Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

Journal homepage: www.elsevier.com/locate/apjr

Document heading 10.1016/S2305-0500(13)60094-9

hCG structure: A logical perspective

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ARTICLE INFO

Article history:
Received 16 October 2012
Received in revised form 20 October 2012
Accepted 25 November 2012
Available online 20 December 2012

Keywords:
hCG
Perspective

ABSTRACT

Introduction: The three dimensional crystal structure of hCG has been publish by Wu *et al*., Lapthorn *et al*., and Lustbader *et al*. The problem is that the crystal is missing 50% of the hCG molecular weight. It is missing the 4 N-linked and the 4 O-linked oligosaccharides and β-subunit amino acid residues 113-145. Here we add back in these missing structures, and considering 11 reported consequences we predict the final structure of hCG, hyperglycosylated hCG, nicked hCG and hCG free β-subunit. **Methods:** The consequences of 11 report are considered. The missing structures are examined by 2 webware programs, Bioinformatics Toolkit (Max Planck Institute), and Bioinformatics and Computational Biology (IIT Delhi). **Results & Discussion:** Structures are proposed for hCG, hyperglycosylated hCG, nicked hCG and hCG free β-subunit, considering the charge effect that the 12 or 19 sialic acid residues may have, movement of α-subunit loop 2 and movement of β-subunit safety belt. **:** Nicking causes a major structural change to β-subunit loop 2.

1. Introduction

Human chorionic gonadotropin (hCG) is a generic term for a group of fascinating molecules. They are fascinating molecules because they are the most acidic glycoproteins known to humans[1,2], and the most glycosylated glycoproteins known to humans[1,3]. Also fascinating because the structures are intricate permitting some hCG variants to bind an hCG/luteinizing hormone (LH) receptor, and other carbohydrate and meric variant structures to bind hCG′s evolutionary cousin, transforming growth factor β (TGF-β2) receptor[4-9].

The group of molecules called hCG shares a common amino acid sequence. There is regular hCG, the hormone, produced in pregnancy by syncytiotrophoblast cells. This acts on the hCG/LH receptor on corpus luteal, trophoblast, and uterine tissues[1]. There is hyperglycosylated hCG, an autocrine produced by cytotrophoblast cells. This promotes blastocyst implantation and placental uterine invasion during pregnancy, it also drives trophoblastic and germ cell cancers by antagonizing a cytotrophoblast cell TGF-β2 receptor[4,10] promoting cell growth and invasion. There is sulfated hCG produced by pituitary gonadotrope cells in men and women[11]. This is a hormone acting on hCG/LH receptor on the corpus luteum, granulosa and theca cells in women and Leydig cells in men.

There is the fetal hCG variant, seemingly made by fetal renal cells[12-14], which act on a fetal hCG receptor, possibly an hCG/LH receptor, to promote fetal organ growth during pregnancy. Finally, there is a hyperglycosylated variant of hCG free β-subunit made by most human cancers[4,10]. This is an autocrine and a major cancer promoter. This antagonizes the TGFβ-II receptor on the cancer cells promoting cancer cell growth and metalloproteinase and collagenase production or cell invasion[4,10]. hCG is a fascinating molecule with such wide reaching functions controlling pregnancy, fetal growth, pituitary endocrinology and cancer cell biology.

hCG is a glycoprotein hormone composed of two subunits α and β joined non-covalently. It is part of a group of endocrines called glycoprotein hormones, hCG, LH, thyroid stimulating hormone and follicle stimulating hormone. Multiple investigators on either side of the Atlantic have examined hCG dimer three dimensional structure using X-ray crystallography techniques, Adrian Lapthorn a chemist in Glasgow[5] Scotland, Hao Wu, a biochemist at Columbia University, New York, USA[15], and Joyce Lustbader a Reproductive Biologist at Columbia University[3,16,17]. I acknowledge and thank these and associated investigators for determining hCG three dimensional structure, for without it the whole concept of understanding hCG structure would be lost. Their models have become the generally accepted structures

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of hCG[3,5,15-17]. The problem has been, that in order to crystallize hCG, Lapthorn *et al*., Wu *et al*. and Lustbader *et al*. had to remove all 4 N-linked oligosaccharides from hCG, and remove the protruding β-subunit C-terminal peptide (residues β112-145) with 4 attached O-linked oligosaccharides. The problem is that hCG is a biologically active very acidic glycoprotein (isoelectric point (pI) 3.5). The three dimensional structural model is for a biologically inactive[18], C-terminal peptide-less, oligosaccharide-less basic molecule of pI ~8.5.

