عنوان مقاله:

Evaluation of glargine insulin expression using Bı Domain of streptococcal protein G tag

محل انتشار:

چهارمین کنگره بین المللی و شانزدهمین کنگره ملی ژنتیک (سال: 1399)

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خلاصه مقاله:

Background and Aim: : Insulin glargine is a long-acting insulin, used in the management of diabetes. It contains ۵۳ amino acids with a molecular weight of ۶۰۶۳ Dalton and is produced in Escherichia coli strain k۱۲. Insulin glargine is different from human insulin as contains glycine instead of asparagine in position ۲۱ of the A-chain and by carboxy-terminal extension of B-chain by ۲ arginine residues. The arginine amino acids shift the isoelectric point from ۵.۴ to ۶.۷, making the molecule more soluble at an acidic pH and less soluble at physiological pH. Studies have shown that the critical and main issue in producing glargine is in its expression and solubility. Some tags are seen to solve these issues; Bı Domain of streptococcal protein G (GB) tag increases protein expression. As result, we fused glargine with GB-tag to enhance its expression.Methods: we obtained the nucleotide sequence of the insulin glargine. The codons were optimized using E.coli codon usage and the gene was inserted into the pUC۵۷ expression vector between two restriction sites of Nde I and Xho I enzymes and then cloned into pET-۲۸a^{+} vector. The recombinant vector was transferred into E. coli strain BL۲۱ (DE۳). As our vector contains kanamycin resistance gene, it was possible to screen recombinant vector using LB agar medium containing kanamycin. To evaluate and determine the best expression condition for GB-Glargine and Glargine, both proteins were expressed in LB and M۹ broth for ۶h, ۹h, and ۱۸ h at ۱۸°C, ۲۵°C a,nd ۳۷°C after inducing the expression by ۰.۱ mM and ۰.۵ mM of Isopropyl β-D-۱-thiogalactopyranoside (IPTG). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the expression difference between Glargine and GB-Glargine proteins.Results: The constructed expression vectors, pET۲۸ insulin glargine were transformed into E. coli BL۲۱ (DE۳) to obtain a recombinant strain, BL۲۱ (DE۳) /pET۲۸-insulin Glargine SDS-PAGE illustrated results of glargine expression in all conditions were the same; glargine protein consisting GB tag also showed the same pattern of expression in different conditions. However, a significant difference was observed between glargine and glargine containing GB tag; in this regard, the expression was seen to be increased when

glargine is fused with GB-tag.Conclusion: The finding of our study shows that GB-tag enhances the expression of glargine like other studies which have used the GB-tag.

کلمات کلیدی:
Insulin glargine, GB-tag, Expression

لینک ثابت مقاله در پایگاه سیویلیکا: