Biochemical, Biological, and Immunological Properties of Chemically Deglycosylated Human Choriogonadotropin*

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A chemical method of deglycosylation of human choriogonadotropin (hCG) was used to assess the role of carbohydrate moiety in the maintenance of quaternary structure and functional parameters such as receptor binding, immunological activity, and in vitro biological response. Treatment of purified hCG with anhydrous HF at 0 °C for 60 min was effective in removing more than 75% of the carbohydrate moiety. This extent of deglycosylation altered its chromatographic characteristics as revealed by retarded behavior on Sephadex G-100 and failure to be retained on concanavalin A-Sepharose. The electrophoretic heterogeneity present in native hCG was markedly reduced by deglycosylation. The deglycosylated hCG was stable in the lyophilized form and retained its quaternary structure as revealed by the fluorescence probe 8-anilino-1-naphthalene sulfonic acid, receptor binding, and immunological activities. Unlike receptor binding and immunological activities, which were fully retained, the ability of the hormone to stimulate cyclic AMP accumulation in vitro in rat interstitial cells was completely abolished.

Human choriogonadotropin is a placental oligomeric glycoprotein having hormonal properties similar to pituitary lutropin. The primary structure of the α and β subunits of the hormone has been established and the sequence of the monosaccharide units in the glycopeptides has been proposed (1, 2). It has long been known that removal of the terminal sialic acid residues in the hormone results in a loss of in vitro biological activity without any alteration of the hormonal responses in vivo systems (3, 4). The role of other sugar moieties in the hormone has been assessed by their sequential removal using specific exoglycosidases isolated from different sources (5-7). Although this approach has provided valuable information on the biological properties of the partially deglycosylated hormone, the method involved prolonged incubation with a series of glycosidases followed by several manipulations to obtain the derivative. Recently, we showed that the chemical method of deglycosylation using anhydrous hydrogen fluoride is effective in the rapid removal of a substantial amount of carbohydrate from ovine pituitary lutropin (8) and its subunits (9). In the present work we have extended this approach to remove oligosaccharide units from hCG in a single step. The data presented here will show that the method is useful in studying the biochemical and biological properties of the deglycosylated hormone.

EXPERIMENTAL PROCEDURES

Hormone Preparations—In preliminary experiments a purified preparation of hCG kindly provided by Dr. Y. T. Tsong (Population Council, NY) was utilized. For later investigations a commercial preparation of hCG (Ayerst Laboratories, Montreal, Canada) was used for purification of the hormone. The crude powder (~3000 IU/mg) was dissolved in 0.025 mM Tris-HCl buffer, pH 7.5, containing 10 mM MgCl₂, 10 mM CaCl₂, 0.5 mM NaN₃, and 0.02% sodium azide (starting con A buffer) and chromatographed on a column of con A-Sepharose (Pharmacia Inc., Canada) at 4 °C (Fig. 1A). After removal of the unadsorbed fraction, the adsorbed glycoprotein was eluted using 0.3 M α-methylglucoside (Sigma) dissolved in the starting buffer. The fractions in tubes 120-150 which showed high activity in a radioreceptor assay specific for lutropin activity were pooled. The solution was concentrated to about 3-5 ml by ultrafiltration using a PM-10 membrane (Amicon Co., Inc., Lexington, MA) and fractionated on Sephadex G-100 (Fig. 1B). The protein in fractions 46-75 were pooled, concentrated, and rerun on Sephadex G-100 (Fig. 2). The final product was recovered by lyophilization and used for all the investigations.

hCG (CR-119) provided by the hormone distribution officer at the National Institutes of Health, Bethesda, MD, was used as standard for comparison of the hCG purified by the above procedure. The purified hCG α and β subunits were also obtained from the same source.

