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Development of Agonists and Antagonists of Glycoproteins Using Site-Directed Mutagenesis and Gene Fusion

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Abstract

One major issue regarding the clinical use of many peptides is their short half-life due to the rapid clearance from the circulation. To overcome this problem previous studies ligate the interested proteins to polyethylene glycol or to IgG-FC fragment. Here we used other strategy, of ligation the signal sequence of O-linked oligosaccharides to the coding sequence of the interested protein. The cassette gene that has been used contains the sequence of the carboxyl-terminal peptide (CTP) of human chorionic gonadotropin subunit (hCG). The CTP contains 28 amino acids with four O-linked oligosaccharide recognition sites.Using this strategy, the CTP sequence was ligated to the coding sequence of follitropin (FSH), thyrotropin (TSH), erythropoietin (EPO), growth hormone (GH) and thus to increase the longevity and bioactivity of these proteins in-vivo. Interestingly, the new analogs of FSH and GH found to be not immunogenic in human and it is already passed successfully clinical trials phase III. Moreover, the European Commission (EC) for treatment of fertility approved FSH long-acting (ELONVA) and it is already used in many countries.

We found that deletion of N-linked oligosaccharides from hTSH and hFSH subunits still bind to the receptor with high affinity and resulted in a significant decrease in the bioactivity. Thus, deglycosylated TSH and FSH variants, behave as potential antagonists that may offer a novel therapeutic strategy in the treatment of Grave's disease, and ovarian hyperstimulation syndrome. In conclusion, addition of O-linked oligosaccharides or deletion of N-linked oligosaccharides could be an interesting strategy for designing new analogs of glycoprotein hormones.

animals lack the potential to synthesize maximum of the vitamins, microorganism have inherent ability to provide those metabolites. With contemporary lifestyle, consumers are becoming more fitness conscious and discerned in their food choices. In this kind of situation, riboflavin-offering LAB offer a clean gain over chemical synthesis through growing the nutritional price of food. The riboflavin biosynthesis in bacteria turned into analysed the usage of comparative analysis of genes, operons and regulatory elements.

Chemical synthesis of a diet is being replaced by fermentation processes due to financial and environmental considerations of the latter. Besides the monetary advantages, additional blessings of the microbial synthesis include the use of renewable sources, environmental-friendly approach and superior nice of the final. version for law of riboflavin biosynthesis is primarily based at the formation of opportunity RNA structure regarding the RFN element (a mononucleotide riboswitch is surprisingly conserved RNA element this is discovered frequently inside the 5' untranslated location of prokaryotic mRNA that encodes for FMN biosynthesis and shipping proteins that is utilized in a later step (lumazine synthase). The 2d and 1/3 enzymatic steps (deamination of the pyrimidine ring of structure and the subsequent discount of the ribosyl side-chain) are controlled by way of some other bi-practical enzyme encoded by means of the first gene of the operon ribG The penultimate step in riboflavin biosynthesis, is catalysed by means of lumazine synthase, the fabricated from the closing rib gene, ribHSo far, records available on entire genomes of numerous microbes has made it clean that riboflavin-producing ability is identified to be strain or subspecies specific. Thus, it may be an attractive technique to bioprospect prolific riboflavin-producing traces from their diversified natural niche and further decorate their capacity to provide this important vitamin with the aid of microbiological and biotechnological interventions.

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