I ask, does this molecule deprived of a C-terminal peptide, without its anionic charge, sialic acid, and without its bulk of sugars, have any reflection on the real life hCG molecule? Missing approximately 50% molecular weight it tells us a limited story. It says nothing about why hCG and sulfated hCG bind the hCG/LH receptor, why nicked hCG does not bind the receptor[19,20], or why or how over-sugarized or hyperglycosylated hCG and its cancer free β-subunit bind a TGF-β2 receptor.

Here, very carefully, the three dimensional structure of aglyco-C-terminal peptide-less hCG is examined. N-linked oligosaccharides and C-terminal peptide with O-linked oligosaccharides are added back in and their effects on hCG structures are considered, and the shape changed accordingly considering 11 critical observations. The differences between hCG and hyperglycosylated hCG, hCG and nicked hCG, and between hCG and hyperglycosylated hCG free β-subunit are considered, and new revised three dimensional models are proposed.

2. Methods

In considering structures, and changes due to the return of the oligosaccharides, hyperglycosylation, free subunits and nicking this article considers for its methods 11 critical observations reported previously:

Observation 1. The molecule hCG contains characteristically contains 12 sialic acid residues, 4 on β-subunit N-linked oligosaccharides, 5 on β-subunit O-linked oligosaccharides, and 3 on α-subunit N-linked oligosaccharides. Hyperglycosylated hCG contains characteristically 19 sialic acid residues, 6 on β-subunit N-linked oligosaccharides, 8 on β-subunit O-linked oligosaccharides and 5 on α-subunit N-linked oligosaccharides[3].

Observation 2. That C1, C2, C3, C5 and C7 preparations of hyperglycosylated hCG dimer dissociate more rapidly (mean dissociation ½ time 18 h) than hCG dimer preparations P1, P2, P3, P4, P5 (mean dissociation ½ time 36 h) suggesting lesser α-β subunit interaction or lesser α-β subunit charge, hydrophobic and hydrogen bond linkages between subunits in hyperglycosylated hCG dimers[1,20].

Observation 3. That antibody B204 radioimmunoassay, specific to free β-subunit and β-core fragment has zero affinity for hCG dimer (P1,P2,P3,P4,P5) but limited detection of C1, C2, C3, C5 and C7 hyperglycosylated hCG dimer, suggesting that hyperglycosylated hCG has limited free β-subunit-like binding sites[19].

Observation 4. Attachment of an N-linked oligosaccharide at α-subunit 52 N-linked oligosaccharide attachment site is critical for hCG biological activity at the hCG/LH receptor[19]. The three dimensional aglyco-C-terminal peptide-less hCG structure is altered somewhat by attachment of this N-linked oligosaccharide[18].

Observation 5. That hyperglycosylated hCG dimer and hyperglycosylated hCG free β-subunit and hCG free β-subunit, but not hCG dimer, function through antagonizing a cancer cell TGF-β2 receptor[4,10]. A structure must be exposed on these hCG variants that facilitated TGF-β2 binding, this must be absent on hCG dimer.

Observation 6. That hCG free β-subunit and not hCG dimer antagonizes a TGF-β2 receptor, indicating that α-subunit covers the TGF-β2 binding site[4,10].

Observation 7. That in trophoblast cells 50% of free β-subunit does not combine with α-subunit. It becomes a free subunit because disulfide bonds are limiting at β93-100 and β26-110 and are incomplete[21,22]. This suggest that these disulfide bridges are not completed on free β-subunit.

Observation 8. No subunit combination is found in non-trophoblastic cancer cells suggesting an absence of disulfide bridges needed for subunit combination or very low subunit production[4].

Observation 9. Eight hCG ß-subunit genes are arranges back-to-back on chromosome 19[23]. Alternative hCG β-subunit genes, varying in sequence by one amino acid are expressed by non-trophoblastic cancer cells[24,25].

