J. Biosci., Vol. 21, Number 6, December 1996, pp 755-764. © Printed in India.

Mapping of assembled epitopic regions of human chorionic gonadotropin reveals proximity of CTPα to the determinant loop ß93-100

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MS received 5 June 1996; revised 21 September 1996

Abstract. Three overlapping assembled epitopes of ßhCG have been mapped using MAb probes and a single step solid phase radioimmunoassay. These epitopes have been shown to be at receptor binding region comprising of the loop region ß Cys93-Cys100. Importance of disulphide bonds in maintaining integrity of these epitopes is assessed. Two MAbs (INN 58 and INN 22) interact with the ß region as well as the α C-terminal peptide, while the other MAb INN 24 interacts with only the ß region. Cross-reactivity pattern with ßhCG and hLH as well as the reported crystal structure of hCG substantiates the epitope identification. The results demonstrate utility of MAbs as probes in investigations on three-dimensional structure of gonadotropins.

Keywords. Epitope mapping; assembled epitopes; human chorionic gonadotropin; monoclonal antibodies; carboxy terminal region; single step solid phase radioimmunoassay.

1. Introduction

Monoclonal antibodies bind specifically to epitopes and some epitopes have been identified for very few antigens, like lysozyme and neuraminidase through X-ray crystallography (Alzavi *et al* 1988; Davis *et al* 1990). This type of identification using X-ray crystal structure is both expensive, time consuming and is unlikely to be of general utility in identifying the regions of interaction, though at present this is the only feasible method for mapping of assembled epitopes. Hence most of the efforts at epitope mapping have been done with linear peptides (Petterson 1992; Weiner and Dias 1992). It suffers from the disadvantage that very small component of the original conformation is identified, as is well documented by the fact that the cross-reactivity of several identified peptides do not reach above 5% of the native antigen (Strasburger *et al* 1989). In most cases linear peptide synthesis fails to identify the region/s as MAbs are frequently directed against the conformation of the protein assembled from different regions of the primary chain by nonpeptidic or noncovalent interactions. Recently we have developed a method for identification of such conformation specific/assembled epitopes (Venkatesh *et al* 1995) and in this paper we report on the identification of the epitopic regions of *ß*hCG, and demonstrate that αC-terminal peptide (αCTP) is in proximity to the *ß* loop region Cys93-Cys100.

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2. Materials and methods

hCG was prepared from early pregnancy urine using affinity chromatography on hCG a/s immobilized on Sepharose. The protein obtained was found to be homogeneous and showed two bands in SDS gel. hCG *ß* was a gift from NHPP program. Iodination was carried out using iodogen (Fraker and Speck 1978). Monoclonal antibodies were gifts from Berger, and their characterization is already reported (Schwarz *et al* 1986). ^{125}I·Na was purchased from Bhabha Atomic Research Centre, Bombay. Plastic wells used for adsorption of MAbs were purchased locally. All other reagents used were of analytical grade.

2.1 *Preparation of MAb coated wells*

Polypropylene micro titre wells were coated with an immunochemical bridge using a procedure similar to the one already described for rabbit antisera (Murthy *et al* 1989) and hybridoma culture fluids (Venkatesh and Murthy 1996). Culture fluids at appropriate dilutions was adsorbed on these wells overnight at room temperature, washed with RIA buffer and stored at 4°C until use. These antibody coated tubes were used within two weeks of adsorption.

2.2 *Single step solid phase radioimmunoassay*

Single step solid phase radioimmunoassay (SS-SPRIA) was carried out by the procedure already described using immobilized MAbs. Briefly MAb coated wells were incubated with 100 µ1 of [^{125}I] hCG (100,000 cpm) and serially diluted standard/modified hCG in a total volume of 250 µl of RIA buffer overnight at room temperature. The wells were washed twice and counted in an LKB multigamma counter.

