

Engineering a Potential Antagonist of Human Thyrotropin and Thyroid-stimulating Antibody*

Fuad A. Fares^{‡§}, Flonia Levi[‡], Abraham Z. Reznick[¶], and Zaki Kraiem^{||}

From the Departments of [‡]Biochemistry and Molecular Genetics and [¶]Anatomy and Cell Biology and the ^{||}Endocrine Research Unit, Carmel Medical Center and the Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, 34362 Israel

Thyrotropin (TSH) and the gonadotropins (FSH, LH, hCG) are a family of heterodimeric glycoprotein hormones composed of two noncovalently linked subunits, α and β . We have recently converted the hTSH heterodimer to a biologically active single chain (hTSH β -CTP α) by fusing the common α -subunit to the C-terminal end of the hTSH β -subunit in the presence of a ~30-amino acid peptide from hCG β (CTP) as a linker. The hTSH β -CTP α single chain was used to investigate the role of the N-linked oligosaccharides of α - and β -subunits in the secretion and function of hTSH. Using overlapping PCR mutagenesis, two deglycosylated variants were prepared: one lacking both oligosaccharide chains on the α -subunit (hTSH β -CTP α_{1+2}) and the other lacking the oligosaccharide chain on the β -subunit (hTSH β -CTP α (deg)). The single chain variants were expressed in CHO cells and were secreted into the medium. hTSH variants lacking the oligosaccharide chains were less potent than hTSH β -CTP α wild-type with respect to cAMP formation and thyroid hormone secretion in cultured human thyroid follicles. Both deglycosylated variants competed with hTSH in a dose-dependent manner. The hTSH β -CTP α_{1+2} variant blocked cAMP formation and thyroid hormone secretion stimulated by hTSH as well as by the antibody, thyroid-stimulating immunoglobulins, responsible for the most common cause of hyperthyroidism, Graves disease. Thus, this variant behaves as a potential antagonist, offering a novel therapeutic strategy in the treatment of thyrotoxicosis caused by Graves' disease and TSH-secreting pituitary adenoma.