Observation 10. Nicking of hCG blocks binding and biological action on hCG/LH receptor[26]

Observation 11. Nicking blocks hCG and β-subunit detection by β2 loop-specific antibodies[19,26].

The following webware was used to aid structure determination.

Bioinformatics Toolkit (Max Planck Institute)

Bioinformatics and Computational Biology (IIT Delhi)

3. Results

Figure 1. shows the published hCG X-ray crystal structure[3,5,15-17]. For study, the β-subunit C-terminal peptide is simply attached, and N- and O-linked oligosaccharides points noted. The proven disulfide bridges are put in plac[5] and all charged amino acids (Asp and Glu -, and Arg and Lys +) are marked as are pertinent hydrophobic sequences. This was the model carefully studies for examining the structure together with the 11 pertinent observations

Addition of the O-linked and N-linked oligosaccharides give the molecule a predominant charge. hCG becomes an extreme anionic glycoprotein by adding as described in observation 1, 12 sialic acid residues to hCG or 19 sialic acid residues to hyperglycosylated hCG. These anionic charges probably forms an anionic charge ring on the surface of the hCG with the 12 or 19 highly charged sialic residues strongly repelling each other (Figure 2). The N-linked oligosaccharides at β13, β30 and α78 are seemingly located on the rim of the aglyco, C-terminal peptide-less molecule, as potentially would be the 4 O-linked oligosaccharides

at β121, β127, β132 and β138. The remaining N-linked sugar attachment site, at α52, is not on the rim. To balance the charge ring, the charges repel the charged sugars attached to α52 pulling them and the α2 loop amino acids away from β-subunit towards the rim (Figure 3). In our model, the α2 loop is slightly moved to accommodate the charge on oligosaccharide on hCG, and moved further to accommodate the larger charge on hyperglycosylated hCG oligosaccharides. This is consistent with observation 4 showing that α52 and the α2 loop are moved by addition of the N-linked oligosaccharide. It is also consistent with observations 2 and 3 which indicate that the final model involves some separation of α-subunit and β-subunit sequences.

Considering observations 5 and 6, that hyperglycosylated hCG has TGF-β2 binding properties and that hCG dimer does not, the movement of the α2 loop further away from the β-subunit on hyperglycosylated hCG probably explains the TGF-β2 binding (Figure 3). As shown in observation 6 it is the α-subunit that blocks TGF-β2 receptor interaction on hCG. Pulling away α2 loop seeming exposes the free β-subunit-like TGF-β2 binding site on hyperglycosylated hCG.

A short sequence links the crystal structure of hCG β-subunit β1-112 with the string of four O-linked oligosaccharides on the C-terminal peptide (β121-138). This C-terminal peptide (β112-β145) comprises primarily Pro and Ser amino acids like a mucopolysaccharide, as shown by protein structure webware this structure holding the charged O-linked oligosaccharides seemingly has no clear folding. The 4 O-linked oligosaccharides contribute to the charge ring of the hCG molecule.

It is predicted from the models that regular hCG has a charge loop (Figure 2), with the N-linked oligosaccharide at α52 somewhat protruding from the proposed folded position at α40-56 (#α 2 loop) adjacent β95-111 (Figure 3). The movement of α40-56 (#α2 loop) which has four cationic amino acids at α42, α44, α45 and α51 likely squeezes the safety belt β90-105 with two repelling cationic amino acids at β95 and β95 back somewhat (see model, Figure 4A). No other changes in structure are predicted. A model is shown in Figure 4 panel A. This model is concordant with observations 2, 3, 4, 5 and 6.

3.1. Hyperglycosylated hCG

Results suggest that N-linked oligosaccharide at α52 protrudes further towards the surface in the hyperglycosylated hCG structure than in the hCG structure (Figure 2). Thus peptide α40-56 is dragged further away from β95-111 in hyperglycosylated hCG than in hCG (Figure 3). The movement of α40-56 (#α2 loop) which has four cationic amino acids at α42, α44, α45 and α51 likely squeezes the safety belt β90-105 with two repelling cationic amino acids at β95 and β95 back somewhat (see model, Figure 4C). All told this further separates the α-subunit and β-subunit explaining observations 2 and 3. It also explains observation 4, showing clear movement of the structure surrounding α52. It also seemingly opens the hCG dimer structure to the cysteine knot explaining observation 5 and 6, like on free hCG β-subunit, permitting binding of TGFβ receptor. A model is presented of the proposed hyperglycosylated hCG structure, Figure 4, panel C.

