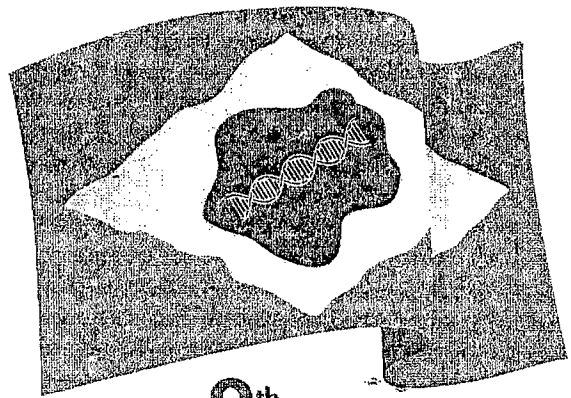

8th PEACe Conference
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P4 POST-TRANSLATIONAL MODIFICATIONS/PRODUCT INTEGRITY

P4.1 Influence of reduced CO2 environment on the secretion yield, potency and glycan structures of recombinant thyrotropin (hTSH) from CHO cells

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A consistent increase of ~ 60% in the secretion yield of CHO-derived hTSH was observed by changing cell culture CO2 conditions from 5% CO2 to air enviroment (0.03 % CO2). The quality of the product obtained under both conditions was analysed for what concerns N-glycan structures, charge isomers and biological activity in comparison with a well known commercial preparation (Thyrogen).

The N-glycans identified in the three preparations were of the complex type, presenting di-, tri- and tetra-antennary structures, with variable level of sialylation. Considering the latter characteristic, which is directly related to in vivo bioactivity, the three preparations have practically an identical percentage (86-88%) of sialylated structures, with some difference in percentage of di- and tri- sialylated glycan. Monosialylated glycans (N2G2S1, N2G1S1 and N2G2S1F) represent the three most abundant structures with 68-69% of all identified forms in the three preparations. The main difference was found in terms of antennarity with 8-10% more N2 structures for hTSH-IPEN produced in the absence of CO2 (-CO2) and 7-9 % more N3 structures for hTSH-IPEN (+CO2) and Thyrogen. Also for what concerns the total percentage of neutral glycans (12-14 %), the three preparations are practically identical. No remarkable difference in charge isomers was also observed between the three preparations, the isoelectric focusing (IEF) profiles showing six well visible bands in the 5.39 - 7.35 pI range, the three major bands focusing at pI 5.85, 6.20 and 6.63. A considerably different distribution, with more acidic forms was observed, though, for two native pituitary preparations of hTSH. When analyzed via a simple and precise single-dose bioassay, a slightly significant difference ($p<0.02$) in activity was found between the two IPEN preparations. hTSH-IPEN (+ CO2) potency was non significantly different from that of Thyrogen, both being 1.6-1.8-fold more potent than the native pituitary reference preparation.

We can conclude that, at least in the case of CHO-derived hTSH, different production processes may not greatly affect its glycan structures, charge isomer distribution or biological activity.

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