Chemically Deglycosylated Human Chorionic Gonadotropin Subunits: Characterization and Biological Properties†

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ABSTRACT: In order to evaluate the importance of carbohydrate side chains on the separate subunits in the biological action of human chorionic gonadotropin (hCG), we have treated each subunit with anhydrous hydrogen fluoride (HF) to remove carbohydrate and tested the products for recombination, immunologic recognition, ovarian receptor binding, and adenylate cyclase activation. The glycoproteins were reacted with HF for 1 h at 0 °C in the presence of anisole scavenger and separated from the reagents by gel filtration. The HF treatment removed 80% of total carbohydrate from the α-subunit and 66% from the β-subunit. Treatment of hCGβ with HF for 3 h removed 85% of the carbohydrate. Chemical characterization, including compositional and sequence analysis after reduction and carboxymethylation, confirmed that the peptide backbone had remained intact and undamaged during the HF reaction. Circular dichroic spectra were unaltered. The deglycosylated hCG bound to antibody with a lowered affinity, suggesting either subtle conformational changes or involvement of the carbohydrate in the antigenic determinant. The HF-treated subunits recombined readily with normal or deglycosylated counterpart subunit. The recombinant hormones (degly-α/normal β, normal α/degly-β, and degly-α/degly-β) bound fully to rat ovarian membrane receptors. By contrast, adenylate cyclase activation was in each case markedly diminished, to levels less than 8% of untreated control. Coincubation with normal α/degly-β recombinant resulted in substantial inhibition of the adenylate cyclase response to normal hCG. These responses were comparable to those found when HF-deglycosylated whole hCG was used. These results support earlier indications that carbohydrate may play a direct role in the biological action of hCG. The impaired adenylate cyclase activity in the presence of normal membrane receptor binding suggests that the carbohydrate may be involved in receptor/adenylate cyclase "coupling", perhaps through interaction with one or more cell membrane lectins. Since the biological effects were observed when either subunit was deglycosylated, the results also enhance the likelihood that the α-subunit, as well as the hormone-specific β-subunit, is directly involved in hormone action.

Human chorionic gonadotropin (hCG),[1] like the other glycoprotein hormones (LH, FSH and TSH), contains a series of carbohydrate side chains on both the α- (common) and β- (hormone-specific) subunits. Accounting for about 30% of hCG molecular weight, they are comprised principally of branched long-chain carbohydrates attached to asparagine residues at positions 52 and 78 in α-subunit and 13 and 20 in β-subunit. There are also four short-chain carbohydrates linked to serine residues in the C-terminal region of the β-subunit of hCG at positions 121, 127, 132, and 138.

The importance of the terminal sialic and galactose residues in determining hepatic clearance and plasma half-life is well recognized (Morell et al., 1971; Zahlten et al., 1981). A number of investigations have more recently indicated that carbohydrate may also have a direct role in glycoprotein hormone action. Typically, these have shown that receptor binding is preserved but subsequent cellular events such as adenylate cyclase activation and steroidogenesis are markedly impaired after the majority of carbohydrate residues are removed from whole hCG by chemical solvolysis (Manjunath & Sairam, 1982; Thotakura & Bahl, 1982; Chen et al., 1982).

To evaluate further the role of carbohydrate in the α- and β-subunits individually, we reacted the separate subunits with hydrogen fluoride to remove carbohydrate. Since the common deglycosylation techniques are sufficiently vigorous to incur the risk of peptide chain cleavage, we applied sequencing techniques not previously used for characterization of deglycosylated subunits to verify the integrity of the peptide chain after HF treatment. Recombinants between HF-treated subunit and normal counterpart subunit were then prepared and their biological and immunological properties compared with those found for deglycosylated whole hCG and unadulterated hCG.

Materials and Methods

Hormone Preparations. hCG purchased in partially purified form from Organon, Ltd. (Oss, The Netherlands), was processed to purity by Sephadex G-100 gel filtration (Pharmacia) and DEAE-cellulose chromatography (Whatman). Subunits were separated by treatment with 10 M urea followed by DEAE-cellulose/3 M urea chromatography and Sephadex G-100 gel filtration (Morgan et al., 1973). Purified hCG (CR-117 and -123), a gift of Dr. Robert Canfield (Columbia University, New York), was used as reference preparation in the biological and immunological assays.

