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Epitope analysis and molecular modeling reveal the topography of the C-terminal peptide of the β-subunit of human chorionic gonadotropin

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Human chorionic gonadotropin (hCG) belongs to a family of heterodimeric glycoprotein hormones that share a common α-subunit and a hormone-specific β-subunit. Among the gonadotropin β-subunits, greater than 85% homology exists between lutropin (hLH)β and hCGβ in their first 114 amino acid residues. However, unlike hLHβ, hCGβ contains a 31-amino acid hydrophilic stretch at its carboxyl end (CTPβ: C-terminal peptide). Although the crystal structure of deglycosylated hCG has been solved, the topography of CTPβ remains unknown. In this study, we have attempted to define the topology of CTPβ using mAb probes. We investigated three epitopes on hCGα, which are hidden in the hCGαβ dimer. However, these epitopes are not hidden in hLH, which has a similar subunit interface to that of hCG, but lacks CTPβ. This suggested that these epitopes are not masked at the subunit interface of hLH or hCG. Hence, we hypothesized that, in the case of hCG, these epitopes are masked by the CTPβ. Consistent with this view, several treatments of hCG that removed CTPβ unmasked these epitopes and enhanced their reactivity with the corresponding mAbs. In order to localise the position of CTPβ on the α-subunit, we used an epitope-mapping strategy [N. Venkatesh & G. S. Murthy (1997) *J. Immunol. Methods* **202**, 173–182] based on differential susceptibility of epitopes to covalent modifications. This enabled us to predict the possible topography of CTPβ. Further, we were also able to build a model of CTPβ, completely independently of the epitope-mapping studies, using a homology-based modeling approach [S. Krishnaswamy, I. Lakshminarayanan & S. Bhattacharya (1995) *Protein Sci.* **4** (Suppl. 2), 86–97]. Results obtained from these two different approaches (epitope analysis and homology modeling) agree with each other and indicate that portions of CTPβ are in contact with hCGα in the native hCG dimer.

Keywords: carboxy-terminal peptide; chemical modification; epitope mapping; glycoprotein hormones; human chorionic gonadotropin.

Gonadotropins, a family of heterodimeric glycoprotein hormones, play a pivotal role in human fertility. The members of this family include human chorionic gonadotropin (hCG), human lutropin (hLH), human follitropin (hFSH) and human thyrotropin. All of them share a common α-subunit within a species and a distinct hormone-specific β-subunit [1]. Among the β-subunits, hLHβ and hCGβ share more than 85% homology. The hCG β-subunit is unique in that it has a hydrophilic stretch of 34 amino acid residues at its C-terminus (CTPβ). For this reason, CTPβ has been considered to be a candidate for the development of hCG-specific antisera and antifertility vaccines [2]. Whereas the crystal structure of deglycosylated hCG is available [3], the topography of CTPβ in the native dimer is as yet unknown. In this paper, we have utilized mAbs as structural probes to gain insight into the structure of CTPβ.

Numerous reports are available on epitope mapping of hCG [4,5]. However, the use of mAbs as structural probes has not been fully exploited because of a lack of quantitative methods for assessment of epitope–paratope interaction [6]. Using a single-step solid-phase radioimmunoassay (SS-SPRIA) developed by us [7] as a quantitative method for assessment of mAb–hCG interactions, we have demonstrated that different covalent modifications affect the reactivity of epitopes with different anti-hCG mAbs to various extents. Based on the fact that epitopes on hCG are differentially susceptible to covalent modifications [8,9], we devised a strategy for mapping assembled epitopes on hCG [7,10]. This strategy has been used to map several epitopes at the receptor-binding region of hCG [10,11]. Besides epitope mapping, we have demonstrated the usefulness of this method for deciphering the proximity of different regions of the α-subunits and β-subunits in the native dimer. These predictions were in agreement with the crystal structure of hCG [11].

In the above studies, mAbs capable of recognizing native epitopes on both the dimer and one of the free subunits were used. It is pertinent to note, however, that one might envisage mAbs that could selectively recognize either the α-subunit or the β-subunit, but not the dimer. These epitopes are disguised in the dimer and unmasked only on dissociation of the subunits. Such epitopes, which are hidden in the native antigen and become unmasked after fragmentation, depolymerization or denaturation, are referred to as cryptic epitopes or cryptotopes [12]. Although studies on the immunochemical mapping of hCG [4,5] are extensive, only a few cryptic epitopes or cryptotopes on hCG have been investigated. Berger *et al*. [13] and Bidart *et al*. [14] have detected two cryptotopes on the hCG

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Abbreviations: hCG, human chorionic gonadotropin; hLH, human lutropin; hFSH, human follitropin; SS-SPRIA, single-step solid-phase radioimmunoassay; CTP, C-terminal peptide.