Chemical Deglycosylation and Recovery of the Product—Purified and moisture-free hCG was subjected to anhydrous HF treatment (8) following our previous protocol with slight modifications. In brief, 50 mg of the hCG powder were treated with 10 ml of anhydrous HF at 0 °C with an effective reaction time of 60 min. Usually about 15 min was allowed for distillation of liquid HF into the reaction vessel at 60 °C and subsequent warm up to 0 °C for initiation of the reaction. Anisole which was used as a scavenger in our previous experiments (8, 9) was omitted in the present work. At the end of 60 min, the reaction was terminated by evacuation of the visible HF using first a water aspirator (15-30 min) followed by a high vacuum pump for about 2 h. The contents of the reaction vessel were then dissolved in 2 ml of ice-cold 0.2 M NaOH to neutralize any traces of residual HF and the pH was readjusted to 7.5 using cold 0.2 M HCl. The mixture was then incubated overnight at 37 °C and chromatographed on Sephadex G-100 in 0.05 M NH₄HCO₃ at 4 °C (Fig. 2A). The protein in fractions 67-90 were pooled and lyophilized. The deglycosylated hormone was kept at 4 °C until use. When further derivatizations were required, solutions were weighed carefully on a Cahn 4700 automatic electrobalance.

Other Materials—8-Anilino-1-naphthalene sulfonate was used as the magnesium salt, obtained from Eastman, was employed without further purification. Bovine serum albumin and cyclic AMP binding protein were obtained from Sigma; collagenase and lime bean trypsin inhibitor were from Worthington and isobutyl methylxanthine was used as a scavenger in our previous experiments (8, 9).

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1 The abbreviations used are: hCG, human choriogonadotropin; ANS, 8-anilino-1-naphthalene sulfonic acid; Fuc, Fucose; conA, concanavalin A; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; DG, deglycosylated.
Fig. 1. Purification of hCG. A, concanavalin A-Sepharose chromatography pattern of commercial preparation of hCG. Concanavalin A-Sepharose column (1.5 x 22 cm) was equilibrated in conA buffer (25 mM Tris-HCl buffer, pH 7.4, containing 10 mM MgCl2, 10 mM CaCl2, 0.5 mM NaCl, and 0.02% Triton X-100). Fractions of 3.8 ml at a rate of 35 ml/h were collected. Fractions 67-90 were pooled. Recovery was 26 mg by weight. B, gel filtration pattern of conA-Sepharose fraction. Sephadex G-100 column (2.5 x 106 cm) was previously equilibrated in 0.05 M NH4HCO3. The adsorbed fraction obtained in A was loaded and eluted with 0.05 M phosphate buffer, pH 7.4, and finally pH 4.95 of 1.4 N. Protein samples for analyses were hydrolyzed in the presence of 4 N HCl at 110 °C for 4 h. Neutral sugars were determined by the phenol-H2SO4 reaction following the modification of McKelvy and Lee (14). For qualitative identification of neutral sugars in DG hCG and native hCG the proteins were hydrolyzed in 2 N HCl at 100 °C for 5 h in evacuated sealed tubes. After removal of HCl by evacuation, the charged components in the hydrolyzate were removed by successive passage through columns of Dowex 50-H+ and Dowex 1-X2 formate. The residue was recovered by flash evaporation and dissolved in 50% ethanol. The neutral sugars present in the solution were separated by thin layer chromatography on microcrystalline cellulose plates (15). The neutral sugars were then identified by spraying with benzidine-periodate reagent (16). Galactose and mannose (Sigma) were used as reference standards. Sialic acid was determined after hydrolysis by a modified fluorimetric assay (17). Ultraviolet absorption spectra of hCG and DG hCG dissolved in 0.05 M phosphate buffer, pH 7.4, were recorded in a Beckman model 25 recording spectrophotometer.

Fluorescence spectra of aqueous solutions of hCG and DG hCG in the presence of the probe anilinonaphthalene sulfonate at room temperature (18) was recorded in a Hitachi-Perkin Elmer MPF-3 spectrofluorimeter.