Deglycosylation. Hydrogen fluoride treatment was carried out with the respective subunits and whole hCG. An aliquot of 5–10 mg of peptide was dried in vacuo over phosphorus pentoxide for 24 h, transferred to the Toho polypropylene distillation apparatus (Toho Industries, Tokyo, Japan), and treated with 10 mL of anhydrous hydrogen fluoride (Matheson, East Rutherford, NJ) in the presence of 2.2 mL (20%) of anisole which was used as a scavenger (Mort & Lamport, 1977). After 1–3-h reaction at 0 °C (maintained by ice bath) the reagents were evaporated. The granular, yellow-brown reaction product was rinsed twice with cold anhydrous ether and then suspended in 10 mL of 10% acetic acid. After several rinses with additional acetic acid, the preparation was centrifuged to remove a small amount of insoluble particulate

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[1] Abbreviations: hCG, human chorionic gonadotropin (choriogonadotropin); LH, luteinizing hormone (lutropin); FSH, follicle-stimulating hormone (follitropin); TSH, thyroid-stimulating hormone (thyrotropin); HF, hydrogen fluoride; TFMS, trifluoromethanesulfonic acid.

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matter, and the supernatant was extracted 3× with diethyl ether in a separatory funnel to remove residual anisole. The preparation was lyophilized and desalted further by gel filtration as described under Results.

Chemical Characterization. Carbohydrate content of untreated and deglycosylated subunits (250–500 μg) was determined by gas chromatography after hydrolysis in 1 M methanolic HCl (20 h, 80 °C) and silylation of hydroxyl groups (Reinhold, 1972). Amino acid analysis was done on the Beckman Model 121MB automatic analyzer following acid hydrolysis in 6 N HCl (100 °C, 24 h).

Reduction and carboxymethylation (Crestfield et al., 1963; Keutmann & Williams, 1977) was carried out with 0.2 M dithiothreitol (Sigma) in 8 M guanidine hydrochloride (pH 8.7, 37 °C, 4 h) followed by 1 M iodoacetic acid (15 min, 20 °C); ^{14}C-labeled iodoacetic acid (New England Nuclear) was included in the second step to introduce a radioactive label into the product. The reagents were separated by Sephadex G-25 gel filtration (Pharmacia).

Automated Edman degradation (Edman & Begg, 1967; Niall, 1973) of reduced, carboxymethylated subunit was done on the Beckman Model 890C sequencer with the 0.1 M "Quadrol" program (Beckman Instruments, Inc.) in the presence of Polybrene (Sigma) to retard extractive losses of small peptides (Tarr et al., 1978). Phenylthiohydantoins were detected by gas/liquid and thin-layer chromatography (Niall, 1973).

Subunit Recombination. A 3–5-mg sample of deglycosylated subunit was incubated with counterpart subunit at a concentration of approximately 0.5 mM (with α-subunit in 20% molar excess), in 0.1 M ammonium bicarbonate, pH 8.5, for 24 h at 25 °C (Pernollet et al., 1976). The recombinant hormone was isolated by gel filtration on Sephadex G-100 (Pharmacia) with the same buffer for elution.

Assay Procedures. Binding to hCG receptors was assayed as described previously by Lee & Ryan (1973) by using a 2000*g* membrane-rich fraction from superovulated rat ovaries. Assays were carried out in 40 mM Tris-HCl buffer, pH 7.4, at hormone doses in the range 10–500 ng/mL. The radiolabeled hCG ligand was prepared by a modification (Lee & Ryan, 1973) of the method of Greenwood et al. (1963). Relative potencies and standard errors were computed from the dose–response curves according to the procedure of Rodbard et al. (1978).

Adenylate cyclase activity was measured as previously described by McIlroy et al. (1980) by using a similar rat ovarian membrane fraction with graded hormone doses of 0.1–1 μg/mL incubated for 20 min at 30 °C.

Radioimmunoassays for hCG were carried out by the double-antibody technique described by Faiman & Ryan (1967). The labeled hormone, prepared as indicated above, was purified native hCG. The antisera employed were prepared against native hCG (R2001, R2005) hCGβ (R1-10), and hCGα (SA6, obtained from the Hormone Distribution Program NIAMDD). Data were analyzed by the logit log transformation described by Rodbard et al. (1978).