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α-subunit and mapped them to the dimer interface, using synthetic peptides spanning different regions of hCGα.

In the present study, we have investigated three cryptic epitopes (designated 3, VM11 and H11G11) on the α-subunit, which are masked in the heterodimer by CTPβ. Delineation of these cryptic epitopes enabled us to predict the topography of CTPβ. We also constructed a three-dimensional model for CTPβ using a homology-based modeling approach [15], independent of the epitope-mapping studies. Both epitope analysis and homology modeling suggest similar conformational features of CTPβ.

EXPERIMENTAL PROCEDURES

hCG was prepared by immunoaffinity chromatography from early pregnancy urine. Iodination-grade hCG dimer, hCG α-subunits and β-subunits, hLH and hFSH were kindly donated by A. F. Parlow, NHPP, Bethesda, MD, USA. All reagents for chemical and enzymatic modifications were purchased from Sigma Chemical Company, St. Louis, MO, USA. Carrier-free [^{125}I] was purchased from BARC, Bombay, India. Iodination was performed using the iodogen method [16] and the specific activities of hCG and its α-subunit were 50 000 c.p.m.·ng^{-1} and 35 000 c.p.m.·ng^{-1}, respectively [17]. Plastic tubes for RIA were procured locally.

mAbs used in the present study were obtained from three different fusion experiments. VM11 and VM14 were raised as reported previously, using hCG as an immunogen [9]. Culture supernatants of hybridoma-producing mAb 3 were kindly provided by R. Dighe, Indian Institute of Science, Bangalore, India. The characteristics of mAb H11G11 are reported by Nagy *et al*. [17]. All immunoassays were performed in duplicate.

Characterization of mAbs

Direct binding method. The mAbs were immobilized to plastic tubes through an immunochemical bridge [a mouse IgG–rabbit anti-(mouse IgG) bridge] as described previously [18]. Briefly, tubes were coated overnight with 600 μL normal mouse serum (diluted 1 : 500 in distilled water), washed three times with RIA buffer (0.05 M phosphate buffer containing 0.025 M EDTA, 0.9% NaCl and 0.1% bovine serum) and then incubated with anti-(mouse IgG) (diluted 1 : 2000 in RIA buffer) for 4 h at room temperature. The mAbs from hybridoma supernatants were captured using these tubes. Direct binding assays were performed in triplicate using these mAb-coated tubes. Briefly, mAb-coated tubes were incubated with 100 000 c.p.m. of various iodinated probes (hCGα, hCG, hLH, hFSH) overnight at room temperature. The contents were aspirated the next day, after which the tubes were washed three times with RIA buffer and counted for bound radioactivity in an LKB multigamma counter (Pharmacia).

Cross-reactivity measurements

Immunological cross-reactivity of mAbs towards the free subunits as well as other glycoprotein hormones were measured by SS-SPRIA developed in our laboratory [7]. Tubes coated with the desired mAb (as described above) were incubated overnight at room temperature with 100 μL aliquots of varying dilution of displacing agents (hCG, hCGα, hCGβ, hLH) ranging from 10 to 100 ng per tube and 100 000 c.p.m. of [^{125}I]-labeled hCG in a total volume of 600 μL. At the end of the incubation period, bound radioactivity was measured after the tubes had been washed once with RIA buffer. Non-specific binding was determined in the presence of excess (1 μg per tube) of unlabeled hCG. Relative cross-reactivities were determined from ED_{50} values obtained from displacement profiles.

Epitope mapping

Chemically or enzymatically modified derivatives of hCG and its α-subunit were prepared as detailed in our earlier reports [8,11]. CD spectra of the modified derivatives indicated that there are no gross conformational changes on treatment with the modifying reagents [9]. Further, lack of local conformational alterations at the epitopic region as the result of modification reactions was ascertained using the epitope-protection strategies described previously [9]. Relative immunoreactivities of the modified derivatives with respect to the native hormone were quantified using SS-SPRIA as explained above for cross-reactivity measurements. As indirect effects of modifications were ruled out, losses of immunoreactivity were attributed to the presence of the amino acid residue modified at the epitopic region. Relative involvement of the modified residues in the epitope–paratope interaction were assessed on the basis of the extent of immunoreactivity reduction. Correlating the immunoreactivity patterns with the primary sequence and disulfide constraints, we were able to map the epitopes 3, H11G11 and VM11 using deductive logic as illustrated previously for several other epitopes [8,10,11].