3.2. Nicked hCG

A nick in the charge-hydrophobic loop (#β2 loop) on β-subunit opens a major structure change. This explains observations 9 and 10 or why nicking makes such a major change in β-subunit structure and why it ablates hCG/LH receptor biologic activity[19,20,26]. Residues β39-43 are an arm ending in a charged Arg residue (Arg 43). The sequence

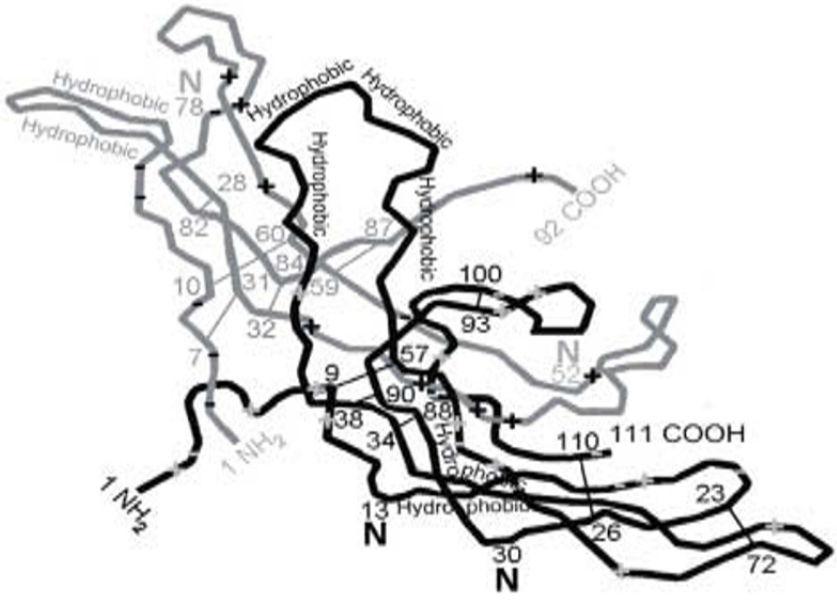

Figure 1. The crystal structure of hCG (3,5,15-17) considering disulfide linkages (added), charge amino acids (added), and hydrophoblic sequences (added).