Protein concentrations were determined either by amino acid composition (see above) or by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Circular Dichroic Studies. Circular dichroic spectra were measured on a Beckman CD spectropolarimeter with a light path of 1.0 cm for recordings between 320 and 250 nm and 0.02 cm between 250 and 210 nm. Thirty-two scans were recorded for the high-ultraviolet region and 16 for the low-ultraviolet region. Scan speed was 50 nm/min.

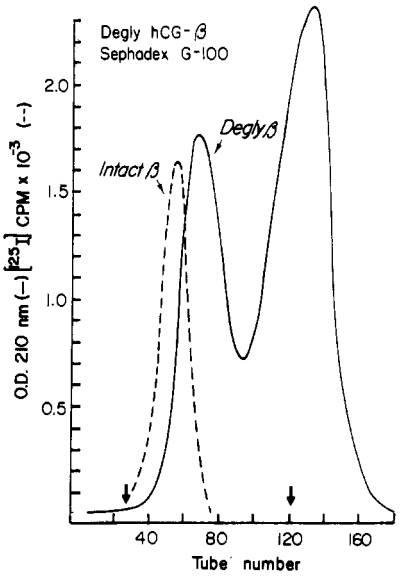

FIGURE 1: Purification of HF-treated, deglycosylated hCG β-subunit by Sephadex G-100 gel filtration. Fraction size was 1 mL; arrows denote void and salt volumes of column, respectively. Elution position of labeled, untreated hCGβ is located by iodine counts. Large peak in the salt region contained exclusively cleaved carbohydrate and unextracted anisole from HF reaction.

Table I: Carbohydrate Analyses of hCG Subunits[a]

	α-subunit		β-subunit		
		HF-		HF treated[c]	
carbohydrate	control	treated[b]	control	1 h	3 h
mannose	7.73	0.33	4.42	1.02	0.15
galactose	3.99	0.29	6.99	2.50	0.71
fucose	0.0	0.0	1.10	0.11	0.0
N-GalNAc	0.0	0.0	3.28	3.40	3.17
N-GlcNAc	7.6	3.85	6.46	2.85	1.30
sialic acid	5.52	0.24	13.25	2.15	0.25

[a] Data expressed as mg of carbohydrate/100 mg of subunit. [b] Mean of two preparations. [c] Mean of four preparations.

Lyophilized subunits were dissolved in 50 mM phosphate buffer, pH 7.4, containing 0.15 M NaCl to make the solutions of the following molarities: native hCG, 43.9×10^{-6}; degly-α/normal β, 52.0×10^{-6}; normal α/degly-β, 51.7×10^{-6}; degly-α/degly-β, 16.7×10^{-6}. No corrections were made for protein contents, and molarities were calculated by using the following assumed molecular weights: native hCG, 40 000; degly-α/β, 36 000; α/degly-β, 34 000; degly-α/degly-β, 30 000.

Results

Isolation of HF-Treated Subunits. The lyophilized HF-treated, ether-extracted β-subunit was dissolved in 1 mL of ammonium bicarbonate buffer (0.2 M, pH 8.7); a trace of white granular precipitate was removed by centrifugation and the supernatant passed over a 1.2 × 90 cm column of Sephadex G-100, eluted with the same buffer. The treated β-subunit eluted later than labeled intact subunit marker (Figure 1), consistent with the reduction in molecular size. The treated α-subunit was processed by gel filtration on a Bio-Gel P-2 column eluted with 0.1 M acetic acid. Overall recovery of either deglycosylated subunit was 40–50% by dry weight.

Carbohydrate Content of Treated Subunits. Table I summarizes the carbohydrate content of the respective deglycosylated subunits and untreated controls. Eighty percent of the total carbohydrate was removed from the α-subunit after 1 h, including 96% of the mannose comprising the core region