Molecular modeling

The BIOSYM software (Biosym Technologies Inc., San Diego, CA, USA) INSIGHT II and HOMOLOGY modules were used for modeling using the Silicon Graphics Crimson/Elan system. Overlapping fragments of the CTPβ were used to search the sequences of the structures available in the Protein Databank (release no. 79) [19]. The CTPβ was modeled on the basis of the consensus from the searches and the possible secondary-structure predictions. The details of the modeling, minimization and dynamics will be published elsewhere. The model was energy minimized using the DISCOVER module of the BIOSYM software. CVFF potentials were used. The co-ordinates for the hCG were from the PDB entry 1HRP [3]. Energy minimization was carried out for the CTPβ in the context of the rest of the β-subunit in 16 110 cycles of steepest descent followed by 12 000 cycles of conjugate gradient minimization. Subsequently, the CTPβ was energy minimized in the context of the complete hCG including the α-subunits and β-subunits in 1500 cycles of the steepest descent and 3000 cycles of conjugate gradient minimization. The final derivatives were less than 0.01 kcal·mol^{-1}·A^{-1}.

RESULTS

Characterization of mAbs

To determine the specificity of the three mAbs, we initially tested their ability to bind to various iodinated probes (hCG, hCGα, hCGβ and hLH) (Table 1). None of the mAbs recognized [^{125}I]hCGβ. However, they all exhibited severalfold higher binding to the free hCG α-subunit than to the dimer hCG. Thus, we predicted that the epitopes for these mAbs are located at the α-subunit interface. However, these mAbs exhibited enhanced binding to [^{125}I]hLH similar to that with [^{125}I]hCGα (Table 1).

Conclusions based on direct binding are often prone to errors due to differences in binding ability and specific activity of the different labeled probes. Hence, to obtain more quantitative

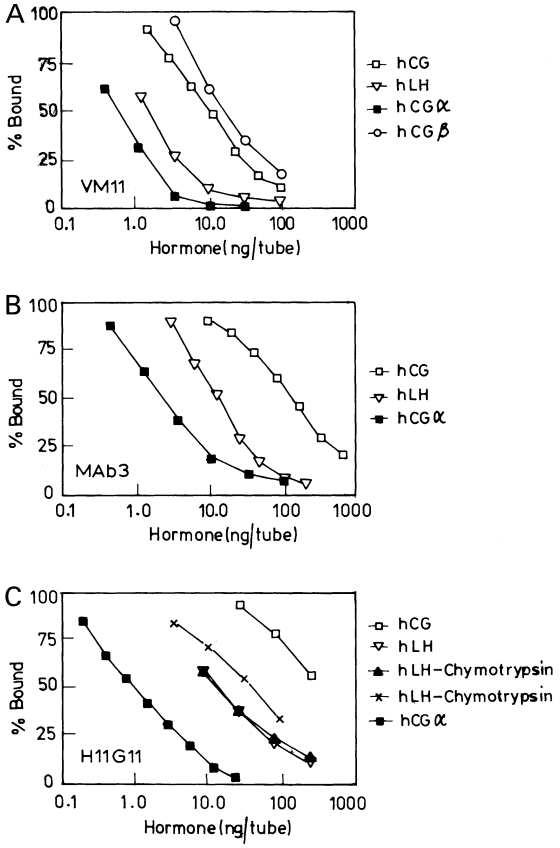

Fig. 1. Inhibition of [^{125}I]hCGα binding to immobilized mAbs VM11 (A), 3 (B) and H11G11 (C) by hCGα, hCGβ, hCG, hLH and chymotryptic digests of hCG and hLH. The data represent the mean values of three independent assays.

comparisons of cross-reactivity, we tested the ability of unlabeled hLH, hCGα, hCGβ and hCG to inhibit the binding of the [^{125}I]hCGα subunit to the three mAbs in competitive displacement RIAs (Fig. 1). The sensitivity of SS-SPRIA using VM11 was the highest and detected at less than 0.1 ng per tube. hCGα showed 50% inhibition at 0.8 ng per tube whereas about threefold higher amounts of hLH and 30-fold higher amounts of hCG were required to obtain 50% inhibition of the maximal binding. A 10-fold higher reactivity to hLH than to hCG was also exhibited by mAb 3. However, the amount of hCGα required to achieve 50% inhibition in RIA using mAb 3 was 10-fold higher than using mAb VM11. This may reflect the differences in affinity constants between the two mAbs. Interestingly, hCGβ displaced the bound tracer from VM11

