Identity and Integrity of a- and b-Subunit of Human Thyrotropin Prepared by Prolonged Acetic Acid Treatment

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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 a-hTSH and ß-hTSH respectively. These values agree with those obtained by analyzing the preparation before dissociation, a difference of -1.8% for a and +1.3% for ß 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 a-hTSH and ß-hTSH subunits were incubated together in 0.2 M sodium phosphate buffer, pH 7.0, at 25ºC 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.

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