2.3 *Chemical modifications of hCG*

All chemical modifications were done by the standard procedure as already described (Venkatesh *et al* 1995). Amino group modification by acetylation and trinitrobenzene sulphonic acid (TNBS), tyrosine modification by tetranitromethane (TNM), histidine modification by diethyl pyrocarbonate (DEPC) were carried out with 200 µg of protein. Carboxyl group modification was carried out at pH 5·0 in the presence of 100-fold excess of diaminoethane to eliminate the possibility of intermolecular/intramolecular crosslinking. Methionine peptide bond cleavage was done by CNBr treatment. All enzymatic digestions of hCG were done at neutral pH (0·2 M sodium bicarbonate) for 2 h at hCG concentration of 2 mg/ml and of enzymes at 1 mg/ml. Digestions were terminated by adding suitable inhibitor, diluted to a final concentration of 10 µg/ml in RIA buffer and stored frozen until assay. In all modifications the concentration of the reagent used was in excess of the concentration required to obtain maximal inactivation.

3. Results

Quantification of the binding activity of the modified/proteolyzed hCG was carried out by SS-SPRIA (figure 1, as a representative case). Modification of hCG by TNBS

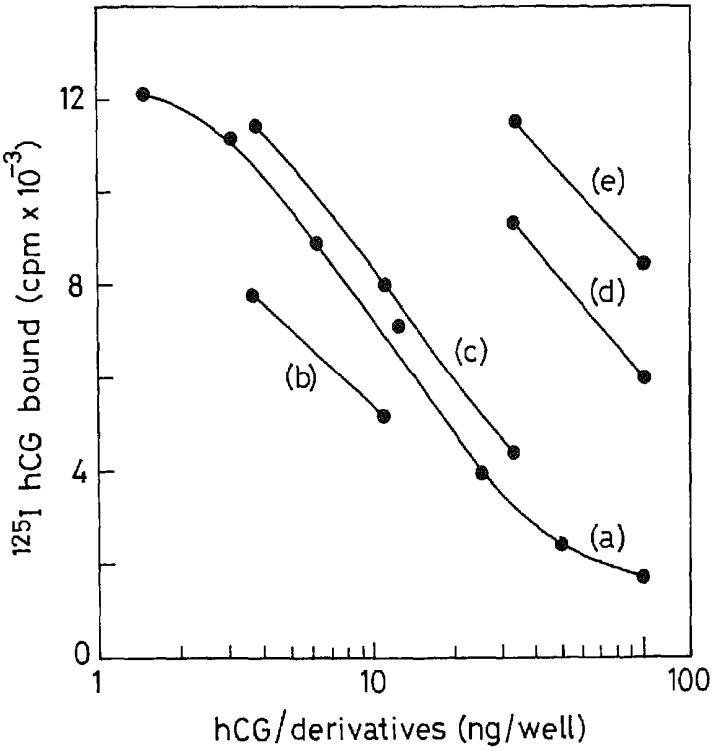

Figure 1. SS-SPRIA using MAb 58 immobilized on plastic wells through immunochemical bridge. Curve a, b, c, d and e present displacement pattern by hCG and modified hCGs.

increases the activity by 100% (curve a and b). However modification by water soluble carbodiimide (CDI) or enzyme digestion by trypsin results in loss of activity of 80–95% (curve d and e), while modification of tyrosines (curve c) brings about marginal loss of activity.

Retention of activity of the subunit for various modifications/enzyme digestions (table 1, columns 1, 2 and 3) show differential loss of activity of epitopes. Likewise loss of activity in modified hCG $\alpha\beta$ dimer are variable (table 1, columns 4, 5 and 6).

Identification of the position of amino acids at the core, proximity or distant to an epitope has been done based on the results presented for inactivation of the ß subunit (from table 1) in table 2. It is seen that all the three epitopes have an R, D/E at the core region. Even though the core region appears to be same they are not identical epitopes as seen from their cross-reactivity pattern as well as differences in the pattern of inactivation on modifications (table 1, columns 4, 5 and 6).

4. Discussion

Identification of amino acid residues near epitopic region has been done from the inactivation profile of the (binding activity) epitope after modification. Chemical modification data has been taken for identification of amino acid residues at the epitope, and enzyme digestion data has been used as supportive evidence. This has been necessitated by

Table 1. Retention of binding activity of modified hCG *β* and hCG *αβ* to different MAbs.