Table 1. Comparison of binding of [^{125}I]hCGα, [^{125}I]hCG and [^{125}I]hLH to mAbs 3, VM11 and H11G11. Tubes coated with the indicated mAbs were incubated with 100 000 c.p.m. of the iodinated probes (hCGα, hCG, hLH, hFSH), overnight at room temperature. After a washing step, the bound radioactivity was measured in an LKB multigamma counter. Counts represent means of duplicate values in each case.

mAb	[^{125}I]hCG	[^{125}I]hLH	[^{125}I]hCGα
3	4500	24 600	36 000
VM11	24 600	32 300	34 100
H11G11	300	6800	8900

Table 2. Immunoreactivities of chemically or enzymatically modified derivatives of hCG with mAbs 3, VM11 and H11G11. Relative immunoreactivities of the modified derivatives with respect to the native hCG were quantified using SS-SPRIA. Tubes coated with mAbs were incubated overnight at room temperature with 100 μL aliquots of varying dilutions of either native or modified hCG and 100 000 c.p.m. of [^{125}I]hCG in a total volume of 600 μL. After washing, the bound radioactivity was measured. Results represent mean values from three independent assays and are expressed as % immunoreactivity.

Treatment	mAb 3	mAb VM11	mAb H11G11
None	100	100	100
Chymotrypsin	1000	500	500
Trypsin	1000	5000	< 1
Formic acid	1000	450	500

with ED_{50} values comparable with that obtained with hCG, indicating that VM11 interacts with the hCG β-subunit, in addition to the core region in the hCG α-subunit. Of the three mAbs, H11G11 appears to be the most specific for free α-subunit. Half-maximal inhibition of binding of [^{125}I]hCGα to H11G11 required more than 1 μg hCG per tube, while 1.25 ng hCGα per tube was adequate to obtain more than 90% inhibition. Together, these results clearly indicate that there are two regions, one corresponding to mAbs 3 and VM11, and the other to H11G11, both unique to the free α-subunit.

Masking of epitopes on hCGα by CTPβ

The higher reactivity of these mAbs to hLH (lacking CTPβ) prompted us to assess the their ability to bind hCG analogs in which the last 34 amino acids of the β-subunit were removed by chemical or enzymatic cleavage. As expected, the immunoreactivities of all three mAbs were found to be increased on treatment of hCG with chymotrypsin and formic acid (Table 2, Fig. 1C). Trypsin treatment enhanced the activity of mAbs 3 and VM11, but not H11G11. In fact, H11G11 did not bind the tryptic digest of hCGα at all (Table 3), indicating that this epitope is localized near a trypsin-susceptible site. The

Table 3. Immunoreactivities of derivatives of hCG α-subunit with mAbs 3, VM11 and H11G11. Immunoreactivity measurements were made as described in the legend for Table 2. Values represent means from three independent assays using two independent preparations of each derivative and are expressed as % immunoreactivity. TNBS, trinitrobenzenesulfonic acid; TNM, tetranitromethane; PG, phenylglyoxal; CDI, carbodiimide; LAP, leucine aminopeptidase; DEPC, diethylpyrocarbonate.

Derivative	3	VM11	H11G11
hCGα	100	100	100
TNBS	35	30	1
TNM	72	60	10
Trypsin	72	72	2
Chymotrypsin	80	100	80
Formic acid	65	100	100
CNBr	14	21	21
CDI	3	2	< 1
LAP	100	100	100
Acetyl	100	100	8
Acetyl-PG	60	100	5
Acetyl-DEPC	100	100	1

