

Biogenesis of immunological epitopes: A study of micro-domain folding and subunit association of hCG

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Immunological epitopes may refer to a definite conformation of a polypeptide chain. We attempted to use this close relationship, between the conformation of limited structural areas and the expression of certain epitopes, to follow the folding of the hCG subunits, in particular of the hCG- β subunit during biosynthesis. Moreover, we have investigated the subunit association and the progress of disulfide bridge formation by the loss of free thiol groups to react with [³H]N-ethylmaleimide ([³H]NEM) during the folding kinetics of the subunits.

Methodology: We have used a panel of well-defined MCAs to follow the subunit folding in definite structural areas and subunit association (9 against the hCG- β , 3 specific for $\beta_{\text{core fragment}}$ (β_{cf}), 7 against the α -subunit, 3 quaternary-structure specific MCAs). The biosynthesis of hCG and the subunits were carried out as pulse-chase kinetics in JEG-3 choriocarcinoma cells labeled with [³⁵S] Met/Cys. Immunoprecipitations obtained with the MCAs were analysed by 1D- and 2D-electrophoreses, single band isolation, and in some cases by MALDI-TOF. In some experiments free thiols were blocked *in vivo* with [³H]NEM in [³⁵S]Met/Cys pulse-labeled cells. The ³H/³⁵S labelling ratio was determined in the single bands after isolation.

Results and Discussion: Most epitopes if not all seem to be expressed during the first minutes of a pulse-chase experiment. For that reason, we used short pulse (1-5 min) and chase periods (0-20 min). The decrease of the temperature from 37°C to 25°C allowed a more detailed analysis without any obvious qualitative changes. With β -subunit specific MCAs that react with free β and $\alpha\beta$ dimers as well as an α -subunit was coprecipitated even at a pulse of 1 min originating of $\alpha\beta$ dimers. The $\alpha\beta$ dimers must have been formed even when the disulfide bridge formation have not completed formed as indicated by a high ³H/³⁵S ratio of single β -bands isolated from [³⁵S]Met/Cys cells treated with after the ³⁵S-labelling [³H]NEM treated cells. Because these complexes were dissociated during immunoprecipitation and elution from the Protein-A agarose we isolated the $\alpha\beta$ dimer complexes by reversible cross-linking with Dithiobis(succinimidylpropionate, DSP) and with several other techniques. The kinetics of epitope formation indicated that the folding of structures giving raise to the β -epitopes in the hairpin loops L1, 3 seems to be much faster than epitopes formed presumably by residues of the β -strands 3 and 4 or epitope structures partially located in the β 1 strand. Cooperative effects on the epitope formation seem to exist even between epitopes closely neighbored in the same micro-domain. A β_{cf} epitope seemed to be transiently expressed during processing of the intact β -subunit.

Conclusions: The folding of hCG subunits during biosynthesis can be followed by means of the formation of immunological epitopes. The formation of $\alpha\beta$ dimers occurs from the very early and does not require the formation of the entire disulfide bridges.

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