Modifications of/ by (group affected)	hCG *β**			hCG*αβ***		
	Retention of activity in SS-SPRIA with immobilized MAbs					
	INN 24	INN 58	INN 22	INN 24	INN 58	INN 22
None	100	100	100	100	100	100
TNBS (Amino)	84 ± 10	194 ± 33	233 ± 20	**35**	**36**	**17**
TNM (Tyr OH)	79 ± 5	81 ± 7	87 ± 12	**52**	**42**	**38**
CDI (COOH)	9	15 ± 1	8	31	25	25
HCOOH (Term. pep)	142 ± 26	110 ± 11	98 ± 8	110	100	**65**
CNBr (Meth)	148	108 ± 11	99 ± 6	110	100	**72**
Trypsin (K, R)	8 ± 2	7 ± 1	4	5	6	4
Chymotryp (F, Y)	97 ± 12	73 ± 11	103	87	**37**	**36**
LAP (N-term)	112	87 ± 7	127 ± 17	105	92	100
CPAse (C-term)	121	89 ± 10	107 ± 11	100	84	81
DTT (Cys-Cys)	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1	< 0·1
Modification of derivatives						
Acetyl *αβ***				100	100	100
Acetyl *αβ* + Phe.Gly.				28	18	32
Acetyl *αβ* + Trypsin				5	8	7
Cross-reactivity†						
hCG				100	100	100
hLH				6	200	< 1
hCG *β*				100	72	60

*All *β* modification results were done in several assays. The values obtained are of assays the same derivative, run at the same time. SD indicates values obtained in atleast 3 assays while those presented otherwise were duplicates of two determinations.

**Acetylated hCG was taken as control for all these reactions to eliminate any possibility of amino group reaction of hCG with these reagents. Acetylated hCG shows nearly 100% retention of activity.

***Data presented in bold letters in columns 4, 5 and 6 shows modifications where the subunit and the dimer differ quantitatively.

†All cross-reactivity determinations were done using freshly prepared solutions of hLH and hCG obtained fresh from NHPP.

the observation of Birken *et al* (1986) that subunits of hCG are not hydrolyzed completely by trypsin and there are regions which have been identified as nonaccessible for enzymatic digestion (Birken *et al* 1987). Assessment of amino groups released after extensive digestion of hCG with trypsin and chymotrypsin (Venkatesh *et al* 1995) matches well with the observation of Birken *et al* (1987).

Utilizing the above approach amino acid residues close to the three epitopes have been identified as shown in table 2. The core region of all the epitopes (where loss of activity after specific side chain modification is more than 70%) shows presence of trypsin digestible arginine, and carboxyl group. K., M and Y are away from the epitopes. Arginine peptide bonds that are susceptible for tryptic digestion in hCG *β* are R43, R74, R95 and R114 (Birken *et al* 1986; Murthy and Venkatesh 1996). Of these arginines R43 and R74 do not have a carboxyl group near and are away from disulphide bridge. Even when the sequences

Table 2. Identification of amino acid residues on the basis of loss of activcity of hCG *β* at epitopes.

	INN 24	INN 58	INN 22
Arginine-SC	+	+	+
Arginine-PB	+	+	+
Lysine-SC	–	+	+
Lysine-PB	–	ND	ND
Tyr-SC	–	–	–
Tyr-PB	–	–	–
Phe-SC/PB	–	–	–
Met-PB	–	–	–
D/E - SC	+	+	+
His-SC	–	–	–
Cys-Cys	+	+	+
Residues at			
(a) Core	R, D/E	R, D/E	R, D/E
(b) Proximal	–	K	K
(c) Distant	K, M, Y	M, Y	M, Y

Residues whose modification results in > 70% loss of activity is considered to be at the core region, those between 30-70% are considered to be at the proximal region and the others as distant. An increased activity seen with lysine modification with TNBS is also considered proximal. Sidechain (SC) contributions are ascribed from chemical modification data while those of the peptide bond (PB) adjascent to K, R, F and Y by enzyme digestion data. [+]Proximity of the residues to the epitope, and- residues to be away from the epitopic region. ND, not done.