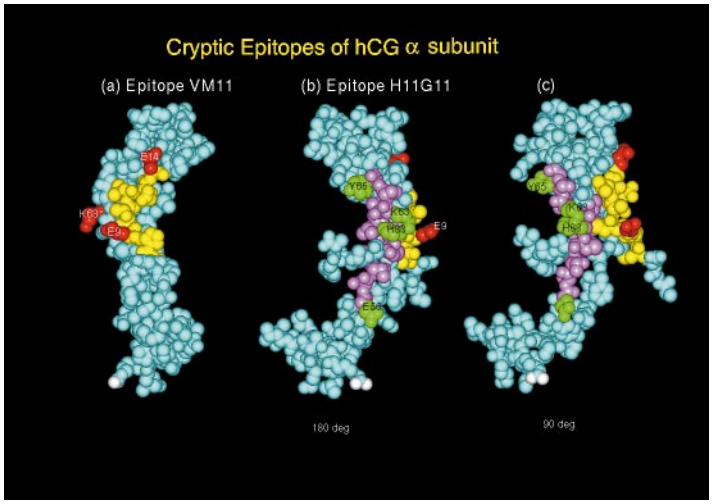

Fig. 2. Structure of cryptic epitopes in hCG α-subunit. Color scheme: α-subunit, blue: backbone of epitopes VM11 (a) and 3, yellow; back of epitope H11G11 (b,c), pink; side chains identified by chemical modification are shown in red for VM11 and 3, whereas those of H11G11 are colored green. The relative orientation of the epitopes are shown by rotation of hCGα with respect to A42 (imino nitrogens are colored white).

possibility that these epitopes represent conformations unique to hLH was ruled out by the fact that treatment of hLH with chymotrypsin (Fig. 1C) or formic acid had no effect on the binding of these mAbs. It should be noted that the cleavage sites are not different between hCG and hLH. Moreover, it is unlikely that any unique conformation, if present, would be rendered stable to such multiple cleavages. In addition, the crystal structure of hCG suggests that the folding pattern of hLHβ is likely to be similar, as there are only 12 amino acid substitutions in the first 112 amino acid residues of hCG. These results strongly suggest that the C-terminal region of hCGβ sterically blocks the binding of these mAbs to the α-subunit.

Epitope mapping

In an attempt to derive topological information on the last 34 amino acids of hCGβ, we characterized the three cryptic epitopes on hCGα at a molecular level. To identify the key residues in contact with these mAbs, we prepared several chemically or enzymatically modified derivatives of the free hCG α-subunit and determined their immunoreactivities with the mAbs using a quantitative SS-SPRIA [7]. The extent of loss or gain in immunoreactivity of the various derivatives with respect to the native α-subunit (Table 3) indicated the relative importance of different residues in the epitope–paratope interaction (Table 4). The immunoreactivity profiles with hCGα derivatives were nearly the same for mAbs 3 and VM11, while that of H11G11 was quite different. By a careful analysis of the sequence data and the available information on disulfides, we deduced the epitope specificities of these mAbs, as illustrated with several other mAbs in our earlier reports [8,10,11]. Crystal structure also provided additional information for mapping these regions. Both epitopes VM11 and 3 were localized to the region α 7–14 (Fig. 2a, backbone is colored yellow). However, unlike epitope VM11, epitope 3 showed marginal loss of immunoreactivity on treatment with phenylglyoxal, suggesting the involvement of R67 in the interaction with the mAb 3. Glutamic acid residues at positions 7 and 14 are most crucial for the binding ability of mAbs 3 and VM11 (Fig. 2a, side chains are highlighted in red), while K63 seems to be relatively less important. The epitopic regions of VM11 and 3 are stabilized by disulfides 28–82, 10–60 and 7–31. Stabilization by M29 is justified by the loss in activity on CNBr treatment. In contrast with these mAbs, binding of H11G11 required several other amino acids in addition to glutamic acid and lysine. The epitopic region of mAb H11G11 is encompassed by residues α 56–65 and α 82–84 (Fig. 2b, backbone is shown in pink). The striking loss on carbodiimide modification (Table 3) implies that, in addition to E56, residue E9 may also be required. All three cystine knot disulfides impart conformational rigidity to this region. M71 plays a similar role in the stabilization of H11G11, as does M29 in case of epitopes 3 and VM11.

Table 4. Relative involvement of amino acid residues in the epitope–paratope interaction.

Region	3	VM11	H11G11
Core	D/E	D/E	K,E/D,H,R,Y
Proximal	K,M	K,M	M
Distant	R,H,F,Y	R,H,F,Y	F

DISCUSSION

Among the gonadotropins, hCG is unique in that it has an extension of 34 amino acid residues at the C-terminus of the β-subunit (CTPβ). While the crystal structure of deglycosylated hCG is available, the structure of CTPβ in the native dimer is yet to be defined. In the present study, we aimed to elucidate the topography of CTPβ in the native dimer employing two different approaches namely, epitope analysis and molecular modeling.

