19. PAST, PRESENCE AND FUTURE OF THYROID-STIMULATING HORMONE (TSH) SUPERACTIVE ANALOGS

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INTRODUCTION

In recent years many new engineered protein therapeutics are being developed and tested in clinical trials (Marshall et al. 2003). Many early protein drugs showed limited applicability due to short half-life or low affinity to their receptors. Recombinant DNA technology permitted engineering protein molecules with specific chemical and biological characteristics. It is now anticipated that engineered hormone analogs will largely exceed clinical efficacy of existing products, including recombinant human TSH (rec-hTSH; Thyrogen^{TM}, Genzyme). Preclinical development of superactive TSH analogs can be divided into three phases: the TSH structure-function phase, the discovery phase and the optimization phase. It is apparent that detailed structure-function studies of TSH provided important foundation for rational modifications of human TSH molecule. This chapter describes the past, presence and future of superactive analogs of TSH with high receptor binding affinity. Such superactive analogs of TSH are expected to provide not only more efficacious diagnostic methods, but should also serve as indispensable tools in management of thyroid carcinomas with low TSH receptor number, impaired coupling and deficient ligand binding.

TSH STRUCTURE-FUNCTION

TSH is a key protein controlling thyroid function through its interaction with TSH receptor in thyroid. TSH is a member of glycoprotein hormone family produced by basophiles in the anterior pituitary (Pierce and Parsons 1981; Hearn and Gomme

2000). Human TSH is a heterodimer composed of two non-covalently linked subunits, α- and TSHβ-subunit. α-Subunit contains 92 amino acids and a sequence identical in all human glycoprotein hormones. TSHβ-subunit contains 118 amino acids and is unique for human TSH. The high-resolution structure of homologous to TSH human chorionic gonadotropin (hCG) has revealed that both subunits contain a central cystine-knot motif and three loops: two β-hairpin loops (*L*1 and *L*3) on one side of a cystine-knot and a long loop (*L*2) on the other (Lapthorn et al. 1994). The long loop in the α-subunit includes two-turn α-helix. The cystine-knot is made up of three central disulfide bonds, where one of the disulfide bonds threads through a ring formed by two other disulfide bonds. Similar to other glycoprotein hormones (LH, FSH and hCG), TSH hetero-dimers are stabilized by a unique segment of the β-subunit termed "seat-belt", because it wraps around the α-subunit. In light of the common α-subunit and 38% sequence identity between the hCGβ- and hTSHβ-subunit, homology modeling of hTSH was performed and showed expected similarities in the global conformation of these two hormones (Szkudlinski et al. 1996). Accordingly, assignment of disulfide bonds in bovine TSH β-subunit revealed bonding analogous to hCG (Fairlie et al. 1996). Thus, in hTSH β-subunit, three disulfide bonds (2–52, 27–83 and 31–85) form cystine-knot motif that determines the core structure, two disulfide bonds (19-105, 88-95) are involved in "seat-belt" formation and one (17–67) links two β-hairpin loops. Such structural features result in an increased interaction between two subunits and provide stability of heterodimer in physiological conditions.

Three carbohydrate chains constitute 15–25% of TSH molecular weight. The human α-subunit contains two carbohydrate chains linked to asparagine 52 and asparagine 78, and the human TSH β-subunit contains one carbohydrate chain attached at asparagine 23. Such asparagine-linked oligosaccharides are complex-type structures displaying notable hormone-, species-, source- and production-dependent differences in their core and terminal residues. Differences in oligosaccharide structure result in physiological heterogeneity of pituitary and recombinant TSH (Szkudlinski et al. 1993). It has been very well established that co-translational attachment of site-specific oligosaccharide chains is highly important in subunit folding, dimerization, TSH dimer secretion, stability, plasma half-life and bioactivity.

As reviewed previously, TSH contains several important domains that are tightly conserved among different species or homologous hormones (Grossmann et al. 1997; Szkudlinski et al. 2002). Even minor modifications of such domains result in decreased expression, impaired receptor binding and bioactivity. These domains located within a "composite binding domain" proposed by Lapthorn et al. (Lapthorn et al. 1994) include: α-helix (α40–46), αLys51, αAsn52, the α-carboxyl terminus (α 88–92), α33–38, "the Keutmann loop" (TSHβ31–52) and the "seat belt" in the β-subunit (TSHβ88–105) (Szkudlinski et al. 1996; Grossmann et al. 1997) (Figure 1). In addition to the stabilizing role of the "seat-belt", recent studies involving β-subunit chimeras have shown that this region is critical in conferring glycoprotein hormone specificity (Grossmann et al. 1997). Additional functionally critical residues have been identified