Antigenic Determinants on Human Choriogonadotropin α-Subunit

I. CHARACTERIZATION OF TOPOGRAPHIC SITES RECOGNIZED BY MONOClonAL ANTIBODIES*

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Immunochemical studies were designed to localize antigenic regions recognized by two monoclonal antibodies directed against the α-subunit of human choriogonadotropin (hCG-α) and to provide information on the three-dimensional structure of hCG and its α-subunit. Monoclonal antibody HT13 bound to a region accessible on both hCG and the free α-subunit, whereas monoclonal antibody AHTZO recognized a site localized only on the free α-subunit. By studying the cross-reactivity of these antibodies to homologous proteins, we found that antibody HT13 did not bind to equine or ovine lutropin, whereas AHTZO was capable of binding to both subunits. This observation suggests that AHTZO recognized a structurally related antigenic determinant on α-subunits of different species. To delineate the portions of hCG-α contributing to the antigenic determinants of AHTZO and HT13, we performed competitive inhibition assays using reduced and carboxymethylated hCG-α, deglycosylated hCG-α, hCG-α minus the 5 COOH-terminal residues (hCG-α core 1), or disulfide-bridged peptides comprising residues 1–35 and 52–91 of hCG-α (hCG-α core 2). Reduced and carboxymethylated hCG-α did not inhibit the binding of 125I-labeled hCG-α to both antibodies, whereas deglycosylated hCG-α was as active as hCG-α, suggesting that antigenic determinants of both antibodies are mainly discontinuous and do not reside on the oligosaccharide part of the α-subunit. hCG-α core 1 had the same capacity as intact hCG-α to inhibit the binding of 125I-hCG-α to both antibodies, indicating that the 5 COOH-terminal residues of hCG-α do not participate in the antigenic determinants. hCG-α core 1 was as potent as hCG-α in inhibition experiments performed with HT13, whereas, in striking contrast, hCG-α core 2 did not compete with 125I-hCG-α for binding to AHTZO, suggesting that the peptides released after proteolysis of the α-subunit by trypsin participate in the epitope of AHTZO and are not included in the antigenic determinant of HT13. In an attempt to elucidate the amino acid residues constituting the antigenic sites of HT13 and AHTZO, hapten inhibition experiments were carried out using as competitive inhibitors five different synthetic peptides spanning the primary structure of hCG-α. None of these peptides inhibited the binding of 125I-hCG-α to HT13. In contrast, two peptides analogous to regions 23–43 and 39–59 of hCG-α exhibited significant potency in competing with 125I-hCG-α for binding to AHTZO. As no inhibition of binding to AHTZO was observed with the peptide analogous to region 41–59 of hCG-α, we were led to believe that residues 33–41 constitute part of the antigenic site recognized by AHTZO. Taken together, these data strongly suggest that the antigenic determinant recognized by AHTZO involves several epitopic residues included in region 36–41 of hCG-α, namely Ala-Tyr-Pro-Thr-Pro-Leu. Finally, “two-site” immunoradiometric assays performed with both antibodies and either native or modified hCG-α confirmed that the epitope of AHTZO, localized in the central portion of hCG-α, which is highly conserved among homologous subunits, is located in regions distinct from those constituting the antigenic determinant of HT13.

Several aspects of the structure of glycoprotein hormones are of considerable interest with respect to their immunological properties and functions. This family of hormones includes the placental hormone choriogonadotropin and the pituitary hormones lutropin, follitropin, and thyrotropin. The glycoprotein hormones consist of two noncovalently associated, glycosylated peptide chains, α and β (1). Within a species, each hormone possesses the same α-subunit which is apparently coded for by a single gene on chromosome 6 (2, 3) and has an identical amino acid sequence. The unique β-subunit confers the biological specificity of the intact heterodimer (4); the isolated subunits are inactive in terms of the functional roles assigned to the glycoprotein hormones (5). The structural and topographical features of these molecules have been probed by selective chemical and enzymatic reactions (6). However, models describing the tertiary structure of the subunits have not yet been obtained since crystallographic approaches have been unsuccessful.