are screened through the disulphide bonds as shown in figure 2, R43 has no carboxyl group in proximity. However R74 has D21 in proximity through the disulphide bridge C72–C23. However the proximity of R74 and D21 has K20 nearby. Stability of these epitopes to TNBS modification rules out the R74 as part of the epitope. Thus R74, D21 and R43 are eliminated from the epitopic region and hence point to R94 and R95 as epitopic arginines. This conclusion is further substantiated by the observation that D99 is brought very close to these arginines through disulphide bond *β* C93–C100. This region does not have in its primary sequence K, M, Y or digestable F at proximity. Thus the core region of all the three epitopes are identifiable with the peptide loop C93–C100 in hCG *β*. Cross-reactivity profile of hLH and hCG*β* are infact different for each MAb, and even in the core region the specificities of recognition are different as seen by their cross-reactivity with hLH (MAb INN-22 and INN-24 do not recognize hLH while INN-58 recognizes hLH with almost same avidity as hCG, table 1). This has been utilized in identifying amino acid residues which cannot be identified by chemical modification. The core of the epitopic regions based on chemical/ enzymatic modification data as well as cross-reactivity data is shown in figure 2.

Even though core region of all the epitopes are same in *β* subunit, they are not identical as seen by the extent of losses of activity on modification, as well as by the differences in the extent of inactivation of these epitopes in hCG *αβ* (table 1, columns 4, 5 and 6). One of the feature that is observed in all the three epitopes is that while modification of K and Y do not lead to loss of epitope structure in the *β* subunit, the same modifications in the *αβ* dimer

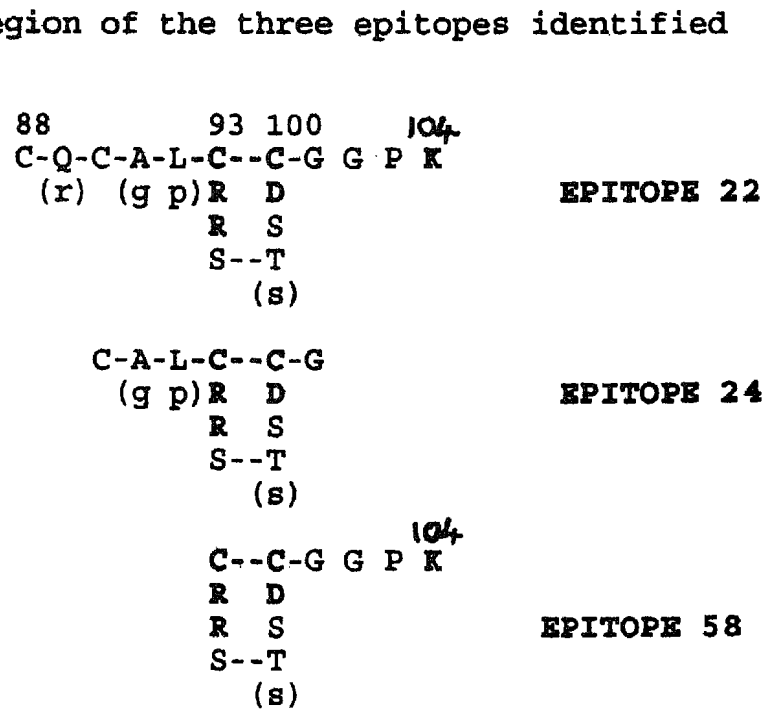

Figure 2. Schematic presentation of the core regions of epitopes 22, 24 and 58 (not drawn to scale). Replacement of amino acids in *ß*hLH is indicated in parentheses and small letters. Replacements are in residues 89, 91, 92 and 97. Amino acid residues presented in bold are identified by the modification (based on table 2).

results in significant loss of binding activity. Formic acid treatment of the dimer α*ß* results in loss of binding for MAb 22, but not for MAbs 24 and 58. Chymotryptic digestion of the dimer causes significant loss of structure for epitope 58 and 22, but not for 24. These results clearly demonstrate that the epitopes, though are specific for the *ß* subunit have contributions coming from the a subunit or indicate the proximity of a subunit sequences to the epitopes.