Receptor Binding and Radioimmunoassays—Receptor binding activity of the hormone was assessed (8, 9) by using homogenate of adult rat testes or pseudopregnant rat ovaries as the receptor(s). Radioimmunoassay was performed using a specific antiserum (batch 2) supplied by the hormone distribution officer at the National Institutes of Health. For the above experiments NIH-hCG (CR119) labeled with 125I by the lactoperoxidase method (8, 9) and having a specific activity of 50-80 pCi/μg was employed as the tracer. The radioimmunoassay was performed in disposable glass tubes (10 x 75 mm). All reagents for the assay were diluted in 0.05 M phosphate buffer, pH 7.5, containing 0.01 M EDTA, 0.154 M NaCl, 0.02% sodium azide, and 3% normal rabbit serum. To the tubes containing different concentrations of the samples (100 μl), labeled hCG (CR119, 50,000 cpm) and 100 μl of antiserum (batch 2, diluted 1:33,000) were each

Fig. 2. Chromatographic characteristics of DG hCG. A, gel filtration pattern of DG hCG. Sephadex G-100 column (2.5 x 106 cm) was previously equilibrated in 0.05 M NH4HCO3. 50 mg of HF-treated hormone were loaded on the column and eluted with the same buffer. Fractions of 3.8 ml at a rate of 35 ml/h were collected. Fractions 67-90 were pooled. Recovery was 26 mg by weight. Broken line indicates elution pattern of hCG on same column. Inset, calibration of proteins on Sephadex G-100 column (1.5 x 107 cm) equilibrated at 4 °C in 0.05 M phosphate buffer, pH 7.5, containing 0.5 M NaCl. Fractions of 1.9 ml at 20 ml/h were collected. a, BSA (68,000); b, ovalbumin (45,000); c, pepcin (35,000); d, ovine prolactin (22,100); and e, lysozyme (14,200). Fucose was determined as described earlier (8).
Deglycosylation of Choriogonadotropin

added in 100-μl aliquots. The tubes were agitated and incubated at room temperature. After 24 h, the bound and free hormones were separated by adding 50 μl of a goat second antibody to rabbit γ-globulin (courtesy of Dr. F. Labrie, Laval University, Quebec). The tubes were incubated overnight and centrifuged at 2,900 × g for 10 min at room temperature. The supernatant containing the free labeled hormone was removed by aspiration and the pellet was counted in a Beckman γ-200 counter.

Biological Activity—The biological characteristics of the hormone before and after deglycosylation were assessed using testicular interstitial cells incubated in vitro. These cells were prepared from adult rat testes (19) using collagenase for dispersion. The cells (0.5 ml) were suspended in Dulbecco’s modified Eagle’s medium containing 37 °C under 95% O2-5% CO2. The reaction was terminated by immersing the tubes in a water bath at 80 °C for 10–15 min. The debris was separated by centrifugation and the clear supernatant was stored before and after deglycosylation were assessed using testicular interstitial cells incubated in vitro. These cells were prepared from adult rat testes (19) using collagenase for dispersion. The cells (0.5 ml) were suspended in Dulbecco’s modified Eagle’s medium containing

RESULTS

The biochemical and other characteristics of hCG purified for the current studies were identical with that of the CR119 preparation used as a reference in this investigation. These included electrophoretic properties, ampicillin composition, receptor binding, immunological potency, and in vitro biological activities.

Chromatographic Characteristics—Treatment of hCG with anhydrous liquid HF altered its gel filtration behavior. The treated hormone was retarded and thus emerged much later than the native hCG. The elution volume (Ve) under identical conditions of the experiment increased from 243 to 300 ml (Fig. 2A). Such a behavior would be expected due to a reduction in molecular weight as well as the degree of hydration following removal of carbohydrate residues by the treatment (see below). Similar results were obtained in our previous studies with ovine lutropin and its subunit after chemical deglycosylation (8, 9).

Chemical deglycosylation also markedly altered the binding of the hormone to affinity column containing conA-Sepharose (Fig. 2B). The DG hCG was completely excluded as compared to the native hCG which was strongly adsorbed and eluted by the competing glycoside. These results indicate that mannose residues which are implicated for binding of glycoproteins to the lectin conA (21) are lacking in DG hCG. Thus, the presence of untreated native hCG in DG hCG preparations is highly unlikely.

The molecular weight as estimated by gel filtration (Fig. 2A) was 74,000 for hCG and 42,600 for DG hCG.

Electrophoretic Properties—In polyacrylamide gel electrophoresis at pH 8.9 (Fig. 3A), the mobility of the DG hCG toward the anode was greatly reduced as compared to native hCG suggesting that the hormone was rendered less acidic. It may also be noted that electrophoretic heterogeneity present in the native hCG (lanes 2, 3, and 6, Fig. 3) which has been partly attributed to variation(s) in the oligosaccharide chain was greatly reduced after deglycosylation. As expected, at pH 4.5, the DG hCG migrated more rapidly than the native hormone (Fig. 3B).