Recent advances in the use of synthetic peptides as probes of antigenic structure and function have led to some progress in the determination of local conformations in several proteins (7, 8). In previous papers (9, 10), we have shown that monoclonal antibodies and synthetic peptides are potent tools.
for the investigation of the three-dimensional structure of hCG. Two topographic antigenic determinants were characterized using several experimental approaches, including (i) production of monoclonal antibodies directed to hCG, its β-subunit, and synthetic peptides analogous to different regions of this subunit; (ii) competitive inhibition studies with synthetic peptides; and (iii) recombination experiments with subunits from different species. The aim of this study was to extend the analysis of antibody-binding sites present on hCG and to study antigenic regions located on hCG-α. Thus, we have delineated residues within the discontinuous epitope recognized by a monoclonal antibody directed against the free α-subunit (11). Our results provide valuable information about the structure-activity relationship of both hCG-α and evolutionarily variant molecules. In the accompanying paper (47), we report the production and characterization of a site-specific monoclonal antibody (FA36) to the COOH-terminal region of hCG-α. Taken together, these studies allow us to propose a model localizing the regions of subunit-subunit and hormone-receptor interactions on hCG-α.

**MATERIALS AND METHODS**

Purified hCG (CR125, 11,900 IU/mg) and hCG-α (CR123) were generously supplied by the National Hormone and Pituitary Program and the Center for Population Research of the National Institute of Child Health and Human Development, National Institutes of Health. Native subunits of equine lutropin (eLH) and ovine lutropin (oLH) were separated by Sephacryl S-200 chromatography following overnight incubation in 8 M guanidine hydrochloride as previously described (12). Na141 (carrier-free) was obtained from the Commissariat à l’Énergie Atomique (Saclay, France). hCG-α labeled with 125I (100 μCi/μg) and 125I-labeled monoclonal antibody HT13 (12-15 μCi/μg) were prepared by the IODO-GEN method (13).

**Preparation of Synthetic Peptides from Modified hCG-α**—The peptides were synthesized with an automatic Applied Biosystems Model 430A apparatus and purified as described (9). The cysteine corresponding to residues 28, 60, 82, and 84 were protected with the hydroxyacridine-resistant acetamidomethyl cysteine (14). The following peptides were constructed according to the sequence described by Morgan et al. (15): hCG-α-(1-22), -(23-43), -(43-59), -(41-59), and -(59-92).

**Endo-β-N-acetylglucosaminidase F (EC 3.2.1.96; Boehringer Mannheim)** was utilized to remove oligosaccharide chains from hCG-α under non-denaturating conditions as described by Lee et al. (16). About 100 μg of glycoprotein was incubated at 37°C with 0.125 unit of enzyme in 0.05 M potassium phosphate buffer, pH 7.0, containing 10 mM EDTA and 2 mM o-phenanthroline (Sigma). The deglycosylated product (DG-hCG-α) was then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Reduction and S-carboxymethylation of hCG-α (RCM-hCG-α) was performed as described by Mise and Babi (17).

**COOH-terminally shortened hCG-α (hCG-α core 1)** was obtained according to the procedure described by Merz (18). Amino acids were released from the COOH terminus of the native subunit by digestion with carboxypeptidase A (EC 3.4.17.1; Sigma). Digestion was performed at 37°C for 2 h, and the enzyme/substrate ratio was 1:10 (w/v). The resulting products were fractionated by reverse-phase HPLC on a C8 RP900 column; and after lyophilization, hCG-α core 1 was analyzed for amino acid composition.

The tryptic core (hCG-α core 2) was prepared from the hCG-α as described earlier (19). The protein (20 mg/ml) was dissolved in 0.2 M ammonium bicarbonate, pH 7.8, and the methodology included two additions of trypsin (1:1-tosylamido-2-phenylethyl chloromethyl ketone-treated, 224 units/mg; Worthington), one at the start (1:100, w/v) and one at 45 min (1:100 w/v) for a final 1:50 (w/v) ratio of trypsin to substrate. The lyophilized tryptic products were separated by gel filtration on Sephadex G-100 and then chromatographed on a DEA Sephacryl A-50 column. The tryptic core was further purified for these studies by reverse-phase HPLC on a Vydac C8 column using a mobile phase of 0.1% trifluoroacetic acid and acetonitrile. The preparation was subjected to amino acid analysis and amino acid sequence analysis and found to be identical to that reported earlier (19).