Loss of activity in hCG α*ß* by HCOOH and chymotryptic digestion indicates a role for α-Y88, Y89 in paratope interaction. Removal of Y89 by chymotryptic digestion (table 1) causes 60% loss of activity and suggests Y89-S92 as partly involved in interaction with MAb INN-22. In contrast epitope 58 loses activity on chymotryptic digestion, but not on HCOOH treatment. This indicates a role for Y89 in the interaction with paratope, but probably excludes any role for other amino acids (H90-S92). In contrast, epitope 24 does not lose activity either by HCOOH treatment or by chymotryptic digestion, thus excluding all the amino acid residues (Y88-S92) from interacting with the paratope. CTP α, thus forms part of epitopes 22 and 58. This conclusion is further substantiated by the observation that *ß* cross-reactivity is in the order MAbs 24 > 58 > 22, in accordance with the involvement of the CTP, where its involvement is none with epitope 24 and most with epitope 22 (table 1).

Even though experimental data indicate marginal roles for Y89 of CTPα and little role for K 91 by enzymatic removal of these residues, chemical modification of these residues causes significant loss of activity in all the three epitopes. Chemical modification unlike

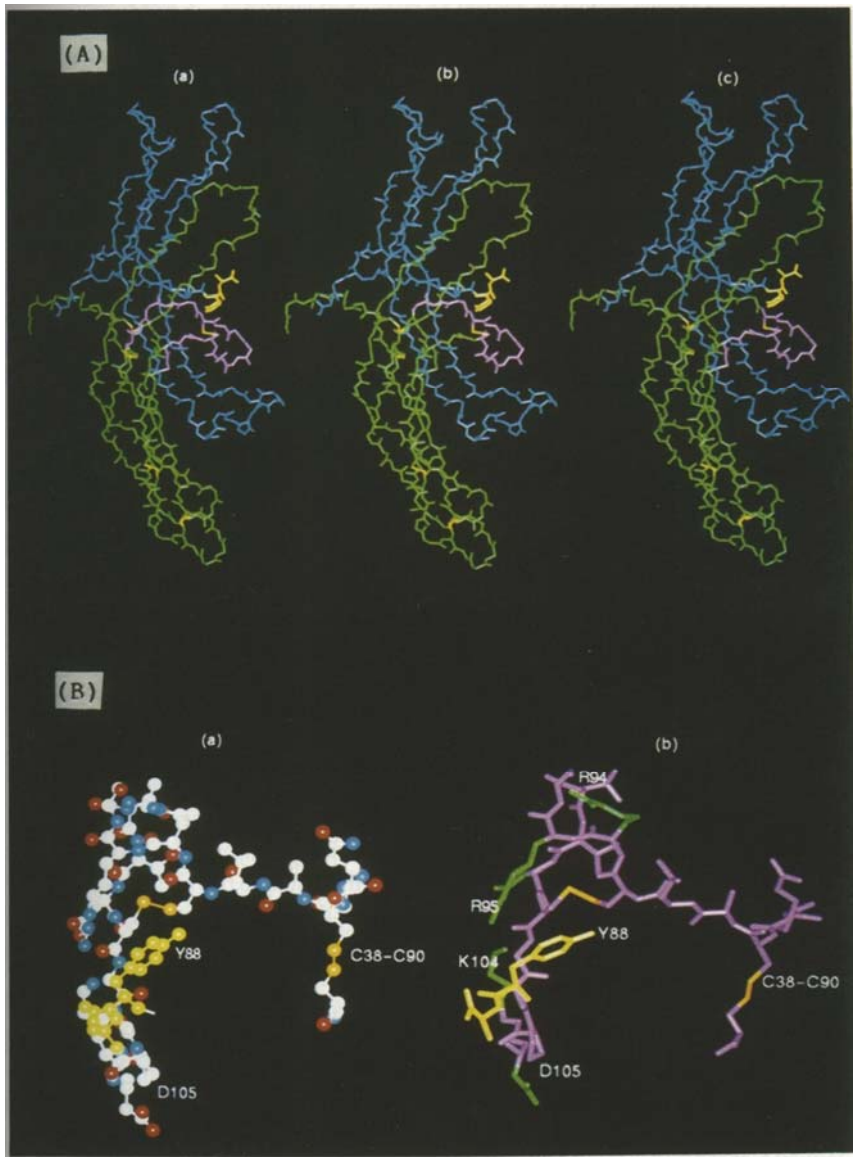

Figure 3. Pictorial representation of the epitopes in the X-ray structure of hCG [using insight II (biosym)]. **(A)** C-a trace of hCG dimer highlighting the epitopic regions of 22(a), 24(b) and 58(c) in pink. Colour code: dark yellow, disulphide bonds; green, *β* subunit; blue, α subunit. Residues Y88 and Y89 of CTP α are projected as ball and stick (coloured yellow). **(B)** Proximity of α CTP to the determinant loop *β*93-100 in ball and stick model (a) and stick model (b). Colour code: yellow, CTP α containing Y88, Y89; pink, *β* epitopic region (in the ball and stick model the atoms N, O and C are coloured blue, red and white respectively)

enzymatic removal results in increase in bulk of the side chain and hence points to the presence of these residues (Y 89, K 91) at/close to epitopic region. This is also justified by X-ray crystal structure of hCG. It is seen that distance between the OH of Y88 and N of R-95 (of *β*) is 4·16 A by the X-ray structure of hCG (see figure 3). Thus in chemical

modification dimension of the side chain is increased significantly and thus the noninteracting side chains of K91 may get bulkier to hinder the access of the paratope to the core of the epitope in the *ß* subunit (C93-C100) sterically, and hence shows loss of activity. Similarly modification of Y by TNM increases the bulk of the Y88 and Y89 and causes inactivation. Increased activity of epitope 22/58 after TNBS modification of *ß* subunit may indicate the proximity of K104 to the epitope, but may not form interacting region. On reaction with TNBS, hydrophobic side chain may add to the hydrophobic interaction between MAb-INN-22 and modified epitope 22 (by TNBS).