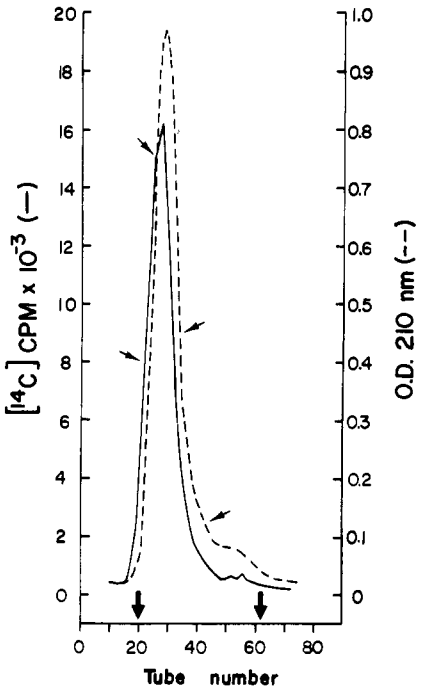

FIGURE 2: Sephadex G-100 gel filtration of deglycosylated hCG β-subunit after reduction and carboxymethylation. Optical density and ^{14}C counts from *S*-(carboxymethyl)cysteine positions are plotted, and small arrows designate fractions taken for amino acid analysis to confirm homogeneity (see text). Fraction size was 0.8 mL. Heavy arrows locate void and salt volumes of column, respectively.

of the chain. Remaining carbohydrate was almost exclusively *N*-acetylglucosamine.

Deglycosylation of the β-subunit appeared less complete; 66% of total carbohydrate was removed after 1 h. Remaining carbohydrate included about 20% of the mannose in the Asn-linked chains as well as 45% of the *N*-acetylglucosamine and all of the *N*-acetylgalactosamine. After 3 h the residual mannose was reduced to 4% and *N*-acetylglucosamine to 22% with no reduction in *N*-acetylgalactosamine content.

Analysis of Effect of HF on the Peptide Chain. Aliquots of 1.5 mg of the 1- and 3-h HF-treated β-subunit were reduced and carboxymethylated to open up disulfide linkages. The products were each subjected to chromatography on a Sephadex G-100 column (0.9 × 90 cm; 0.2 M ammonium bicarbonate buffer), and the elution was monitored by optical density and *N*-[^{14}C]-(carboxymethyl)cysteine radioactivity. In each case the peptide appeared close to the position expected for full-length subunit (Figure 2). Aliquots across the peptide peak were analyzed for amino acid composition and peptide content; all agreed with one another and with the expected amino acid content of the intact subunit. The only nonstoichiometric composition was found in a fraction at the tail of the peak, representing less than 3% of the total protein; this low-level heterogeneity was also seen on chromatography of untreated starting material.

Edman degradation of the reduced, carboxymethylated (degly)β-subunit through 12 cycles showed an identical pattern of phenylthiohydantoin derivatives to that found on parallel degradation of reduced, carboxymethylated unmodified β-subunit. Similar evidence for homogeneity was obtained after reduction and carboxymethylation, gel filtration, and compositional analysis of α-subunit. More limited quantities of this subunit did not enable us to carry out Edman degradations of the deglycosylated α.

Subunit Recombination. Following incubation of either deglycosylated subunit with normal counterpart subunit (see Materials and Methods), gel filtration on Sephadex G-100 yielded a principal protein optical density peak eluting slightly later than labeled unmodified hCG (Figure 3). The amino acid composition of this product in both cases was consistent with full-sized recombined hCG.

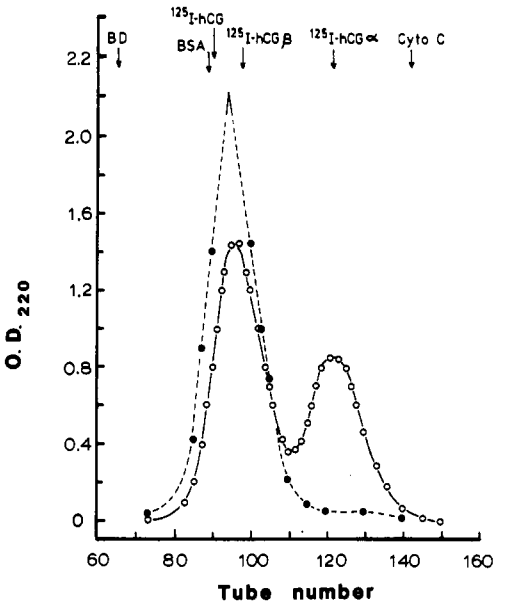

FIGURE 3: Gel filtration of hCG recombinants on Sephadex G-100 (1.6 × 80 cm) in 0.1 M ammonium bicarbonate buffer, pH 8.5: (○) degly-β (0.31 μmol) + α(.47 μmol); (●) degly-α (0.35 μmol) + β(0.36 μmol). Elution positions of several marker peptides are designated at the top. Fraction size was 2.3 mL. Material from major peak at tubes 85–105, representing fully recombined hCG (see text), was pooled for further biological studies. The smaller peak consisted of uncombined α-subunit, used in slight excess in the incubation with degly-β.