**Production and Characteristics of Monoclonal Antibodies**—Immunizations using either hCG or hCG-α, cell fusions, and screening assays were performed as previously reported (11, 14). Protein A affinity chromatography was used for the purification of monoclonal antibodies. The physicochemical characteristics of monoclonal antibodies utilized in this study and raised against either hCG or its α-subunit, namely HT13 and AHT20, are shown in Table I. Monoclonal antibody HT13 recognizes an antigenic determinant present on the α-subunit, either free or associated with the β-subunit. In contrast, the monoclonal antibody designated as AHT20 has a unique profile of binding to free hCG-α (11).

**Competitive Inhibition Assays and Immunoradiometric Assays**—Purified monoclonal antibodies were utilized in competitive inhibition assays performed as previously described (9, 10). Briefly, 100 μl of 125I-hCG-α (30,000 cpm), 100 μl of monoclonal antibody (30–120 ng/ml), and 50 μl of competitive peptide, native subunit, or related or modified subunit were incubated simultaneously in 0.01 M phosphate buffer, 0.14 M NaCl, pH 7.2 (P/NaCl), containing 1% bovine serum albumin at 4°C for 18 h. Polyethylene glycol (M, 6,000) was used to precipitate all of the immunoglobulin plus bound 125I-hCG-α. The half-maximal inhibitory dose was determined for each molecule tested (9).

A forward “two-site” monoclonal immunoradiometric assay (MI-IRMA) was utilized to determine the location of antigenic determinants recognized by antibodies AHT20 and HT13 on either native or modified hCG-α. This MI-IRMA was developed using conditions similar to other MI-IRMAs already described (14). Briefly, antibody AHT20 was coated on polysyrene beads, and the antibody-coated beads were incubated with either native or modified hCG-α.

**RESULTS**

**Competitive Inhibition Assays Using hCG, Native α-Subunit, and Structurally Related Molecules**—In order to determine the fine specificity of two monoclonal antibodies directed against hCG-α, we first assessed their reactivity with native hCG, its α-subunit, and the evolutionarily related molecules eLH-α and oLH-α. We have previously shown that monoclonal antibodies HT13 and AHT20 exhibit different patterns of binding to hCG and hCG-α. Whereas HT13 recognizes an antigenic determinant present on both the α-subunit and the α/β-dimer, AHT20 binds to a site localized to the free α-subunit (10, 11). Studying the cross-reactivity of these antibodies to homologous proteins, we found that AHT20 was capable of binding to eLH-α and oLH-α; this

| Immunogen   | Monoclonal antibody | HT13 | AHT20 |
|-------------|---------------------|------|-------|
| hCG-α       | hCG-α               | hCG  |
| Isootype    | IgG                 | IgG  |
| Epitope specificity | Free hCG-α | Free hCG-α and hCG-α (α/β-dimer) |

| Kᵦ (lbers/mol) | 5.0 × 10⁶        | 1.3 × 10⁹ |

*The isotype of each antibody was determined using rabbit anti-mouse immunoglobulin isotypes (IgG1, IgG2a, IgG4, IgM, and IgA).

Affinity constants were measured by incubating purified monoclonal antibodies with 125I-labeled hCG-α in the presence of increasing levels of unlabeled subunit. Kᵦ was calculated from binding data by Scatchard analysis.

**TABLE 1**

**Characteristics of monoclonal antibodies directed against hCG-α**

1 The abbreviations used are: hCG, human choriogonadotropin; hCG-α, the α-subunit of hCG; eLH, equine lutropin; oLH, ovine lutropin; hCG-α core 1, hCG-α minus the 5 COOH-terminal residues; hCG-α core 2, disulfide-bridged peptides comprising residues 1-35 and 5-91 of hCG-α; M-IRMA, monoclonal immunoradiometric assay; HPLC, high pressure liquid chromatography; PCM-hCG-α, reduced and carboxymethylated hCG-α; DG-hCG-α, deglycosylated hCG-α.
suggests that antibody AHT20 recognizes a structurally related antigenic determinant present on α-subunits of different species. In contrast, antibody HT13 did not bind to eLH-α and oLH-α (Fig. 1).

**Competitive Inhibition Assays Using Modified α-Subunits—**

To delineate the portions of hCG-α contributing to the antigenic determinants recognized by antibodies AHT20 and HT13, we used modified α-subunits as inhibitors in competitive inhibition experiments. First, RCM-hCG-α no longer inhibited the binding of 125I-labeled hCG-α to both monoclonal antibodies, proving that the respective epitopes comprise regions remote from each other in the primary structure, but close in three-dimensional space (Fig. 2). Moreover, DG-hCG-α was as active as native hCG-α, indicating that the lack of oligosaccharide residues did not modify the immunological reactivity of the α-subunit with AHT20 and HT13 antibodies.