We investigated the epitope specificities of three mAbs capable of recognizing the free hCGα, but showed weak/no binding to the dimer hCG. Initially, we expected that these mAbs were directed towards the α-subunit interface. However, they also recognized hLH, which has a similar subunit interface to that of hCG but lacks CTPβ. Thus, we attributed their inability to recognize the dimer hCG to the presence of CTPβ in hCG. In support of this view, any treatment of hCG that resulted in the removal of CTPβ enhanced the activity of the mAbs. Thus it is evident that the epitopes VM11, 3 and H11G11 are sterically blocked by CTPβ. Delineation of these epitopes would provide useful insights into the positioning of

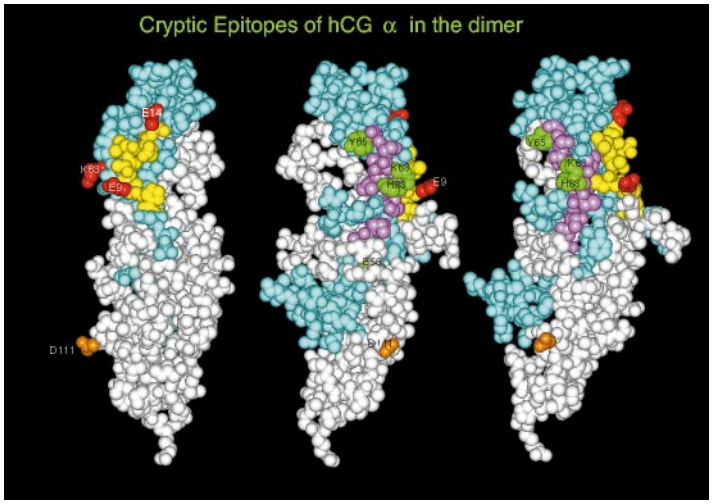

Fig. 3. Cryptic epitopes of hCGα in the dimer hCG. Atoms are colored in the same way as in Fig. 2. D111 of β-subunit is shown in orange. E56 of the α-subunit is blocked by amino acid residues 103–105 of the β-subunit. Rotation of the dimer with respect to D111.

CTPβ over the hCG α-subunit. To this end, we mapped the crucial amino acid residues contributing to the specificity of the mAbs. The epitopes 3 and VM11 encompassed a region in the α-subunit within E7 and E14, while the region comprising α 56–67, α 82–84 and E9 contributed to the binding of H11G11. These regions appear (on the three-dimensional structure of the α-subunit) to be highly solvent accessible and therefore available for antibody binding, thus substantiating our identification of these epitopes. The regions corresponding to epitopes 3 and VM11 (Fig. 2A, backbone shown in yellow) and to epitope H11G11 (Fig. 2B, backbone shown in pink) are related by an angle of about 180 °. This has been shown by rotation of the α-subunit to 90 ° and 180 ° with respect to R42 (Fig. 2C, imino hydrogens are colored white). The side chains of amino acid residues identified by chemical modification are colored differently. Figure 3 shows the relative spatial arrangement of these three epitopes in the dimer. While all the residues of epitopes 3 and VM11 are completely exposed in the dimer (shown in yellow), residue E56 of epitope H11G11 is blocked by amino acids P103 and K104 of the β-subunit. This explains the higher reactivity of mAb H11G11 with the α-subunit than with hLH. In contrast, mAbs 3 and VM11 exhibited comparable activity with hCGα and hLH. Noteworthy is the absence of residues beyond D111 of the β-subunit in the crystal structure of hCG (Fig. 3).

On the basis of our epitope-mapping studies, we hypothesized that, in the dimer hCG, CTPβ spans these three cryptic epitopes. The contact region of CTPβ on the α-subunit can be further narrowed on the basis of our results from epitope mapping of another mAb, designated VM14 which binds both the α-subunit and the dimer hCG (unpublished data). Interestingly, this mAb recognized a region encompassing the residues α 60–67, α 82–84 and E9, thus sharing its epitope with that of H11G11. However, unlike epitopes 3 and VM11, epitope VM14 is not disguised in the dimer. MAb VM14 had a cross-reactivity of only 20% with hLH; hCG derivatives lacking CTPβ bound poorly to mAb VM14, which suggests that CTPβ forms a part of the interacting region for mAb VM14. Thus, by comparing the epitope specificities of mAbs VM11, 3 and VM14, it can be deduced by a process of elimination that CTPβ contacts the region α 56–60. Based on the epitope analysis, we have schematically represented a hypothetical trace of CTPβ over the amino acid residues α7-α14 and α56-α60 (Fig. 4A).