Upon SDS-gel electrophoresis both hCG and DG hCG (Fig. 3C) showed two bands corresponding to the α and β subunits. However, α and β subunits in DG hCG migrated faster than the corresponding bands in hCG indicating a reduction in molecular weight. The estimated molecular weights for hCG subunits were 23,000 (α) and 35,000 (β). The estimated molecular weight for DG hCG subunits were 17,600 (α) and 23,200 (β).

Spectral Characteristics — The UV absorption spectra of hCG and DG hCG were similar except for a slight but perhaps significant enhancement in absorbance at 276 nm for the latter (Fig. 4A). The fluorescence probe ANS has been extensively used for the analysis of the conformational differences between native hCG and its α and β subunits and in recombination studies (18). As seen from Fig. 4B, there appear to be no conformational differences between hCG and DG hCG. Both proteins enhanced the fluorescence of ANS at 475 nm. Although both in the above studies equal concentrations of DG hCG and hCG were used (on a weight basis). DG hCG enhanced the fluorescence slightly more than that caused by hCG. This increase could partly be due to the alteration of protein:carbohydrate ratio in favor of the latter in DG hCG.

Amino Acid Composition—Amino acid analysis of hCG and DG hCG did not reveal significant differences in their composition (Table I). The number of each amino acid residue in both preparations correspond well with values computed for hCG from structural determinations (1).

Carbohydrate Composition—Treatment of hCG with an-
**Deglycosylation of Choriogonadotropin**

![Graph](http://www.jbc.org/)

**TABLE I**

<table>
<thead>
<tr>
<th>Component</th>
<th>hCG</th>
<th>DG hCG</th>
<th>hCG by structure (Ref. 1)</th>
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</thead>
<tbody>
<tr>
<td>Lys</td>
<td>10.7 ± 0.05</td>
<td>10.7 ± 0.1</td>
<td>10.7 ± 0.4</td>
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<tr>
<td>His</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Arg</td>
<td>13.5 ± 0.3</td>
<td>14.2 ± 0.2</td>
<td>14.3 ± 0.6</td>
</tr>
<tr>
<td>Asp</td>
<td>18.4 ± 0.2</td>
<td>18.4 ± 0.2</td>
<td>18.1 ± 0.6</td>
</tr>
<tr>
<td>Thr</td>
<td>18.8 ± 0.15</td>
<td>18.3 ± 0.12</td>
<td>17.0 ± 0.25</td>
</tr>
<tr>
<td>Ser</td>
<td>17.6 ± 0.25</td>
<td>18.1 ± 0.22</td>
<td>18.3 ± 0.6</td>
</tr>
<tr>
<td>Glu</td>
<td>19.4 ± 0.15</td>
<td>19.5 ± 0.32</td>
<td>19.5 ± 0.25</td>
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<tr>
<td>Pro</td>
<td>27.9 ± 0.7</td>
<td>30.7 ± 0.63</td>
<td>29.3 ± 0.2</td>
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<tr>
<td>Gly</td>
<td>12.8 ± 0.1</td>
<td>13.5 ± 0.2</td>
<td>13.3 ± 0.42</td>
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<tr>
<td>Ala</td>
<td>11.9 ± 0.36</td>
<td>13.0 ± 0.2</td>
<td>12.2 ± 0.11</td>
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<tr>
<td>Half-cystine</td>
<td>20.7 ± 0.5</td>
<td>21.3 ± 0.3</td>
<td>22.7 ± 0.63</td>
</tr>
<tr>
<td>Val</td>
<td>16.3 ± 0.9</td>
<td>16.4 ± 0.4</td>
<td>16.8 ± 1.0</td>
</tr>
<tr>
<td>Met</td>
<td>3.4 ± 0.2</td>
<td>3.0 ± 0.1</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Ile</td>
<td>6.1 ± 0.0</td>
<td>5.9 ± 0.21</td>
<td>5.6 ± 0.15</td>
</tr>
<tr>
<td>Leu</td>
<td>16.6 ± 0.25</td>
<td>16.9 ± 0.5</td>
<td>16.2 ± 0.5</td>
</tr>
<tr>
<td>Tyr</td>
<td>5.9 ± 0.1</td>
<td>ND</td>
<td>6.0 ± 0.23</td>
</tr>
<tr>
<td>Phe</td>
<td>6.2 ± 0.1</td>
<td>6.5 ± 0.3</td>
<td>6.1 ± 0.15</td>
</tr>
</tbody>
</table>

*Mean of two analyses.