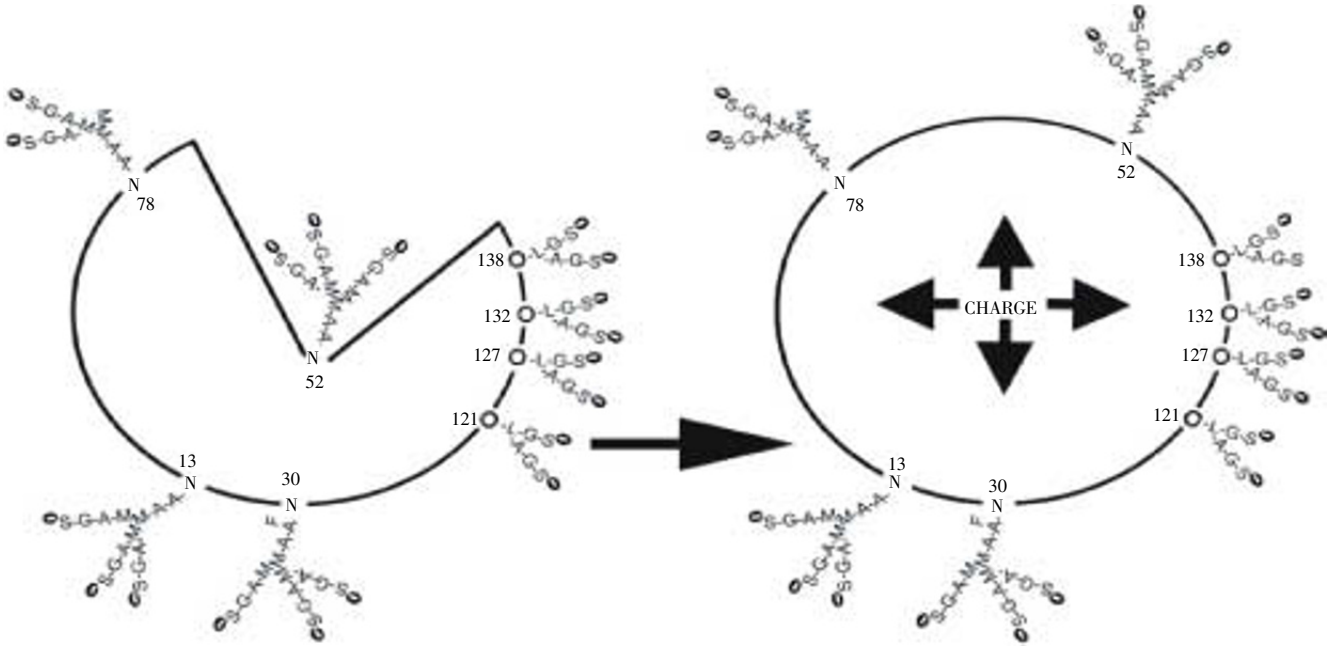

Figure 2. Anionic charge ring of hyperglycosylated hCG showing oligosaccharides in positions shown in Figure 1. Figure illustrates the move in oligosaccharide at α52 to outer ring of molecule.