We also built a model for CTPβ in the dimer hCG, totally independent of the results of the aforementioned epitope-mapping studies. The topography of CTPβ modeled using the knowledge-based consensus approach is shown in Fig. 4B. This model shows that CTPβ covers part of epitopes 3, VM11 and H11G11, confirming the localization of the CTPβ from the epitope-mapping studies. The CTPβ loops out over the hCG

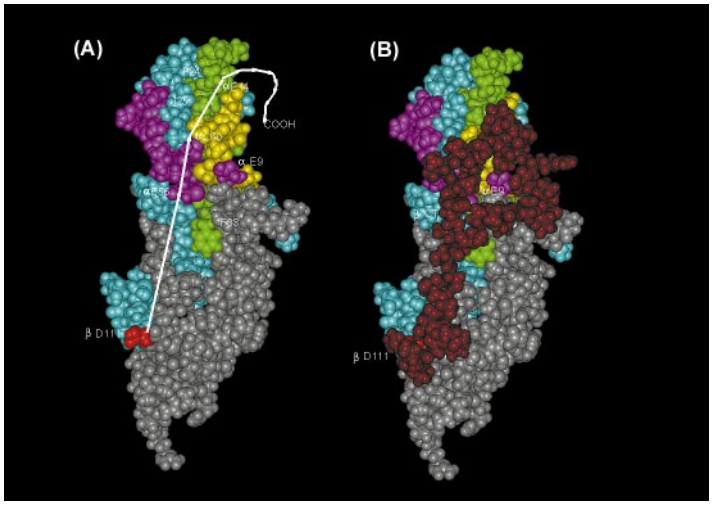

Fig. 4. Agreement between the prediction based on epitope analysis and an independent modeling approach. (A) A space-filling model of hCG schematically representing the tentative path of CTPβ over hCG α-subuint based on mapping cryptic epitopes. (B) Space-filling model of hCG showing the modeled CTPβ. Color code: α-subunit, blue; β-subunit, purple; epitope 3 and VM11, pink; epitope H11G11, yellow. Side chains of the exposed residues within α18–33 are colored green. A hypothetical backbone of CTPβ is indicated as a white line on the CPK model and in green outside the frame of hCG.

overlaying αE9. Several prolines in the CTP give it a twisted conformation. Interestingly, the path of CTPβ in this model (Fig. 4B) is similar to that predicted independently (Fig. 4A) on the basis of delineation of cryptic epitopes VM11, 3 and H11G11. The main difference between the tentative prediction based on epitope-mapping studies and the independent model building is in the location of the last few residues of CTPβ.

Until now, only two epitopes unique to free hCGα and masked in hCG dimer have been localized at the molecular level. A region between amino acid residues 33 and 41 was assigned to an α-specific mAb (AHT20) by Bidart *et al*. [14]. This region forms a part of the subunit interface and is justified by the crystal structure of hCG. Ryan *et al*. [1] characterized two mAbs, designated E502-2 and E511-3, which are specific to the free α-subunit. Interestingly, like our set of mAbs, these two mAbs recognized hLH more efficiently than hCG. The relative cross-reactivities were 100% (hCGα), 1.67% (hCG) and 30% (hLH) which is similar to mAb 3 (hCGα: 100%; hCG: 3%; hLH: 33%). Based on the ability of overlapping synthetic peptides to inhibit the binding of [^{125}I]hCG to these mAbs, their epitopes have been localized to α 18–34. The ED_{50} values were 1×10^4 and 3×10^{10} for the peptide and hCGα, respectively. The amino acid residues 26–33 are buried in this region and are not accessible to the solvent. Some of the residues between positions 19 and 25 are likely to be masked by CTPβ. Interestingly, this region (Fig. 4, shown in green) is located between the two crypic regions of hCGα (epitope H11G11 and epitopes 3 and VM11), justifying the predicted topography of CTPβ.

In conclusion, the topography of CTPβ in the hCG dimer has been predicted using two independent approaches (epitope analysis and molecular modeling). Concordance of the results obtained from the two different approaches emphasize the possible utility of epitope analysis by SS-SPRIA using various mAb probes, as illustrated here, in deciphering the topology of certain regions which may not have been crystallographically determined.