Based on these observations epitopes 24, 22 and 58 are represented as shown in figure 3. Proximity of the Y88 and R95/C93 is seen clearly in the model of the X-ray structure and also explains all the experimental data. The core region represented in pink colour is exposed to the solvent in the *αß* dimer, clearly indicating that the epitopes are accessible to respective paratopes for interaction. The figure also shows proximity of Y88 and Y89 (coloured yellow) which explains the involvement of CTP α in epitopes 24 and 58.

The method discussed above has several features which make it very useful as a routine method for epitope mapping. It identifies assembled epitopes with a simple method and does not need an expensive infrastructure, and is adoptable for batch procedure/processing. Its potential to demonstrate the importance of the disulphide bonds in conferring the stability to the conformation of the epitope is very obvious. In addition other regions of the molecule, away from the core region, are also identifiable as is shown in involvement of CTPα in the above epitopes, an information almost impossible to be obtained by any other method. Even though CTPα is part of epitope 58, cross-reactivity of α subunit with MAb INN-58 is insignificant. Both RCMhCG and CTPa fail to displace [^{125}I] hCG in MAb SS-SPRIA and indicates the limitation of the linear peptide synthetic approach for identification of such epitopes. It is also observed by Dirnhofer *et al* (1994) that linear peptides synthesized fail to show cross-reactivity with several of these MAbs. Quantification of extent of changes in the binding activity on modification also provides in several instances data on the proximity of regions. Thus in this instance proximity of epitope region *ß*93-*ß*100 to α-Y88 is very clear. Justification of this data is seen by the crystallographic structure, indicating the usefulness of this approach in picking up the proximity of regions away from each other, sometimes from different subunit.

The validation of the method probably may have to await the synthesis of disulphide linked peptides to demonstrate cross-reactivity. However there are several evidences of validation. The method has identified α CTP (Y88/Y89) to be close to C93-C100 of the *ß* chain, a data substantiated by X-ray structure (Lapthorn *et al* 1994). The three epitopes investigated above should be very closely placed as their core region is all the same, and is clearly demonstrated by the IRMA method (Schwarz *et al* 1986). All the three epitopes have their core at the receptor binding region and hence we can predict that all the three MAbs should inhibit the binding of the [^{125}I] hCG to its receptor. Infact the data obtained on receptor binding inhibition (RBI) assay clearly demonstrates that all the three MAbs are positive inhibitors in RBI assay (table 3). The core region identified should be exposed to the solvent in the dimer and is infact seen in figure 3. These observations provide a clear indication that the above method of epitope mapping is a valid approach for identification of assembled epitopes, and also indicates the feasibility of a batch approach for epitope analysis for proteins.