**DISCUSSION**

The glycoprotein hormone hCG consists of a high percentage of carbohydrate. Brief treatment of the hormone with anhydrous HF at 0°C for 60 min resulted in the removal of approximately 75% of the total carbohydrate units. As this was achieved in a single step, this method of deglycosylation has advantages over the enzymatic approaches using sequential treatment with various glycosidases (5). Despite prolonged incubation for 7 days sequentially with exoglycosidases, the overall removal of carbohydrate was about 57% as calculated from published data (5). Another distinct advantage of the chemical deglycosylation procedure lies in the improved recovery of the product in a lyophilized and stable form. For example, in two experiments about 23 mg of the DG hCG were recovered in powder form starting from 50 mg of the purified hCG. The overall recovery after applying corrections for the loss of carbohydrate (Table II) and increased protein content is of the order of 68% after purification. This compares favorably against 22% recovery as calculated from the data of Moyle et al (5). It may be possible to achieve further deglycosylation by performing the HF treatment for either a longer time or at slightly elevated tempera-

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*Inubation for 5 min at room temperature, the fluorescence spectra was recorded with excitation wavelength set at 360 nm. Addition of either native hCG in 50 μl above buffer were mixed. After incubation for 5 min at room temperature, the fluorescence spectra was recorded with excitation wavelength set at 360 nm. Addition of either native hCG or hCG β subunits to the samples did not alter the fluorescence in both instances.*

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Hydrous liquid HF had a marked effect on its oligosaccharide moiety as revealed by the carbohydrate analysis of the product (Table II, compare columns 2 and 4). The treatment resulted in a loss of 87% of the neutral sugars, more than 90% reduction of the sialic acid content, and complete loss of fucose. Qualitative analysis of the neutral sugars in DG hCG revealed the presence of mannose and the absence of galactose. Among the hexosamines, only glucosamine was reduced by about 50% but galactosamine residues appeared to be unaffected by the treatment. The native hormone contained about 44.4 g of carbohydrate/100 g of the polypeptide and after HF solvolysis this was reduced to 9.6 g for the same amount of polypeptide. Thus, this accounts for a loss of about 78% of the carbohydrate residues in the molecule.

**Receptor Binding and Immunological Activities**—Partial deglycosylation of hCG by the chemical method resulted in an increase in receptor binding activity (Fig. 5, A and B and Table III). In both binding assays using rat testicular and rat ovarian membrane, DG hCG effectively competed with the labeled hCG for the receptor sites (Fig. 5, A and B). Although our data in Fig. 5, A and B are shown on a weight basis, the DG hCG was more active than hCG on a molar basis also. Data pooled from several experiments on different preparations of DG hCG are shown in Table III.

The radioimmunoassay for hCG was highly specific and conformation-dependent. Thus, the α and β subunits of hCG were recognized very poorly by the antibody (about 1% cross-reaction on a weight basis). In such an assay, DG hCG was as active (Fig. 5C and Table III) as native hCG indicating that the loss of a substantial proportion of carbohydrate had no influence on immunological reactivity (Table III).

**Biological Activity in Vitro**—Native hCG at low concentrations caused a marked increase in the accumulation of cyclic AMP in rat interstitial cells incubated in vitro. The isolated α and β subunits had very weak activity in this assay (Fig. 6). In contrast to native hCG, different preparations of the partially deglycosylated hormone at a wide range of concentrations up to 200 times higher than the maximal stimulatory doses of native hCG failed to evoke an increase of the cyclic AMP.

