (+6) on the displayed ionization pattern corresponding to the molecular weight of SCI-57 (6412.6554 Da). The RP-HPLC of the PSCI-57 shows the presence of a peak with similar retention time (rt) and UV spectroscopic profile to the insulin standard (SCI-57 rt=12.96 and insulin rt=12.70 min). The collected SCI-57 peak had ELISA signal. The total protein amount in CLE from transformed plants was higher compared to untransformed plants. Conclusions: Our results suggest the feasibility to produce insulin analogue SCI-57 by transient expression in Nicotiana benthamiana. Further work is being undertaken to evaluate the biological activity by glucose uptake by insulin-sensitive and insulin-resistant murine and human cultured adipocytes.

Keywords : insulin analogue, mass spectrometry, Nicotiana benthamiana, transient expression
Conference Title : ICPP 2017 : International Conference on Protein and Proteomics
Conference Location : Toronto, Canada
Conference Dates : June 15-16, 2017