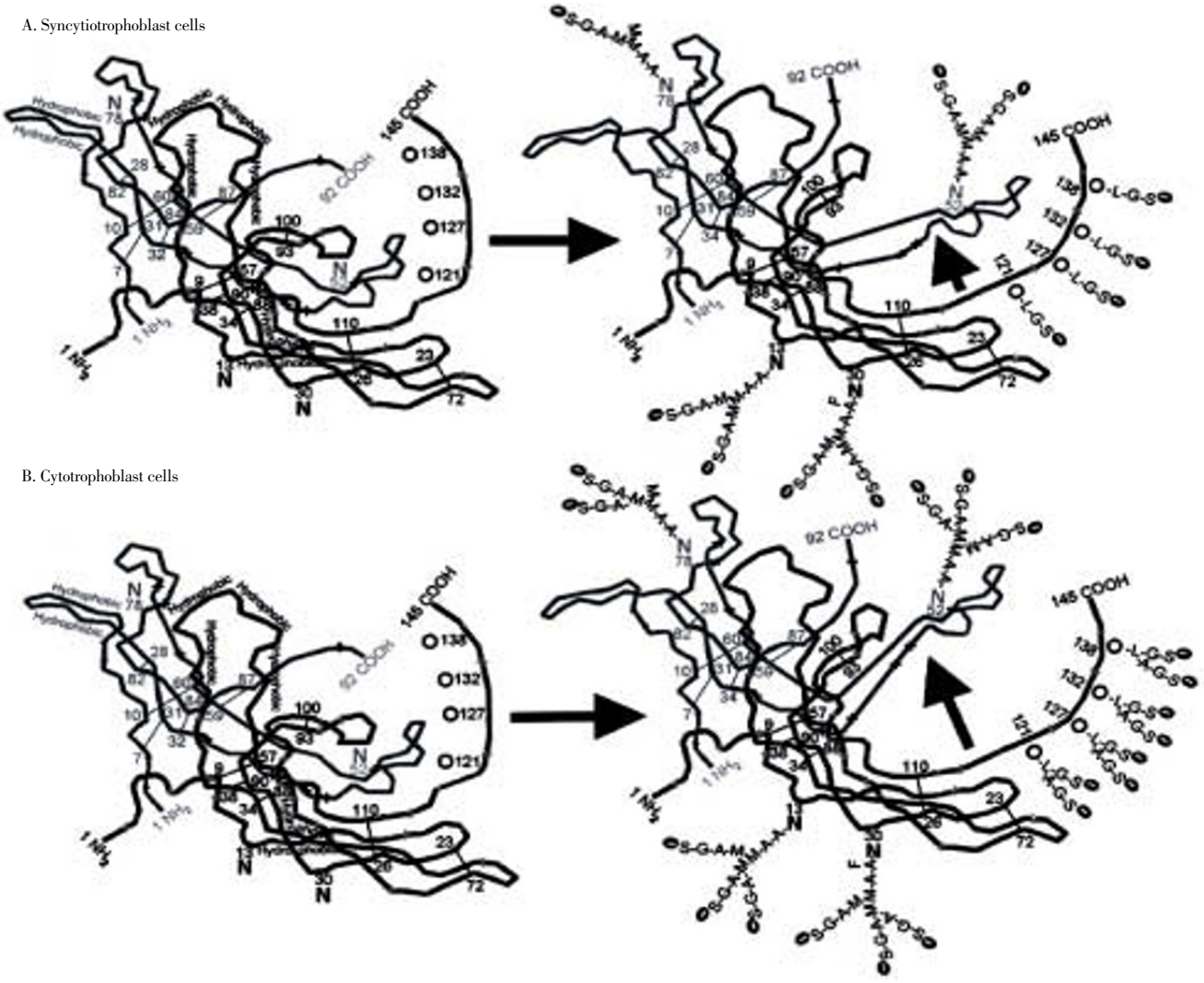

Figure 3. Action of charge on position of oligosaccharide at α52 and associated peptide sequences.