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REFERENCES

1. Ryan, R.J., Keutmann, H.T., Charlesworth, M.C., McCormick, D.J., Milius, R.P., Calvo, F.O. & Vutyavanich, T. (1987) Structure-function relationships of gonadotropins. *Recent Prog. Horm. Res.* **43**, 383–429.
2. Stevens, V.C. (1996) Progress in the development of human chorionic gonadotropin antifertility vaccines. *Am. J. Reprod. Immunol.* **35**, 148–155.
3. Lapthorn, A.J., Harris, D.C., Littlejohn, A., Lustbader, A.W., Canfield, R.E., Machin, K.J., Morgan, F.J. & Isaacs, N.W. (1994) Crystal structure of human chorionic gonadotropin. *Nature (London)* **369**, 455–461.
4. Bidart, J.M., Birken, S., Berger, P. & Krichevsky, A. (1993) Immunochemical mapping of hCG and hCG-related molecules. *Scand. J. Clin. Lab. Invest.* **216** (Suppl.), 118–136.
5. Berger, P., Bidart, J.M., Delves, P.S., Dirnhofer, S., Hoermann, R., Isaacs, N.W., Jackson, A., Klonisch, T., Lapthorn, A., Lund, T., Mann, I., Roitt, I., Schwarz, S. & Wick. G. (1996) Immunochemical mapping of gonadotropins. *Mol. Cell. Endocrinol.* **125**, 33–43.
6. Goldberg, M.E. (1991) Investigating protein conformation, dynamics and folding with monoclonal antibodies. *Trends Biochem. Sci.* **16**, 358–362.
7. Venkatesh, N. & Murthy, G.S. (1996) Dissociation of monoclonal antibody-antigen complexes: implications for ELISA procedures. *J. Immunol. Methods* **199**, 167–174.
8. Venkatesh, N., Nagaraja, G. & Murthy, G.S. (1995) Analysis of a conformation-specific epitope of the alpha-subunit of human chorionic gonadotropin: study using monoclonal antibody probes. *Curr. Sci. (India)* **69**, 48–56.
9. Venkatesh, N. & Murthy, G.S. (1997) Stability of assembled epitopes on human chorionic gonadotropin: analysis using monoclonal antibody probes. *Int. J. Biochem. Mol. Biol.* **42**, 853–863.
10. Venkatesh, N. & Murthy, G.S. (1996) A novel method for mapping assembled epitopes in batches; identification of three epitopes at the receptor binding region of human chorionic gonadotropin. *Curr. Sci. (India)* **70**, 55–62.
11. Venkatesh, N. & Murthy, G.S. (1997) Immunochemical approach to the mapping of an assembled epitope of human chorionic gonadotropin: proximity of CTPα to the receptor binding region of the β-subunit. *J. Immunol. Methods* **202**, 173–182.
12. Van Regenmortel, M.H. (1989) The concept and operational definition of protein epitopes. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **323**, 451–466.
13. Berger, P., Klieber, R., Panmoung, W., Madersbacher, S. & Wolf, G. (1990) Monoclonal antibodies against the free subunits of human chorionic gonadotrophin. *J. Endocrinol.* **125**, 301–309.
14. Bidart, J.M., Troalen, F., Ghillani, P., Rouas, N., Razafindratsita, A., Bohuon, C. & Bellet, D. (1990) Peptide immunogen mimicry of a protein-specific structural epitope on human choriogonadotropin. *Science* **248**, 736–739.
15. Krishnaswamy, S., Lakshminarayanan, I. & Bhattacharya, S. (1995) Knowledge based consensus approach to modeling of McrA. *Protein Sci.* **4** (Suppl. 2), 86–97.
16. Fraker, P.J. & Speck, J.C. (1978) Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3α,6α-diphrenylglycoluril. *Biochem. Biophys. Res. Commun.* **80**, 849–857.
17. Nagy, A.M., Vanbellinghen, A.M., Robyn, C. & Meuris, S. (1996) Epitope mapping on intact, heated and reduced molecular variants of human chorionic gonadotrophin. *Mol. Cell. Endocrinol.* **122**, 51–57.
18. Murthy, G.S., Lakshmi, B.S. & Moudgal, N.R. (1989) Radioimuno-assay of polypeptide hormones using immunochemically coated tubes. *J. Biosci.* **14**, 9–20.
19. Bernstein, F.C., Koetzle, T.F., Williams, G.J., Meyer, E.E., Jr, Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. & Tasumi, M. (1977) The Protein Data Bank: a computer-based archival file for macromolecular structures. *J. Mol Biol.* **112**, 535–542.
20. Ryan, R.J., Keutmann, H. T., Charlesworth, M. C., McCormick, D. J., Milius, R. P., Calvo, F. O., & Vutyavanich, T. (1987) Structure-function relationships of gonadotropins. *Recent Prog. Horm. Res.* **43**, 383-429.