Table II: Receptor Binding and Adenylate Cyclase Activity of Recombined Deglycosylated hCG Subunits and Deglycosylated Native hCG

preparation	N^a	relative potency[b] receptor binding	relative potency[b] adenylate cyclase
degly-α/β (recombinant)	3	2.09 ± 0.15	<0.08
degly-β/α (recombinant)	4	0.92 ± 0.12	<0.07
degly-α/degly-β (recombinant)	1	0.68 ± 0.07	<0.07
deglycosylated hCG	1	1.00 ± 0.07	<0.01

[a] N = number of preparations tested. [b] Mean potency ± standard error, using incubated CR-117 hCG as standard.

Ovarian Membrane Binding and Adenylate Cyclase Activation. Results of testing for ovarian membrane receptor binding and adenylate cyclase activity by the recombinants and whole hCG are compared in Table II. When assayed against incubated CR-123 native control, the 1-h treated preparations bound fully to membrane receptors, and binding by degly-α/native β was even moderately enhanced. The displacement curves were parallel for all types of recombinant as illustrated by Figure 4.

By contrast, activation of adenylate cyclase by all of the deglycosylated preparations was markedly diminished (Table II). Responses were nonparallel, with small but finite elevations above basal levels at higher doses (0.5–1 μg/mL). Potencies could be estimated only at half-maximal activation and were in all cases less than 8% of control.

Coincubation with Native hCG. In order to discern any inhibitory properties, a series of adenylate cyclase assays of native hCG were done with coincubated degly-β/native α recombinant (Table III). In most experiments, there was

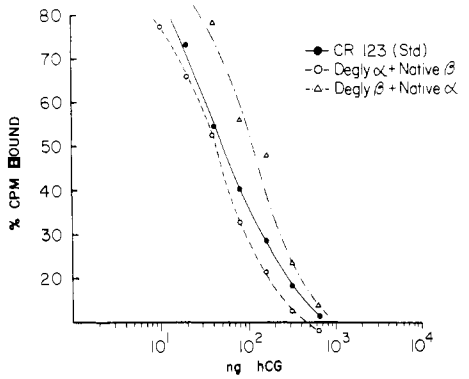

FIGURE 4: Displacement curves for deglycosylated hCG recombinants in the ovarian membrane receptor binding assay. For clarity in showing parallelism among the preparations, doses are plotted by mass before correction for molar concentration.

Table III: Effects of hCGα–degly-β (A) on the Rat Ovarian Cyclase Response to Native hCG[a]

preincubation (30 min, 20 °C)	assay (20 min, 30 °C)	cAMP [pmol mg^{-1} (20 min)$^{-1}$][b]
none	buffer	106 ± 5
none	hCG	290 ± 25
none	hCG + A (0.3 μg)	258 ± 23
none	hCG + A (1.0 μg)	222 ± 20
buffer	buffer	146 ± 5
buffer	hCG	374 ± 11
A (0.3 μg)	hCG	231 ± 7
A (1.0 μg)	hCG	210 ± 15

[a] Native hCG preparation CR-117 (1.0 μg). Hormone doses based on 1.0 mL volume. [b] ±SE.

evidence for decreased cyclic AMP production when native hCG standard CR-117 was coincubated with incremental doses of modified hCG up to a 1:1 molar ratio. This effect was more evident, however, when the target tissue was preincubated with the deglycosylated preparation. Response to normal hCG added 20 min later was progressively diminished by increasing amounts of deglycosylated preparation in the preincubation medium. Conversely (data not shown) preincubation with CR-117 overcame any effect of subsequently added deglycosylated hCG.