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![Graph](http://www.jbc.org/)
was the radioreceptor assay using rat testicular homogenate. About were added at the same time. In this figure as well as in total binding in the absence of the unlabeled hormone was calculated as ratio of the remaining sugar (column 4) to initial sugar (column 2) multiplied by its initial number of residues/mol of hCG (column 3).

\[
\text{Binding of hCG and DG hCG to gonadal receptors and specific antibody. A, inhibition curves of hCG and DG hCG in the radioreceptor assay using rat testicular homogenate. About 50,000 cpm }^{131} \text{I-hCG (CR119) was incubated with 25 mg of wet weight equivalent of tissues for 2 h at 37 }^\circ \text{C in a total volume of 0.5 ml. The total binding in the absence of the unlabeled hormone was } 17\% \text{ of added radioactivity. Nonspecific binding in presence of } 1 \mu g \text{ of hCG was } 5.5\%. \text{ Labeled hormone and unlabeled samples (where present) were added at the same time. In this figure as well as in B and C, data from one of several assays are shown. B, inhibition curves of hCG and DG hCG in the radioreceptor assay using pseudopregnant rat ovarian homogenate. About 66,000 cpm }^{131} \text{I-hCG was incubated with } 10 \mu g \text{ of wet weight equivalent of tissue incubated as above. Total binding in the absence of unlabeled hormone was } 14.2\% \text{ of added radioactivity. Nonspecific binding in presence of } 1 \mu g \text{ of hCG was } 4.7\%. \text{ C, radioimmunological activity of hCG and DG hCG estimated by gel filtration (42,000) or by SDS-PAGE (41,000) is much higher than the value of 27,200 expected from structural determinations (1). This abnormally high molecular weight has been attributed to the presence of covalently bound carbohydrate. Such an anomalous behavior is common with glycoproteins. The abnormal behavior of hCG was considerably reduced but not eliminated by deglycosylation which is still incomplete. The apparent molecular weight for DG hCG estimated by gel filtration (42,000) or by SDS-PAGE (41,000) is still higher than the value of 27,200 expected from the loss of carbohydrate. The presence of sugar residues remaining attached to the polypeptide could be responsible for these higher estimates.}

\[
\text{Receptor binding, immunological, and biological activity of hCG and DG hCG}
\]

Numbers in parentheses indicate the number of assays.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Activity*</th>
<th>hCG</th>
<th>DG hCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioreceptor assay a. Rat testicular homogenate</td>
<td>185.9 ± 10.3 (10)</td>
<td>178.8 ± 17.5 (10)</td>
<td></td>
</tr>
<tr>
<td>b. Rat ovarian homogenate</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Radioimmunoassay</td>
<td>98.2 ± 6.6 (8)</td>
<td>98.2 ± 6.6 (8)</td>
<td></td>
</tr>
<tr>
<td>Bioassay (in vitro)</td>
<td>0.00 (6)</td>
<td>0.00 (6)</td>
<td></td>
</tr>
</tbody>
</table>

* Activity relative to native hCG expressed on a molar basis. The molecular weight of DG hCG was assumed to be 27,200 as calculated from the loss of carbohydrate (see Table II and "Discussion").
The chemically deglycosylated hCG appears to be quite stable in the lyophilized form at 4°C. Two preparations have been evaluated for 12-16 months and found to be unaltered in all respects including receptor binding, immunological, and biological activities. Deglycosylation had no effect on the specificity of binding of the hormone to its specific receptor. This was evident from the failure of DG hCG to affect the binding of ovine follitropin to its own receptor in the testis (data not shown).

The extent of loss of the different monosaccharide units as a result of chemical deglycosylation (Table II) appear to be consistent with the proposed structure of the oligosaccharide moiety of hCG. This hormone consists of four asparagine (N-glycosidic)-linked and four serine (O-glycosidic)-linked carbohydrate units (2). Recently, two revised structures as shown (Structure 1) have been proposed for the N-glycosidically-linked moiety (22) and O-glycosidic linkages (23) (Structure 2).

