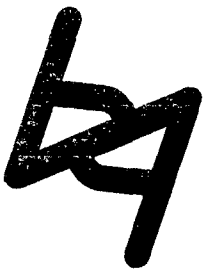

The Brazilian Biochemistry and Molecular Biology Society - SBBq

XXXVIII Annual Meeting of SBBq

Program

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Identity and Integrity of α- and β-Subunit of Human Thyrotropin Prepared by Prolonged Acetic Acid Treatment

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Abstract

Alpha- and beta- subunits, prepared by efficiently dissociating, during 16 hours, a recombinant thyrotropin (hTSH) preparation with 0.4 M acetic acid and isolating them by RP-HPLC, were analysed for what concerns their identity and integrity. Identity was evaluated by MALDI-TOF mass spectrometry (MALDI-TOF MS). A relative molecular mass of 14021 and of 15851 was obtained for α-hTSH and β-hTSH respectively. These values agree with those obtained by analyzing the preparation before dissociation, a difference of -1.8% for α and +1.3% for β being observed. Integrity of the subunits was evaluated by their capacity of self reassembling and of restoring the in vivo bioactivity of the hormone. When α-hTSH and β-hTSH subunits were incubated together in 0.2 M sodium phosphate buffer, pH 7.0, at 25°C and under gentle shaking, a complete reassociation occurred after 4 days, forming an heterodimer. In an *in vivo* mouse bioassay, the T_4 levels of the animals treated with the reassociated hormone were non-significantly different ($p > 0.05$) from those obtained when the original preparation was administered (2.71± 0.63 µg/dL versus 2.84± 0.23 µg/dL, n=6, respectively). In conclusion, subunits prepared by prolonged acetic acid treatment maintain their original molecular mass and can perfectly restore the biological activity of the reassociated heterodimers.

Keywords: α- and β-subunits; hTSH; MALDI-TOF-MS; Biological Assay.

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Comparison Between CHO-derived Thyrotropin Containing α2,6 Sialic Acid Linkages (hIsr-hTSH) and the Conventional Recombinant Product

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In this work two different recombinant thyrotropin (hTSH) preparations were compared for what concerns N-glican structures, biological activity and charge heterogeneity. One of them (hIsr-hTSH) was derived from a CHO cell line with a dual-sialic acid linkage introduction (61% of α2,3 and 39% of α2,6) which had been genetically modified by the introduction of rat α2,6-sialyltransferase cDNA. The other thyrotropin (r-hTSH) was derived from a conventional CHO cell line capable of expressing only α2,3 sialic acid linkages. Concerning the N-glycan structures both preparations presented complex structures (di-, tri- and tetra-antennary), sometimes fucosylated and with variable levels of sialylation. The most remarkable difference was the presence of ~16% more tetra- and ~8% more tri-sialylated structures in hIrs-hTSH than in r-hTSH. These differences, however, did not influence the biological activity. When hIsr-hTSH and r-hTSH were analyzed via an *in vivo* bioassay based on hTSH stimulation of thyroxin (T_4), hIrs-hTSH was shown to be equipotent with r-hTSH ($p < 0.05$). Concerning the distribution of charge isomers, when hIrs-hTSH and r-hTSH were evaluated by isoelectric focusing, no remarkable differences were observed. In both preparations, about six components with pI between 5.20 and 7.35 were found. In conclusion, the genetic modification in the carbohydrate moiety introduced in hIsr-hTSH does not seems to influence significantly the bioactivity and charge isomers distribution of this recombinant glycoprotein, although differences were observed in N-glycan structures and may exist in its pharmacokinetics.

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Keywords: Thyrotropin; α2,6 sialic acid linkage; α2,3 sialic acid linkage, N-glycans.

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