Radioimmunoassays. The results given in Table IV indicate that HF deglycosylated hCG showed dose–response curves that were parallel to intact hCG when assayed with anti-native, anti-α, or anti-β sera. The minor variations in slope were not statistically significant. However, the potency of deglycosylated hCG relative to native hCG was reduced 30–60% in the assays for native hCG and hCGα. The potency in the hCGβ assay was not significantly affected.

Circular Dichroic Studies. The circular dichroic spectra of native hCG showed minima at approximately 210, 270, and 285 nm; the various recombinants of deglycosylated α- and β-subunit, with each other or intact counterparts, showed

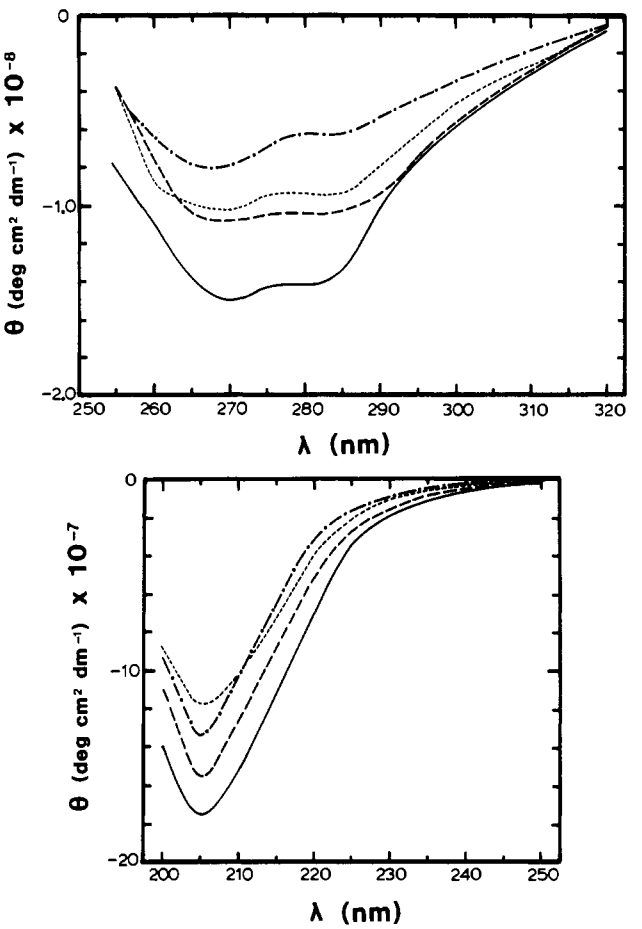

FIGURE 5: Circular dichroic spectra of hCG recombinants in the near- (top) and far- (bottom) ultraviolet ranges: native hCG (—); degly-α/β (- - -); α/degly-β (···); degly-α/degly-β (- · -).

minima at the same wavelengths (Figure 5) although there were variations in magnitude.

The differences in magnitude could be explained by weighing errors, variations in water or salt content of the dried materials, or erroneous assumptions concerning molecular weights (see Materials and Methods). Nonetheless it is possible to conclude from these data that there are no major changes in the conformation of the peptide chains following deglycosylation of the hCG subunits.

Discussion

Our findings showing normal ovarian receptor binding and impaired adenylate cyclase activation strengthen previous evidence that carbohydrate plays a major role in glycoprotein hormone–target cell action. Furthermore, the data indicate that the α-subunit as well as the β-subunit is directly involved in mediating cellular events after receptor binding; the biological effects of the "hybrid" recombinants of treated individual subunits were comparable to those found with deglycosylated whole hCG by ourselves and others (Manjunath

Table IV: Radioimmunoassay of HF Deglycosylated hCG Compared to Native hCG[a]

assay	slope: native hCG	slope: deglycosylated hCG	relative potency
anti-hCG native R2001	−1.21 ± 0.20 (3)	−1.07 ± 0.09 (3)	0.408 ± 0.269 (3)
anti-hCG native R2005	−0.92 ± 0.05 (4)	−1.08 ± 0.11 (4)	0.601 ± 0.312 (4)
anti-hCGβ R1-10	−0.84 ± 0.12 (5)	−1.02 ± 0.18 (5)	0.871 ± 0.293 (5)
anti-hCGα SA6	−0.97 ± 0.34 (3)	−1.09 (2)	0.69 (2)

[a] Protein was determined by the procedure of Lowry et al. (1951). All assays included six dose levels of standard and six of unknown. Results are given as means ± standard deviation for (*n*) number of separate assays.

