The Subunits of Human Pituitary Thyroid-stimulating Hormone

ISOLATION, PROPERTIES, AND COMPOSITION*

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SUMMARY

The subunits, α and β, of human thyroid-stimulating hormone (TSH) from the pituitary have been obtained by the same procedures utilized for the preparation of bovine TSH subunits, namely, dissociation in 1 M propionic acid and separation by gel filtration. The amino acid composition of human TSH-α indicates that this subunit is identical or nearly so with the α subunits of human luteinizing hormone and human chorionic gonadotropin. The NH₂-terminal residue is valine and it appears that human TSH-α is shortened at its amino end by 7 residues relative to bovine TSH-α; a similar relationship has been shown by others for human LH-α relative to the ovine or bovine subunit. The β subunit of human TSH differs markedly in amino acid composition from those of the two human gonadotropins, particularly with respect to tyrosine, isoleucine, valine, proline, arginine, and lysine. The carbohydrate analyses for the 2 subunits are given. A major difference between human and bovine TSH is the presence of sialic acid in the former, nearly all in the α subunit.

Immunological comparisons by double diffusion show that antisera to the intact hormone or its α subunit cross-react with homologous TSH and with the human gonadotropins but that the antiserum against the β subunit is specific for thyroid stimulating hormone.

The human subunits were shown, by gel electrophoresis and bioassay, to form intra- and interspecies recombinants with complementary subunits from other glycoprotein hormones.

Studies in several laboratories (1-3) have demonstrated that the glycoprotein hormones, thyroid-stimulating hormone and luteinizing hormone, consist of two dissimilar polypeptide chains, designated α and β (4), with oligosaccharide moieties bound to each. In both hormones the α subunit has an identical amino acid sequence. The amino acid sequences of the β subunits differ from each other and from the α sequence although there is marked homology among all three (5). Combination of an α chain with a β (hormone-specific) chain results in a molecule having the hormonal activity, either thyroid or gonad stimulating, of the parent hormone of the β subunit. The β subunits are immunologically distinct and many antisera to intact LH β or TSH reflect primarily determinants on their β chains (e.g., 4, 6-9).

The above observations were made with bovine and ovine hormones; more recently interest has centered on the human hormones, in part because of the desirability of obtaining purified antigens for radioimmunoassays and receptor-binding studies.

The gonadotropin of the human placenta, HCG, also consists of 2 subunits and comparison of preliminary amino acid compositions of the α subunits of human TSH, LH, and CG suggested that these subunits are also very similar (4). Evidence of the similarity of the α chain of HCG with those of bovine TSH and LH was obtained by the formation of active hybrid molecules between HCG-α and bovine TSH-β or LH-β as well as the reverse (10). Proof that the α chains of HCG and human LH are identical or nearly so is found in recent reports of their amino acid sequences (12-14); the data also show that while there are interspecies differences, major features of the sequence of these two human hormones and those of domestic animals have been conserved.

Although the preliminary data on the amino acid composition of the subunits of human TSH (4) enabled recognition of similarities between the α chains of the three human hormones, details of the preparation and properties of human TSH subunits have not appeared. These are presented here together with the carbohydrate and amino acid compositions, NH₂-terminal residues, and immunological properties.

1 The abbreviations used are: TSH, thyroid-stimulating hormone (thytrotropin); LH, luteinizing hormone (interstitial cell-stimulating hormone); HCG, human chorionic gonadotropin; FSH, follicle stimulating hormone; α and β, the 2 subunits of the glycoprotein hormones; Fd-1-dimethylaminonaphthalene-5-sulfonyl. In tables and in figure legends: h, human; b, bovine.

2 It has come to our attention that the gonadotropin potencies of recombinations with HCG-β and LH-β reported were expressed in terms of weight of β chain present rather than the total weight of recombinant (α plus β) and thus represent the increase in potency achieved by recombination relative to the potency of β chains alone (see also Reference 11).

3 Preliminary reports of some data concerning amino acid composition and immunological response have appeared (4, 6).
EXPERIMENTAL PROCEDURE

Details of the method of preparation of human TSH in this laboratory (15) have not been published. The procedure is modified from that of Stockell-Hartree (16). The starting material was a "gonadotropin powder" supplied by the National Pituitary Agency; two 10-g batches were available. One 10-g sample of the gonadotropin powder was extracted overnight with an ammonium acetate-acetic acid buffer, 0.004 M with respect to ammonium ion, pH 5.5, and the extract applied to a CM-cellulose column (Whatman CM-52). After elution of nonabsorbed material with the above buffer, the TSH and LH were eluted with 1 M ammonium acetate, pH 7.15, and recovered by freeze-drying after dialysis against water. The TSH-LH fraction was then chromatographed on DEAE-cellulose (Whatman DE-32); the material was placed on the column in 0.005 M sodium glycinate, pH 9.5. Initial elution was with 0.05 M sodium glycinate, pH 9.5. A gradient was then applied that was prepared with a Varigrad apparatus (Technicon Instruments) with 160 ml of the following concentrations of sodium glycinate, pH 9.5, in the first five chambers: 0.1 M, 0.1 M, 0.15 M, 0.2 M, and 0.25 M. After completion of the gradient, 0.25 M sodium glycinate, which was 0.5 M with respect to NaCl, pH 9.5, was used to complete elution. The separation of LH and TSH was monitored by double diffusion with antisera against human LH and TSH (15). After dialysis and freeze-drying, the TSH-containing fractions were rechromatographed on CM-cellulose (Whatman CM-52). The initial eluting buffer was 0.01 M PO4, pH 6.3; the TSH was eluted after the nonabsorbed fractions with 0.01 M PO4-1 M NaCl, pH 6.3. Gel filtration on Sephadex G-100 was performed on one preparation to remove any aggregated material. The amount of aggregate was negligible and this step was omitted for the second batch.

