5th Conference on Recombinant Protein Production

A comparative view on host physiology

130- 8h

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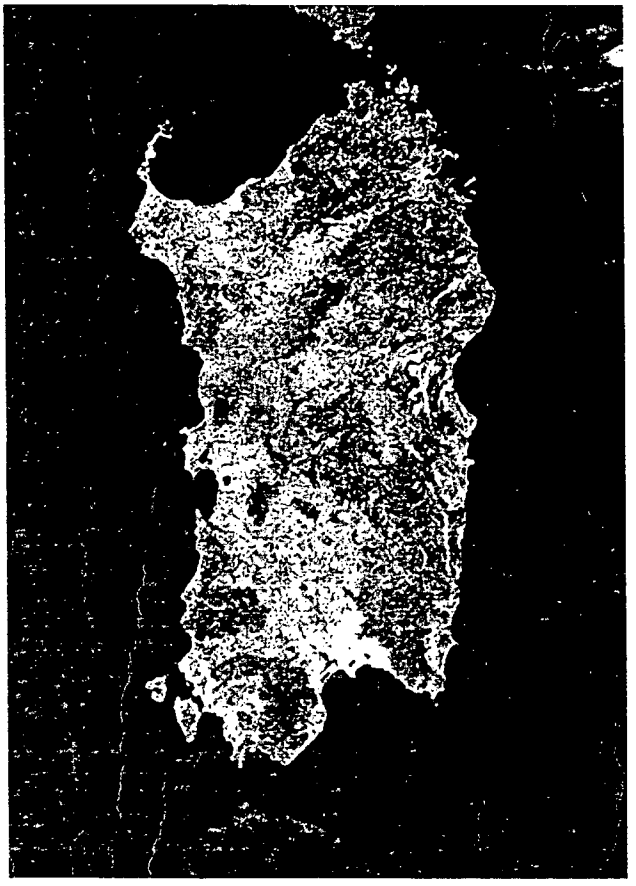

Co-Chairs

Diethard Mattanovich	Enrico Berardi	Jeff Cole
Austria	*Italy*	*United Kingdom*

ABSTRACT BOOK

13662

P1

Stable expression of a human-like sialylated recombinant thyrotropin in a chinese hamster ovary cell line expressing α2,6-sialyltransferase

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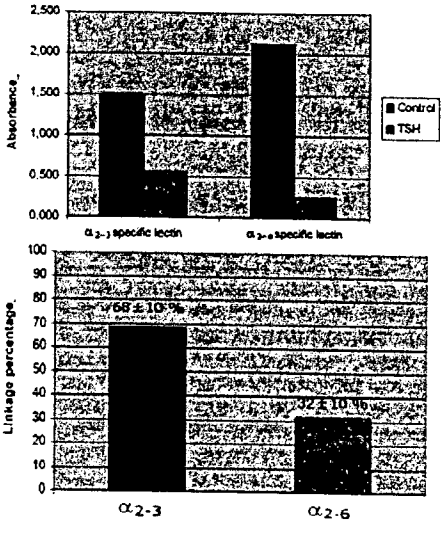
Recombinant thyrotropin (r-hTSH), expressed in CHO cells, lacks α2,6-sialic acid-galactose linkages at the termini of the carbohydrate chains, unlike pituitary-derived hTSH (p-hTSH) that contains approximately 70% of α2,3- and 30% of α2,6- linked sialic acid residues. In this work, a genetic modification in the hTSH-producing CHO cell line by the introduction of rat α2,6-sialyltransferase cDNA was carried out, generating a human-like r-hTSH (hlr-hTSH) more similar to the native hormone, with respectively 61 ± 10 % of α2,3- and 39 ± 10 % of α2,6-linked sialic acid residues. The best clone, isolated from this "human-like" CHO cell line co-transfected with the dicistronic expression vectors pEDdc-α and pEDdc-β hTSH and submitted to gene amplification with up to 8 μM methotrexate (MTX), presented a secretion level of 2.1 μg hTSH/10^6 cells/per day, which is useful for production purification and characterization.
The relative molecular masses (Mr) of the heterodimer and of the α- and β-subunits of purified hlr-hTSH, obtained by MALDI-TOF mass spectrometry, were 29187, 14038 and 15243 respectively, which are in good agreement with previously determined values for r-hTSH.The carbohydrate structures of this r-hTSH were of the complex type, presenting 57.1% of di-, 18.8% of tri- and 23.2% of tetra-antennary structures, partly fucosylated and with variable levels of sialylation. The most abundant structures were the monosialylated biantennary N-linked sugar chains, representing ~ 45% of all identified forms, followed by tri- and tetra-sialylated N-linked sugar chains (~17.5% each). About 86% of oligosaccharides were sialylated, with 5.47moles sialic acid/mole protein, being the sialic acid: galactose ratio = 0.73. "Human-like" r-hTSH, analyzed via an *in vivo* bioassay based on hTSH-induced T_4 release, was shown to be equipotent ($p > 0.05$) with the unique commercial preparation of r-hTSH (Thyrogen), and 1.6-fold more potent than p-hTSH-NIDDK($p<0.001$). We are showing below an example of sialic acid linkage analysis for p-hTSH and hlr-hTSH based on specific lectin interaction.

13662

p-hTSH



hIr-hTSH