& Sairam, 1982; Chen et al., 1982; Thotakura & Bahl, 1982).

Normal binding by recombinants of deglycosylated ovine LH subunits to Leydig cell receptors was observed previously by Sairam (1980), and testosterone production was impaired by degly-α/β or degly-α/degly-β. However, the α/degly-β ovine LH preparation did not diminish testosterone production. Kalyan & Bahl (1981) used recombined TFMS-reacted hCG subunits to show normal ovarian receptor binding, but additional biological testing after treatment with TFMS was done only with native hormone. Contrasting results were obtained by Fein et al. (1981), who found recombination, Leydig cell binding, and adenylate cyclase activation all to be markedly impaired after enzymatic deglycosylation of hCG subunits. The explanation for these differing findings is as yet unclear but may reflect differences in residual carbohydrate among the various preparations, or undetected peptide chain damage or cleavage.

The studies with HF-treated hCG reported here have emphasized rigorous confirmation of the integrity of the subunit chains. Amino acid compositions of the subunits following deglycosylation were identical with those of the respective proteins before treatment, but these data alone might not reveal peptide cleavage or fragmentation because of abundant cysteine cross-linking. After reduction and carboxymethylation to open up disulfide bridges, the results of gel filtration and compositional and sequence analysis assured us that the peptide chain had remained intact during HF treatment for up to 3 h.

Similarly, the conformation of the respective subunits was not grossly distorted as shown by the comparable circular dichroic spectra before and after treatment (Figure 5). This is reflected by the ease with which deglycosylated subunits recombined and by the parallel dose–response curves in the immunoassays. In contrast to the equal potency found by immunoassay of HF-treated whole hCG by Manjunath & Sairam (1982), the potencies of our preparations were somewhat lower than native hormone (Table IV). The reduced potencies might indicate subtle conformational changes not detectable in the CD spectra or a small effect of carbohydrate itself on the reactivity of the antisera used in our assays.

The carbohydrate content of the hCG subunits after 1-h HF treatment paralleled closely that found in whole hCG by Manjunath & Sairam (1982) and Chen et al. (1982). Mannose content constitutes an important indicator of the removal of "core" carbohydrates in asparagine-linked chains, and taken together our individually deglycosylated subunits contained similar amounts of residual mannose to the 13% reported in their preparations of whole hormone. We found the mannose to be localized predominantly to the β-subunit (Table I), possible due to the influence of conformation or adjacent sequences on the rate of solvolysis. When HF treatment was extended to 3 h, virtually all remaining mannose could be removed, leaving only the *N*-acetylglucosamine adjacent to asparagine residues. This linkage has been shown to be resistant to even prolonged treatment with HF at higher temperatures (Mort & Lamport, 1977; Manjunath et al., 1982).

Most remaining carbohydrate (*N*-acetylgalactosamine and smaller amounts of galactose and sialic acid) in β-subunit after 1-h treatment could be related to the O-linked short-chain carbohydrates found in the C-terminal region of the molecule (Bahl et al., 1978). Some additional removal of sialic acid and galactose was observed after 3 h, but complete cleavage of serine-linked residues could be achieved only by β-elimination using strong alkali (Downs et al., 1973) (data not shown). A closely similar pattern of retained carbohydrate in β-subunit was found by Kalyan & Bahl (1981) after 1-h treatment with anhydrous TFMS instead of HF.

The mechanism(s) through which the hormonal carbohydrate chains lead to activation of adenylate cyclase remains (remain) unknown. The events concerned appear to be immediately postreceptor, since the nucleotide-binding protein and adenylate cyclase enzyme remain responsive to guanine nucleotides in the presence of deglycosylated whole hCG (Thotakura & Bahl, 1982). The possibility of subtle changes in conformation have been mentioned above. It is tempting to speculate on an alternative possibility that the carbohydrates bind to membrane lectins which are close to, but not necessarily a part of, the receptor that binds the peptide portion of the hormone.