We then studied the influence of amino acid removal on antigen binding to anti-hCG-α antibodies using either hCG-α core 1 or core 2 as inhibitors. The compositions of hCG-α core 1 and core 2 are presented in Table II. Under our experimental conditions, treatment of hCG-α with carboxypeptidase A led to the removal of 5 COOH-terminal residues of the α-subunit (hCG-α core 1). Amino acid sequence analysis of the preparation of the α-tryptic core indicated that it contains the same structure as reported earlier (19), with the deletion of residues 36-51 of the α-subunit (hCG-α core 2).

Competitive inhibition experiments showed that both hCG-α core 1 and core 2 had the same capacity as intact unlabeled hCG-α to inhibit the binding of 125I-hCG-α to HT13. Moreover, hCG-α core 1 was nearly as immunopotent as its parent α-subunit in inhibiting the binding of 125I-hCG-α to AHT20; but in striking contrast, hCG-α core 2 did not compete with 125I-hCG-α for binding to AHT20. These results suggest that:

**Table II**

| Amino acid analysis of hCG-α core 1 and core 2 |
|-----------------------------------------------|
| Amino acid | hCG-α | hCG-α core 1 | hCG-α core 2 |
| Aspartic acid | 6 (6) | 6 (6) | 5.99 (6) |
| Threonine | 8 (8) | 6.04 (6) |
| Serine | 8 (8) | 7.61 (7) |
| Glutamic acid | 9 (9) | 8.04 (8) |
| Proline | 7 (5) | 5.15 (5) |
| Glycine | 4 (4) | 3.72 (4) |
| Alanine | 5 (4) | 2.46 (4) |
| Cysteine | 10 (10) | NA (10) |
| Valine | 7 (7) | 5.33 (6) |
| Methionine | 3 (3) | 0.87 (2) |
| Isoleucine | 1 (1) | 0.51 (1) |
| Leucine | 4 (4) | 2.31 (2) |
| Tyrosine | 4 (4) | 2.51 (3) |
| Phenylalanine | 4 (4) | 4.3 (4) |
| Histidine | 3 (3) | 2.4 (3) |
| Lysine | 6 (6) | 6.21 (3) |
| Arginine | 3 (3) | 2.46 (2) |

* Each was hydrolyzed in 6 N HCl and analyzed on an LKB 4150 α-amino acid analyzer. Numbers in parentheses are from known sequences.

**Competitive Inhibition Assays Using Synthetic Peptides—**

To aid in the mapping of antigenic sites on the surface of hCG-α, we used synthetic peptides spanning the primary structure of hCG-α as competitive inhibitors in hapten inhibition experiments (Fig. 3). Binding of 125I-hCG-α to monoclonal antibody HT13 was not displaced by the various peptides at concentrations greater than 5 × 10⁻⁴ M. In contrast, two peptides corresponding to a central region in the primary structure of the subunit exhibited significant potency in competing with 125I-hCG-α for binding to AHT20. These synthetic peptides had amino acid sequences analogous to regions 23–43 and 33–59 of hCG-α and displayed a half-maximal inhibitory dose of 4 × 10⁻⁵ and 1.8 × 10⁻⁴ M, respectively. As synthetic peptide 33–59 was the most efficient inhibitor in competitive experiments, we assayed a subpeptide corresponding to residues 41–59 of hCG-α, but no inhibition was observed. Finally, the inhibition profile observed with monoclonal antibody AHT20 indicated that a region of hCG-α
corresponding to residues 33–41 might constitute a portion of the antigenic site recognized by this antibody.

**Immunoradiometric Assays of Native and Modified hCG-a**—These were performed to study further the regions of hCG-a which might contribute to the binding of the antibodies. Fig. 4 illustrates a typical experiment using a two-site monoclonal immunoradiometric assay based on the combination of antibody AHT2O coated on a solid-phase support and ^125I-labeled HT13 in the liquid phase. Both native hCG-a and hCG-a core 1 presented a similar pattern of recognition, whereas no significant binding was found when hCG-a core 2 was tested by M-IRMA. This assay was also performed with eLH-a and oLH-a, but no binding was observed with these molecules, consistent with the absence of cross-reactivity of HT13 with the evolutionarily variant molecules. Overall, these data confirmed previous results observed by competitive inhibition experiments and strongly suggest that the epitope of AHT2O, composed in part by residues 36–41, is located in regions distinct from those constituting the antigenic determinant of HT13.