Since there is no loss of N-acetylgalactosamine in DG hCG (Table II) and all of this is present in hCG β subunit (23) linked to serine residues near the carboxyl terminus, it is evident that the O-glycosidic linkages are more resistant to solvolysis by HF under the conditions employed. This has been experienced by other investigators also (24) in their studies with other glycoproteins. Thus, in DG hCG it is reasonable to suppose that only GalNAc attached to each of the four serine residues have remained intact. Since no lactose was found in the acid hydrolysates of DG hCG after thin layer chromatography, this sugar present in O-glycosidically linked carbohydrate moiety must have been removed by HF treatment. From the available data in Table II, one can also speculate that the peripheral NeuAc, Gal, GlcNAc, and Man have also been removed effectively in the N-glycosidically linked oligosaccharide units leaving behind one mannose and two GlcNAc residues attached to the Asn in each of the four such glycopeptides present in the hormone. The presence of about 6% NeuAc in DG hCG (Table II) which amounts to one residue of the sugar may be presumed to be part of the O-glycosidic moiety which is affected to a lesser degree. Since no fucose could be detected, the single residue present in the hormone (hCG β subunit, see Ref. 23) was completely removed.

Chemical deglycosylation of hCG did not affect the polypeptide moiety as revealed by amino acid composition data (Table I). We have also noted earlier (8, 9) in the case of ovine lutropin and subunits that their polypeptide moieties were not altered by HF treatment. This has been confirmed by the recent report in which it was noted that HF treatment of hCG β subunit did not generate any new NH₂-terminal residue not present in the starting material (25). In our present study the exclusion of anisole which is normally used during HF treatment as a scavenger (8, 9, 24) had no deleterious effects on the recovery of amino acid residues such as tyrosine, methionine, serine, or threonine, after hydrolysis of DG hCG (Table I). The full retention of spectral characteristics of DG hCG both in terms of the UV absorption spectrum and enhancement of ANS fluorescence confirm the lack of an effect on tyrosyl and/or other amino acid residues involved in such sensitive interactions.

\[
\begin{align*}
\text{NeuAC} \rightarrow & \text{Gal} \rightarrow \text{GlcNAc} \rightarrow \text{Man} \\
\text{Man} \rightarrow & \text{GlcNAc} \rightarrow \text{GlcNAc} \rightarrow \text{Asn} \quad \text{polypeptide} \\
\text{NeuAC} \rightarrow & \text{Gal} \rightarrow \text{GlcNAc} \rightarrow \text{Man} \\
\text{NeuAC} \rightarrow & \text{Gal} \rightarrow \text{GalNAc} \rightarrow \text{Ser} \quad \text{polypeptide} \\
\text{NeuAc} & \\
\text{STRUCTURE 1} & \\
\text{STRUCTURE 2}
\end{align*}
\]
The receptor binding, immunological, and biological properties of chemically deglycosylated hCG (Table III) appear to be similar to the enzymatically derived preparation in most respects. However, there are some differences which may be worthy of note. The DG hCG studied in this report and having greater than 75% less carbohydrate was more effective in binding to the lutropin receptor (Fig. 5, A and B, and Table III) than that of enzymatically deglycosylated hCG in which all four enzymes had been used to achieve the maximum possible deglycosylation (5).

Another significant difference between chemically obtained DG hCG and enzymatically prepared DG hCG lies in their possible deglycosylation (5).

The present studies confirm and extend previous observations (5-7) that the full integrity of the carbohydrate moiety of DG hCG and enzymatically prepared DG hCG is not required for receptor binding and immunological activities (Table III). However, a derivative with 75% less carbohydrate is devoid of in vitro biological activity (Fig. 6). It is not possible from the present data to precisely define if the alterations have directly interfered with the action of the hormone or if it is a consequence of a subtle change in the hormone or if it is a consequence of a subtle change in the properties of chemically deglycosylated hCG (Table III) appear to

Acknowledgments.—The assistance of Jayasree Sairam and Caroline Chagnon-Labelle is appreciated. We thank Dr. Y. T. Tsong, New York, and Ayerst Laboratories, Montreal, for the supply of human choriongonadotropin preparations and Dr. C. H. Li, San Francisco, for the gifts of ovine prolactin. The standards and human choriongonadotropin radioimmunoassay kits were supplied by the hormone distribution officer at the National Institutes of Health, Bethesda, MD. We are grateful to Dr. P. W. Schiller for allowing us to use the I1F apparatus.

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P Manjunath and M R Sairam


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