5th Conference on Recombinant Protein Production

A comparative view on host physiology

-130- 8h

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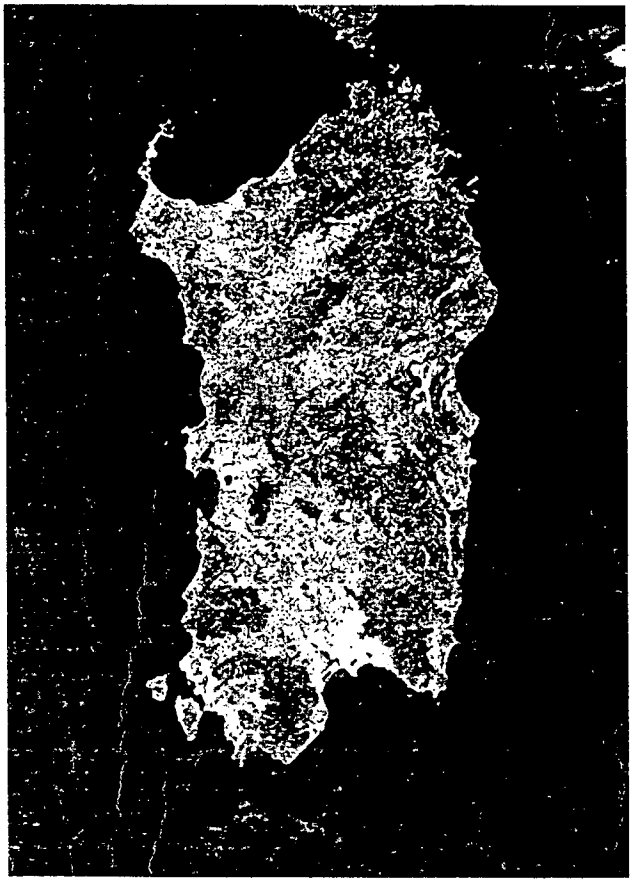

Co-Chairs

Diethard Mattanovich	Enrico Berardi	Jeff Cole
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ABSTRACT BOOK

N-Glycan structures of glycoprotein hormones: a comparison between recombinant and native human pituitary thyrotropin (hTSH)

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The number of biopharmaceuticals that are synthesized by DNA recombinant techniques in different host cells is continuously increasing. Among these are human pituitary glycoprotein hormones, i.e. thyrotropin (hTSH), follicle-stimulating (hFSH) and luteinizing (hLH) hormone, all mostly synthezised in CHO cells. It is well known that while the protein moiety is essentially identical to that of the native hormone, the carbohydrate moiety is different. CHO cells glycosylation machinery, in fact, differently from thyrotropes, lacks galactosyl-transferase, sulfo-transferase and $\alpha_{2,6}$ sialyl-transferase: this strongly influences the N-glycan termini of the biosynthetic drug.

Considering that, talking about hormone action or therapy, an acceptable biological activity cannot be the sole parameter to be considered, it was decided to study and compare the N-glycan structures that are present in either native or recombinant hTSH. We started, therefore, comparing, two recombinant (Thyrogen^R from Genzyme Corporation and our in house preparation of rec-hTSH-IPEN) with a pituitary reference preparation (p-hTSH-NIDDK from the National Hormone and Pituitary Program of USA). Up to 20 different N-glycan structures have been identified in the recombinant preparations, while 25 have been found in the pituitary. Of the latter, whose analysis has been for the moment semi-quantitative, only 3 structures have been found in common with those that are present in the recombinant hormone, the predominant glycoforms presenting sulfated N-acetyl galactosamine termini.

The percentage of $\alpha_{2,3}$ and $\alpha_{2,6}$ sialic acid-galactose linkages were also determined in p-hTSH-NIDDK, obtaining values of 68±10% and 32±10% respectively, which practically confirmed the theoretical values of 72% and 28% calculated by us with basis on pioneering work of Green and Baenziger (1988). More extensive and quantitative studies are in progress in which more than one pituitary preparations of hTSH is going to be analyzed.

In the figure below an example of the N-glycan structures determined for rec-hTSH (Thyrogen) is presented.

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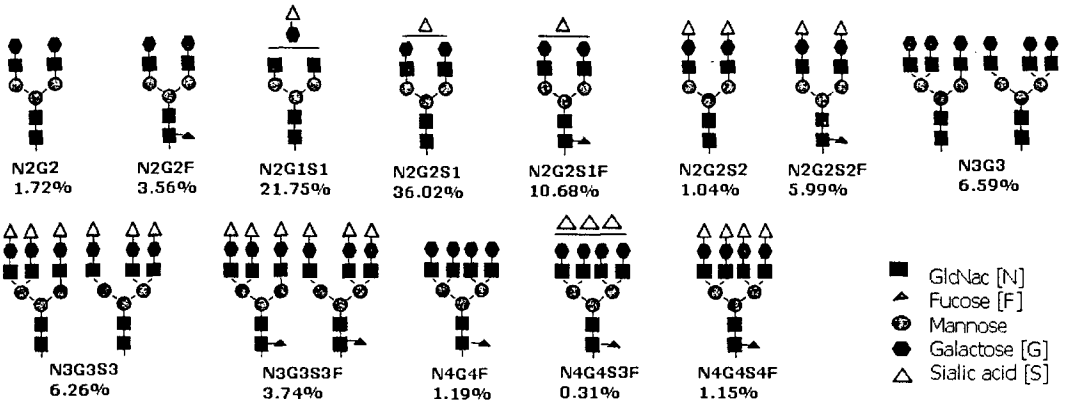
N2G2
1.72%
N2G2F
3.56%
N2G1S1
21.75%
N2G2S1
36.02%
N2G2S1F
10.68%
N2G2S2
1.04%
N2G2S2F
5.99%
N3G3
6.59%
N3G3S3
6.26%
N3G3S3F
3.74%
N4G4F
1.19%
N4G4S3F
0.31%
N4G4S4F
1.15%
GlcNac [N]
Fucose [F]
Mannose
Galactose [G]
Sialic acid [S]