**DISCUSSION**

In this study, we have utilized current immunochemical methods for epitope localization to map the surface of hCG-a. Such methods allow characterization of antigenic determinants in the configuration that they assume in the native protein (20). We have examined the fine specificities of two monoclonal antibodies directed against hCG-a using competitive inhibition experiments and immunoradiometric assays performed with native hCG-a, modified hCG-a, peptides analogous to different regions of hCG-a, and a-subunits from glycoprotein hormones of equine and ovine origin.

The characterization of epitopes recognized by monoclonal antibodies directed at hCG-a has provided valuable information defining the relative orientation of these epitopes and for recognizing regions on the surface of the molecule which are of biological interest (21–24). However, most of the antibodies utilized in previous studies bind to the free a-subunit as well as to the a/b-dimer. Schwarz *et al.* (22) distinguished three distinct epitopes on native hCG-a and hCG which were also found in identical topographical relations to each other on human lutropin, follitropin, and thyrotropin. These authors concluded that only the a-subunit is species-specific. Thotakura and Bahl (25) produced an antibody to the a-subunit displaying high affinity and negligible cross-reactivity with both intact hCG and the a-subunits of glycoprotein hormones from other species. They suggested that the antigenic site might reside in a nonhomologous region of the molecule. Recently, we reported the production and characterization of two monoclonal antibodies displaying different patterns of recognition. One (HT13) binds to hCG-a, hCG, and pituitary glycoprotein hormones, whereas the other (AHT2O) is specific for free hCG-a (11). As RCM-hCG-a was ineffective in competitive inhibition assays performed with both antibodies, our results demonstrate that the corresponding antigenic determinants are composed of residues from different parts of the sequence brought together by the folding of the protein in its native structure (26). Furthermore, the deglycosylation product of hCG-a showed an affinity similar to the native subunit for both antibodies. Taken together, these results are in agreement with previous reports suggesting that antigenic determinants on glycoprotein hormones and their subunits are mainly discontinuous and do not reside on the oligosaccharide parts of the molecules (9, 25, 27, 28).

Removal of five amino acids from the carboxyl-terminal end of the a-subunit (hCG-a core 1) does not affect binding of the monoclonal antibodies. Thus, antigenic determinants associated with free hCG-a and/or the a/b-dimer are not altered by this modification. This observation is supported further by previous results using a rabbit antiserum raised against isolated hCG-a (29) and by experiments based on a monoclonal antipeptide antibody directed against the COOH-terminal part of the molecule (see accompanying paper (47)). Tryptic digestion of native hCG-a generates disulfide-bridged peptides (hCG-a core 2) comprising residues 1–35 and residues 52–91. An earlier report (19) demonstrated the inability of the tryptic core to recombine with native hCG-b. Circular dichroism suggested a significant loss of tertiary structure in the core, which explains the failure of this product to combine with the intact complementary subunit. However, hCG-a core 2 was as effective as native hCG-a in competitive inhibition assays using a monoclonal antibody (A109) recognizing an antigenic determinant present both on the free a-subunit and on the a/b-dimer. It appears that immunological potency is less affected than recombination ability by the loss of region 36–51 of hCG-a. Consistent with these results, we found that monoclonal antibody HT13 presents a similar pattern of recognition as antibody A109 and binds to both native hCG or hCG-a and the tryptic core. In contrast, the loss of region 36–51 hCG-a dramatically affects the binding of AHT2O since hCG-a core 2 did not compete with native hCG-a for binding to this antibody. This unexpected result is partly in contradiction to earlier observations suggesting that the antigenicity of denatured (reduced and S-carboxymethylated) hCG-a is mainly borne by the NH2-terminal portion of the molecule (19, 30, 31). However, the antigenicity of the denatured subunit may be quite different from that of the native a-subunit. From our results, it seems likely that the major part of the antigenic site recognized by AHT2O is localized in the central portion of hCG-a, which is highly conserved among homologous subunits, as substantiated by the immunological cross-reactivity of the AHT2O antibody with these molecules.

