Production of hCG from α subunit mRNA and site-specific mutant β subunit mRNA


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Human chorionic gonadotropin (hCG) is a member of a closely related group of glycoprotein hormones including thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), and luteinizing hormone (LH). All of these hormones consist of two noncovalently linked subunits, α and β chains (1). The α subunit is 92 amino acids long and common to all hormones (2), while the β chain has amino acid sequence unique to each hormone, conferring biological specificity on the individual hormone. Varying degree of sequence homology in amino acids exists among these β subunits. The residues between position 34 and 37 of amino acids, Cys-Ala-Gly-Tyr (3), are the highly conserved fragment and are called as the "CAGY" region.

It has been reported that the point mutation in the CAGY region of TSH subunit caused the congenital thyrotropin deficiency. The point mutation found in the congenital thyrotropin deficiency was at base position 85 from G to A, resulting in the conversion of amino acid from glycine to arginine (4).

In the present study, we made three kinds of site-directed mutant hCG β cDNA that have the same change in the CAGY region as observed in congenital thyrotropin deficiency. The effect of the mutation on the production of hCG was investigated by in vitro transcription and translation system using Xenopus laevis oocytes (5).

The site-directed mutants of hCG β cDNA were
prepared by olygonucleotide-directed in vitro mutagenesis system (Amersham, England). The method and the region of point mutation are schematically illustrated in Fig.1. The mutations at CAGY region were confirmed by the DNA sequence analysis. The mRNA corresponding to each mutant $\beta$ cDNA normal hCG $\beta$ cDNA or normal hCG $\alpha$ cDNA was synthesized in vitro using T3 RNA polymerase. The mRNA of hCG $\alpha$ subunit was microinjected into Zenopus Laevis oocytes with the mRNA of either normal hCG subunit or mutant hCG $\beta$ subunit. The microinjected oocytes were incubated in Barth's medium for 36 hrs. at 20 °C. After incubation, the medium was recovered and the concentration of hCG in the medium was measured by enzyme immunoassay (EIA). These process were showed in Fig.2.

As shown in Fig.3, hCG was produced from the oocytes microinjected with mRNA of both $\alpha$ and normal $\beta$ subunits. However, among the oocytes injected with three kinds of mutant hCG mRNA together with normal hCG mRNA, same mutant 1 and 2 in Fig.3 hardly produced hCG into the medium. The concentrations of hCG produced by these oocytes were less than one tenth of that obtained from the oocyte injected with normal $\alpha$ and $\beta$ hCG mRNA. The point mutation of hCG $\beta$ mRNA that caused the inactivation of hCG production was at base position 106 from G to C or at base position 107 from G to A. The mutation at base position 104 from C to A had no inhibitory effect on hCG production from Zenopus laevis oocytes. The mutation of the base from G to C or G to A means the conversation of the amino acid from Gly to Arg or from Gly to Asp, respectively. Interestingly, the alteration of amino acid from Gly to Asp at CAGY fragment corresponds to the change of amino acid sequence identified in the congenital thyrotropin deficiency.

Theses results indicates that Gly in CAGY region could be essential residue for the production of hCG.

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REFERENCES
Fig. 1

**mutant hCG β DNA**

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**normal**

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**mutant 1**

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**in vitro mutagenesis system**

[Diagram of the in vitro mutagenesis system showing the steps from hCG β cDNA to nicking, annealing, DNA polymerase, digestion by exonuclease III, and repolymerization.]
Fig. 2

METHODS

hCG α, β or mutant β cDNA

T3 Promoter → Bluescript M13+ → T7 Promoter

Amp' → T3 RNA Polymerase

Synthesized RNA

↓

Microinjection of RNA mixture

↓

Xenopus Oocyte

↓

20 °C, 36 Hrs.

↓

Supernatant

↓

hCG EIA
Fig. 3

hCG concentration secreted from mRNA injected oocytes