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Folding and dimer formation of the human Chorionic Gonadotropin (hCG) subunits

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JEG-3 cells were used to study the formation and maturation of the immunological epitopes of the human chorionic gonadotropin (hCG), in particular of the hCG-β subunit.
By means of pulse-chase and pulse kinetics, it was possible to classify the immunological epitopes of the hCG-β subunit in three categories according to the time-point and the velocity of its formation relative to each other: quick- and rapid-appearing epitopes (β2), intermediate epitopes (β4, β11) and late-appearing epitopes (β1, β3, β5, β7, C-1/2).
The association of the GPH-α with the hCG-β subunit was detected even at 1' pulse intervals, thus evidencing that association can be considered as an early event that seems to take place independently of the degree of folding achieved by each of the individual subunits that constitute hCG.
In order to correlate the different β-subunit forms appearing during the maturation process with the formation of disulfide bridges, JEG-3 cell cultures were subjected to double-labeling experiments. Cells were first labeled with [^{35}S]-Cys/Met in a pulse-chase experiment, semi-permeabilized with digitonin and then labeled with [^{3}H]-NEM which caused a blockade and labeling of those free thiol groups that had not formed disulfide bridges at the given time point. The ratio [^{3}H]/ [^{35}S] yielded a measure on the extent of disulfide bridge formation. By maintaining the cell cultures at low temperature (25°C) while performing the double-labeling procedure, the detection capability of early intermediate forms of the hCG-β subunit was enhanced due a decreased β-subunit maturation. High-molecular weight variants could be described for the first time which are possibly complexes with chaperones. Moreover, a much higher number of folding intermediates of the hCG-β subunit could be described than that known until now.
HeLa cells were used to study isoforms of the GPH-α subunit with a high molecular weight (HMW) and the maturation of the GPH-α subunit in an environment where the expression of the hCG-β subunit was not detectable by means of pulse-chase experiments. Gel filtration experiments with a Sephadex G-150 column indicated that the HMW isoforms were not conformational variants of the conventional GPH-α subunit and provided evidence that they represented dimers of GPH-α subunits.
For the first time during the biosynthesis of the hCG-β subunit the expression of the hCG-β core fragment epitope β11 was detected and described to exist on the intact hCG-β subunit without any indication for intracellular proteolysis. This is an important fact with respect to the diagnostic use of the hCG-β core fragment as a terminal degradation product of hCG and for the prenatal diagnosis of Down's syndrome. It is the principal hCG-β-subunit-related molecule found in pregnancy urine samples, while its levels in serum are almost undetectable.