Identification of the epitope recognizing MAb INN-58 done in this study is in variance with those obtained with the peptide synthesis approach earlier (Dirnhofer *et al* 1994). Peptide sequence identified for this epitope by competitive RIA using peptides indicate *ß*45-*ß*52 as forming part of the epitopic region, whereas in the pepscan method none of the peptides reacted

Table 3. Inhibition of [^{125}I]hCG binding to corpus luteum receptor by monoclonal antibodies.

	Dilutions of MAbs used		
MAbs	1/13500	1/40500	1/121500
	(% inhibition in RBI assay)		
INN 22	96·8	82·6	65·9
INN 24	88·3	77·4	51·8
INN 58	95·3	86·8	65·7

MAbs were taken and incubated with [^{125}I] hCG for 90 min and 100 µl of receptor was added. After an hour it was precipated with 5% PEG and pellet was counted.

with the antibody. However our data indicate that receptor binding region *ß*93-100 is the core region of epitope 58. The reason for this variance is not known presently. Considering that this is one of the early report where epitope mapping of conformation specific epitope by two methods are available, it would be difficult to rationalize the results. Based on X-ray crystal structure data, modification approach scores over peptide synthesis approach as the region *ß*45-*ß*52 is removed far away from the receptor binding region (Lapthorn *et al* 1994). Like in the case of hCG identification of conformation specific epitope of cytochrome c does not match with the peptides synthesized (Savoca *et al* 1991; Schwab *et* al 1993; Leder and Bosshard 1994). It is also observed that not a single epitope out of eight identified by Charlesworth *et al* (1991) for hCG αshow cross-reactivity with the peptide region α60-α67, though our data indicates that this forms a major immunodominant region for the two α specific epitopes we have investigated by our method (Venkatesh *et al* 1995; Venkatesh N, Nagaraja G and Murthy G S, unpublished results). Two epitopes FBT 10 and FBT11 (Robert *et al* 1994) which shows specificity to the *ß* hCG have been identified to have *ß*-22, 29, 74, 75, 95 and *ß*1-7, *ß*51 as hypothetical epitopes. All the epitopes investigated (total of 8 nos) have the core region at the *ß*93-*ß*100 (Murthy and Venkatesh 1996). Thus there are discrepencies seen in the results obtained by peptide synthetic method and our method. However comparisons with more number of epitopes and identification of the epitopes for several MAbs by the two methods would have to be done to arrive at a valid generalization.

One of the anamoly that is in variation with the above identification is that these MAbs recognize *ß* core fragment (*ß*cf) of hCG which is lacking residues 93 onwards (Dirnhofer *et al* 1994). We have no explanation at hand presently for this anamoly. One of the observations we have made is that when the *ß*cf is treated with DEPC or TNBS (to modify His and Lys respectively) it looses its ability to bind to MAb 12 (this has K105 and H106 as proximal region for that epitope) (Murthy and Venkatesh 1996). *ß*cf should not have any histidine as per the sequence and hence indicates the possibility that *ß*cf may still have the 93-106 region, as suggested by O'Connor *et al* (1994) based on the failure to identify a free sulphydral in the *ß*cf. More experiments needs to be done to resolve the anamoly.

Acknowledgements

Authors thank Dr Peter Berger, Innsbruck, Austria for providing monoclonal antibodies, Mr N Venkatesh for helpful discussions and insight (Biosym) photographs and

Prof, N R Moudgal for his continued interest in the work. We are grateful to Prof. N W Isaacs, University of Glasgow, UK for providing the X-ray structure coordinates for hCG.

