

**Biological Function of Single Chain Glycoprotein Hormone Mutants**

**Min Kwan-Sik**, Chang Yoo-Min, Chang Sun-Hwa,  
Lee Hyen-Gi, Lee Yun-Gun, Chang Won-Kyong, Cheong Il-Cheong  
Lab, of Cellular Biochemistry, Division of Animal Biotechnology, NLRI

Human chorionic gonadotropin (hCG) is a member of the glycoprotein hormone family which includes FSH, hCG, TSH. These hormone family is characterized by a heterodimeric structure composed a common  $\alpha$ -subunit noncovalently linked to a hormone specific  $\beta$ -subunit. The correct conformation of the heterodimer is also important for efficient secretion, hormone-specific post-translational modifications, receptor binding and signal transduction. To determine  $\alpha$  and  $\beta$ -subunits can be synthesized as a single polypeptide chain (tethered-hCG) and also display biological activity, the tethered-hCG molecule by fusing the carboxyl terminus of the hCG  $\beta$ -subunit to the amino terminus of the  $\alpha$ -subunit was constructed and transfected into chinese hamster ovary (CHO-K1) cells. We also constructed C-terminal deletion mutants (D91, D89, D88, D87, D86, D84, D83) of single chain hCG to determine the biological function (secretion, LH-activity, receptor binding, cAMP production) of these mutants. Between six and eight stably transfected pools of cells expressing wild type and mutant hCGs were selected for neomycin resistant. The hCGs secreted by the stably transfected cells into serum-free media were collected and quantified by radioimmunoassay, as described in protocol (DPC(hCG IRMA)).

LH activity was in terms of testosterone production and aromatase activity in primary cultured rat Leydig cells. The tethered-wthCG was efficiently secreted and showed similar LH-like activity to the dimeric hCG. The D83hCG mutant was not detected in this assay. It is suggest that hCG C-terminal part is very important for hCG secretion. Now, we checking the LH-like activity of these mutant hCGs. These data indicate that the constructs of tethered molecule will be useful in the study of mutants that affect subunit association and/or secretion.

(Key Words) **Tethered-hCG/FSH, recombinant, CHO cells**