Mammalian membrane lectins are known to exist, but their precise functions are yet to be defined (Barondes, 1981). Binding to a second membrane component would allow divalency which in turn would permit lateral mobility and produce complex dissociation kinetics. Multiphasic dissociation kinetics of the hCG–receptor complex are already known (Lee & Ryan, 1973). In this regard, the binding of IgE to mast cell receptors is of interest (Ishizaka, 1982). Binding per se did not lead to activation of adenylate cyclase, calcium influx, histamine secretion, or membrane lipid methylation. However, if the IgE–receptor complexes were cross-linked by an antibody to IgE then all of these processes were activated. Further, they were inhibited by some of the same protease inhibitors which block hCG activation of adenylate cyclase (McIlroy et al., 1980).

Impaired activation of adenylate cyclase in the presence of normal membrane binding suggests that the deglycosylated preparations might constitute inhibitors to glycoprotein hormone action. In our coincubation experiments using α/degly-β recombinant, inhibition was most evident if the ovarian membranes were preincubated with the deglycosylated preparation. The preincubation probably enables the antagonist to offset the slow dissociation rate of native hCG from its receptor (Lee & Ryan, 1973). The antagonistic properties of this recombinant on cAMP production were closely similar to HF-treated whole hCG as found by Chen et al. (1982) using Leydig cells under comparable incubation conditions and hormone doses.

The slight intrinsic activity of the "hybrid" recombinants in the adenylate cyclase system could prove sufficient to compromise the effectiveness of these compounds as antagonists to steroidogenesis, as found in in vivo assays of HF-treated whole hCG (Chen et al., 1982). On the other hand, two recent studies of HF-treated whole hCG have shown receptor binding to be enhanced about 2-fold compared to native untreated hormone (Manjunath & Sairam, 1982; Chen et al., 1982). Although our preparation of HF-treated native hCG was equipotent with untreated native hCG in the rat ovarian binding assay, the recombinant degly-α/native β did show a 2-fold enhancement, perhaps reflecting the more complete removal of carbohydrate from the α-subunit.

The degly-α/β hybrid recombinant might therefore be the most appropriate preparation for further studies of antagonist properties in vivo. Since clearance of deglycosylated whole hCG appears to occur rapidly (Chen et al., 1982), preparations retaining the normal complement of sialic acid in the β-subunit might survive longer in the circulation through greater resistance to hepatic breakdown.

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Registry No. Adenylate cyclase, 9012-42-4.

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Isolation, Properties, and Androgen Regulation of a 20-Kilodalton Protein from Rat Ventral Prostate†

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ABSTRACT: An abundant 20-kilodalton protein has been isolated from the cytosol fraction of rat ventral prostate by ammonium sulfate precipitation, DNA–cellulose chromatography, and gel filtration. The purified 20K protein is a glycoprotein, containing 11% hexose by weight. It contains no fucose, hexosamine, or sialic acid. The 20K protein does not bind androgen. Binding of the 20K protein to DNA is nonspecific, showing affinity toward DNAs of various tissue origins, as well as poly(dA-dT), poly(rI-rC), and phosphocellulose. The 20K protein comprises about 9% of the total cytosolic proteins in rat ventral prostate. Examination of eight different rat organs, including prostate secretion, lateral and dorsal prostates, and rat ejaculate, for the presence of the 20K protein by double immunodiffusion analysis revealed that the protein is a rat ventral prostate specific secretory protein. Hybridization of prostatic poly(A) RNA with a cloned cDNA coding for the 20K protein indicated that the synthesis of the 20K protein is regulated by testosterone at the mRNA level.

The rat prostate, a male accessory sex gland, produces several abundant proteins in response to testosterone stimulation. Parker et al. (1978) have reported the presence of three major androgen-regulated proteins in the postnuclear supernatant fraction of rat ventral prostate, identified electrophoretically as polypeptides of M_r values of 8K, 12K, and 20K. The 12K polypeptide actually contains two polypeptides of M_r 13K and 11K which, together with the 8K polypeptides, constitute the subunit components of PBP[1] (Heyns & De Moor, 1977; Heyns et all., 1978; Lea et al., 1977, 1979; Forsgren et al., 1979) or

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[1] Abbreviations: PBP, prostatic binding protein; $NaDodSO_4$, sodium dodecyl sulfate; TEM, 20 mM Tris-HCl, pH 7.5, 10 mM EDTA, and 1 mM β-mercaptoethanol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

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