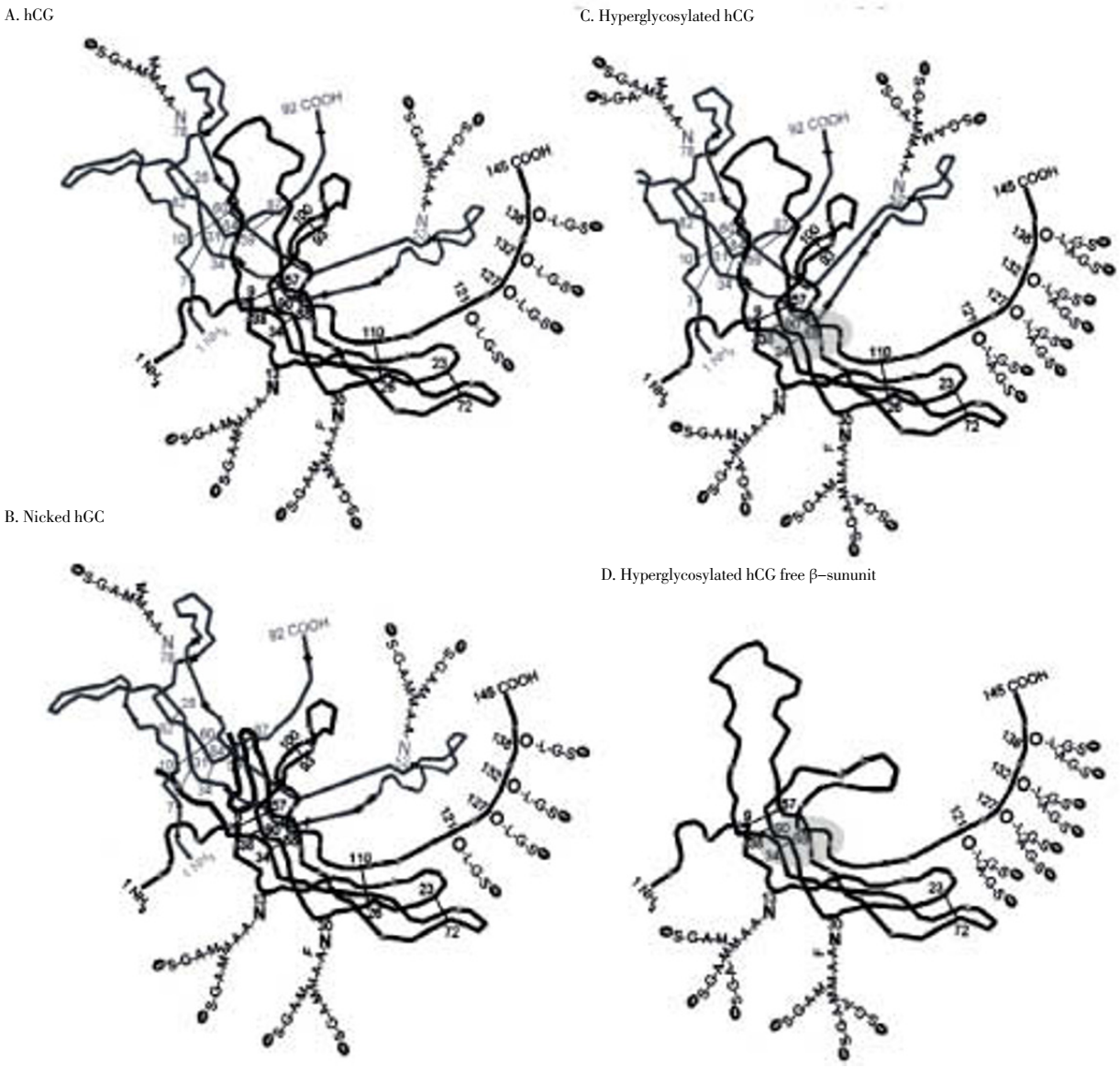

Figure 4. Proposed three dimensional structure of hCG (Panel A), nicked hCG (panel B), hyperglycosylated hCG (Panel C), and hyperglycosylated hCG free β-subunit (Panel D).
Shown in gray shading is the assumed TGF-β2 binding site on hyperglycosylated hCG and its free β-subunit, blocked by α-subunit on hCG and nicked hCG.

β44-55, in contrast, is highly hydrophobic. The charged arm heads for the surface of the molecules, the hydrophobic sequence folds up and buries its self in the middle of the molecule. If a nick occurs at β43-44 with a trypsin-like protease, or β44-45 or β47-48 with leukocyte elastase it opens the loop (#β2 loop) causing a major structure change (Figure 4, panel B). The proposed structure is consistent with observations 10 and 11, with nicking make a dramatic structural change to hCG. Nicking occurs commonly on hCG molecules, and more commonly on hyperglycosylated hCG and hCG free β-subunit molecules[19,20,26].

3.3. hCG free β-subunit

Multiple factors effect hCG free β-subunit conformation. Firstly, the free β-subunit may not fold completely, with absence of β93-100 and β26-110 disulfide bonds, the slow forming or combination rate limits disulfide bonds (observation 7 and 8)[21,22]. Disulfide bonds are incomplete on free β-subunit at 93-100 and at 26-110 in the proposed model, this seemingly leaves lets loop unravel and structurally loosen. Secondly, alternative β-subunit genes may be expressed by cancer cells[24,25] leading to alternative shCG free β-subunit structures, observation 9, (not shown in Figure 4). The proposed structure of hyperglycosylated hCG free β-subunit is shown in Figure 4, panel D. Nicking of free β-subunit likely has similar structural effect on hCG free β-subunit to nicking of hCG.

4. Discussion

A crystal structure has been proposed for hCG dimer missing N-linked and O-linked oligosaccharides and

missing the C-terminal peptide[3,5,15-17]. This molecule is basic with 18 acidic amino acids (Asp and Glu) and 25 basic amino acid (Arg and Lys). It has been estimated that this molecule would have a pI of ~8.5. This article has tried to examine this structure carefully considering the oligosaccharides and C-terminal peptide replaced using protein structure software and webware, and 11 pertinent observations, and tried to estimate the final structures of key hCG-related molecules.

While trying to keep close to the important structural information learned from this aglyco- C-terminal peptide-less hCG crystal structure we have tried to construct likely models for intact complete hCG dimer, hyperglycosylated hCG dimer, nicked hCG dimer and hCG free β-subunit, considering a list of structural and biological observations, Figure 4, panels A-D. In summary, considering the limited resources available and absence of useful structure equipment, only minimal possible changes were made to the representative proposed agylco, C-terminal peptide-less crystal structures[3,5,15-17].

Until such a time as new crystal or other structural techniques are available to determine the structure of highly glycosylated molecules, the proposed models should be considered. Briefly, the biggest changes considered adding back the missing oligosaccharides and C-terminal peptide, are an anionic charge ring and its energy (Figure 2). Nicking refolds the hCG β-subunit structure (loop β2), and basic glycosylation and hyperglycosylation and the charge ring seemingly move the α52 oligosaccharide and loop α2 structure exposing the assumed pertinent cystine knot structure. We ask the reader to consider the proposed model as strictly a proposal, and far from proven.

Conflict of interest statement

We declare that we have no conflict of interest.

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