References

Alzavi P M, Lascombe M B and Poljak R J 1988 Three dimensional structure of antibodies; *Annu. Rev. Immunol.* **6** 555-580

Birken S, Kolks M A G, Amr S, Nisula B and Peut D 1986 Tryptic digestion of the α subunit of human chorionic gonadotropin; *J. Biol. Chem.* **261** 10719-10727

Birken S, Kolks M A G, Amr S, Nisula B and Peut D 1987 Structural and functional studies of the tryptic core of the human chorionic gonadotropin in *ß*-subunit; *Endocrinology* **121** 657-666

Charlesworth M C, Bergert E R, Morris J C, McCormick D J and Ryan R 1991 The antigenic structure of the human glycoprotein hormone α-subunit: III. Solution- and Solid-Phase mapping using synthetic peptides; *J. Endocrinol.* **128** 2907-2915

Davis D R, Padlan E A and Sheriff S 1990 Antibody antigen complexes; *Annu. Rev. Biochem.* **59** 439-473

Dirnhofer S, Madersbacher S, Bidart J M, Ten Kortenaar P B W, Spottl G, Mann K, Wick G and Berger P 1994 The molecular basis for epitopes on the free subunit of human chorionic gonadotropin, its carboxy terminal peptides and the core fragment; *J. Endocrinol.* **141** 153-162

Fraker P J and Speck J C 1978 Protein and cell membrane iodination with sparingly soluble chloramide 1, 3, 4, 6-tetrachloro-3, 6-diphenylglycouril; *Biochem, Biophys. Res. Commun.* **80** 849-857

Lapthorn A J, Harris D C, Little John A, Lustbader J W, Canfield R E, Machin K J, Morgan F J and Issacs N W 1994 Crystal structure of human chorionic gonadotropin; *Nature* (*London*) **369** 455-461.15

Leder L and Bosshard H R 1994 Immunoreactivity of cytochrome c: Antibodies to horse cytochrome c distinguish between sequence-related cytochromes only at the level of the 3-D-structure; *Biochimie* **76** 465-470

Murthy G S, Lakshmi B S and Moudgal N R 1989 Radioimmunoassay of polypeptide hormones using immunochemically coated plastic tubes; *J. Biosci.* **14** 9–20

Murthy G S and Venkatesh N 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.* **70** 55-62

O'Connor J F, Birken S, Lustbader J W, Krichevsky A, Chen Y and Canfield R E 1994 Recent advances in the chemistry and immunochemistry of human chorionic gonadotropin: Impact on clinical measurements; *Endocrine Rev.* **15** 650-683

Petterson I 1992 Methods of epitope mapping; *Mol. Biol. Reports* **60** 149–153

Robert P, Troalen F, Bellet D, Bousfield G R and Bidart J M 1994 Immunochemical mapping of human lutropin; I. Delineation of a conformational antigenic determinant; *Mol. Cell. Endocrinol.* **101** 11–20

Savoca R, Schwab C and Bosshard H R 1991 Epitope mapping employing immobilized synthetic peptides. How Specific is the reactivity of these peptides with antiserum raised against the parent protein?; *J. Immunol. Methods* **141** 245–252

Schwab C, Twardek A, Lo T P, Brayer G D and Bosshard H R 1993 Mapping antibody binding sites on cytochrome c with synthetic peptides: Are results representative of the antigenic structure of proteins?; *Science* **2** 175-182

Schwarz S, Berger P and Wick G 1986 The antigenic structure of human chorionic gonadotropin as mapped by murine monoclonal antibodies; *Endocrinology* **118** 189–197

Strasburger C J, Kostyo J, Vogel T, Bernard G J and Kohen F 1989 The antigenic epitopes of human growth hormone as mapped by monoclonal antibodies; *Endocrinology* **124** 1584–1557

Venkatesh N and Murthy G S 1996 Dissociation of monoclonal antibody-antigen complexes: Implications for ELISA procedures; *J. Immunol. Methods* **199** 167–174

Venkatesh N, Nagaraja G and Murthy G S 1995 Analyasis of a conformation-specific epitope of the alpha subunit of human chorionic gonadotropin; Study using monoclonal antibody probes; *Curr.Sci*. **69** 48–56

Weiner R S and Dias J A 1992 Identification of assembled epitopes on the α subunit of human follicle stimulating hormone; *Mol. Cell. Endocrinol.* **85** 41–52

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