Synthetic peptides have been used as probes of structure-function relationships in follitropin (32) and choriogonad-
tropin (33, 34) with their corresponding receptors. Similar strategies have been successfully utilized for delineating continuous antigenic determinants located on the surface of the hCG β-subunit (9, 35) and the entire α/β-dimer (10). The ability of two synthetic peptides corresponding to portions of hCG-α in inhibiting the binding of 125I-hCG-α to AHT20 substantiates the validity of this approach and has allowed us to identify critical amino acids participating in the epitope of this monoclonal antibody. Since peptides 33–59 and 23–43 are inhibitory, whereas peptide 41–59 and hCG-α core 2 are not, we suggest that a region including residues 36–41 constitutes an essential zone in the interaction of hCG-α with antibody AHT20. The observation that synthetic peptide 23–43 is less potent than peptide 33–59 in the competitive inhibition assay is not clearly understood, as they possess the same sequence at residues 36–41. It is likely that the folding characteristics of each peptide are important in such assays. Circular dichroism measurements indicate the hCG β-subunit to be relatively rigid, whereas the α-subunit is more flexible. Such flexibility might explain the ability of one peptide relative to another to inhibit the binding of the antibody to the subunit. Thus, the antigenic determinant recognized by antibody AHT20 involves several residues included in region 36–41 of hCG-α, namely Ala-Tyr-Pro-Thr-Pro-Leu. Earlier studies provided valuable information about this region. First, there seems to be an absolute requirement on the α-subunit for an aromatic residue (Tyr) at position 37 (6). Residues around this position are also highly conserved in all the known α-subunit sequences. Moreover, Burleigh et al. (36) have shown that there is a drastic reduction in α/β-recombination ability when α-Tyr37 is nitrated. This residue is 1 of the 2 α-subunit tyrosine residues which are not iodinated in the intact hormone (37, 38) and is probably involved in intersubunit interactions. Furthermore, Parson and Pierce (39) and Cole et al. (40) have shown that free α-subunits produced by both the placenta and the pituitary are unable to combine with the dissociated β-subunits of lutropin or hCG. The sequence Pro-Thr-Pro found at positions 38–40 is a potential site for O-glycosylation (41). An O-linked sugar unit was detected at Thr38 of these free α-subunits, and this carbohydrate is responsible for the inability of these subunits to combine with dissociated β-subunits. Thus, it appears that several residues in region 36–41 are highly conserved among related molecules and may be located at the α/β-subunit interface. These observations are consistent with the binding pattern of AHT20 to the free α-subunits of the species tested. Finally, it is noteworthy that 2 invariant Arg residues are located at positions 35 and 42 and are probably involved in subunit-subunit interaction or hormone-receptor interaction (6). Charlesworth et al. (34) postulated recently that region 25–41 is also part of the receptor-binding site(s) associated with or located near the subunit interface. Evidence for this view is provided by structural analyses based on predictive methods (42).

In contrast to our expectations, we failed to find inhibition of the binding to 125I-hCG-α of HT13 by synthetic peptides. Any explanation for this result remains speculative, but the lack of binding may be associated with the nature of the discontinuous epitope recognized by HT13, the choice of sequences for the synthetic peptides, or their conformation in solution. It is well known that monoclonal antibodies elicited against a protein recognize discontinuous antigenic determinants located on the surface of the molecule and that, among these antibodies, an even smaller fraction may recognize a linear array of amino acids which are part of an epitope (20).

From our results, it is likely that HT13 binds to residues specific to hCG-α, i.e. variant residues between species in disulfide-bridged peptides 1–35 and 52–91. We had shown earlier that monoclonal antibody HT13 does not bind to the hCG-receptor complex, suggesting that region(s) of the α-subunit are buried in the complex and/or sufficiently modified by a conformational change to abolish the binding of HT13 (10). These results are consistent with a previous report by Millus et al. (43), who suggest that the α-subunit directly interacts with the receptor, thereby masking immunoreactive sites normally available on the free hormone.

In summary, we have used various related α-subunits, modified molecules, and synthetic peptides corresponding to segments of hCG-α to map the binding sites of two monoclonal antibodies. Current information available on the physico- and immunochemical properties of hCG-α are schematically shown in Fig. 5, which illustrates the antibody-binding region of AHT20. These results provide new information about the antigenic regions and the three-dimensional structure of hCG and its α-subunit.

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