Subunits were prepared as described for bovine TSH (1). The hormone was allowed to stand overnight at room temperature in 1 M propionic acid (0.5 mg of protein per ml). After freeze-drying to remove the propionic acid, the material was immediately subjected to gel filtration on Sephadex G-100 in 0.126 M ammonium bicarbonate to separate the dissociated subunits.

Subunits of HCG were kindly supplied by Dr. Om P. Bahl. Bovine TSH, LH, and their subunits were prepared as described previously (4).

Analytical Methods—The bioassay for TSH was the measurement of the uptake of 14C into the thyroids of 1-day-old chicks (17). LH was assayed by measurement of ovarian ascorbic acid depletion (18).

Disc gel electrophoresis was as described (19). Gel electrophoresis in the presence of mercaptoethanol and sodium dodecyl sulfate was by the method of Weber and Osborn (20).

NH2 termini of human TSH and its subunits were determined by formation of the dansyl derivatives by the method of Gray (21) except that urea and excess reagents were removed by dialysis rather than by gel filtration. The dansyl amino acids were determined by thin layer chromatography on polyamide plates (22).

Amino acid analyses were by the method of Spackman (23). Phenol was added to protect tyrosine during hydrolysis and half-cystine and methionine were determined as cysteic acid and methionine sulfone (24). Neutral sugars were determined by gas-liquid chromatography of their alditol acetates and the amino sugars by amino acid analysis (25). Sialic acid was determined by the method of Warren (26).

Recombination of Subunits—To test for recombination, 50 pg of each subunit were dissolved in 100 ul of 0.012 M sodium glycinate, pH 9.5. The tubes were flushed with N2 and incubated overnight at 37° (1). Aliquots were then removed for bioassay and electrophoresis.

Immunological Procedures—The antisera against human TSH, LH, TSH-α, and TSH-β were made as described previously (6, 15, 19). Anti-HCG-α and anti-HCG-β were a gift of Drs. J. L. Vaitukaitis and G. T. Ross. Tests for cross-reactivity were made by the double diffusion method of Scheidegger (27).

RESULTS

In the initial CM-cellulose chromatography, as reported by Stockell-Hartree (16), much of the FSH activity is unabsorbed while most of the TSH and LH are retained on the column. The yield of the TSH-LH fraction was approximately 1.5 g from the original 10 g of "gonadotropin powder." The results of the chromatography of the TSH-LH fraction on DEAE-cellulose are shown in Fig. 1A. Double diffusion showed that the material in Fractions I and II cross-reacted strongly against anti-human LH but negligibly against anti-human TSH. Fractions emerging subsequent to Fraction I showed an increasing cross-reactivity towards anti-human TSH, with the most intense cross-reaction given by the material in Fraction IV. Some cross-reactivity against anti-human LH was evident in all fractions. The 360 mg obtained as Fraction IV were recho-
Fig. 2. Top, electrophoresis in 7.25% polyacrylamide gels. Samples a to d are Fractions I to IV (Fig. 1A); Samples e and f are Fractions I and II after chromatography on CM-cellulose; Fraction II is hTSH; Samples g and h are hTSH-α and hTSH-β, respectively; Samples i to k are bTSH, bTSH-α, and bTSH-β. Middle, sodium dodecyl sulfate gel electrophoresis in the presence of urea and β-mercaptoethanol, the samples are, from a to f: hTSH, hTSH-α, hTSH-β, hTSH-γ, bTSH, bTSH-α, and bTSH-β. Bottom, electrophoresis in 7.25% polyacrylamide gels. Samples a to d are recombinations of hTSH-α + hTSH-β, hTSH-α + bTSH-β, bTSH-α + hTSH-β, and bTSH-α + bTSH-β. Controls of separate hTSH-α, hTSH-β, bTSH-α, and bTSH-β which were incubated under the same conditions and were subjected to electrophoresis at the same time are shown in the top panel, Gels g, h, j, and k. Samples i to l are recombinations of hTSH-α + bLH-β; hTSH-α + HCG-β; bLH-α + hTSH-β; HCG-α + hTSH-β; the controls of separate bLH-β, HCG-β, bLH-α, and HCG-α are shown in Gels e to h. Gels e and h were run in a separate experiment. Gels f and g as well as samples of hTSH-α and hTSH-β were subjected to electrophoresis together with the recombinations, i to l. The hTSH-α and hTSH-β controls were identical with the equivalent gels shown in the top panel, g and h.
matographed on CM-cellulose; the results are shown in Fig. 1B; the TSH activity is in Fraction II. The electrophoretic patterns of the fractions obtained after both chromatographic runs are shown in Fig. 2, top (Samples a to f). One hundred fifteen milligrams of TSH were recovered from the 10 g of starting material. Its potency was 10 to 15 units per mg when compared to the International Standard for thyrotropin (bovine), the LH contamination was 0.17 times the National Institutes of Health ovine LH standard, S-1. In the processing of the second 10-g batch, stepwise rather than gradient elution was used for the chromatography on DEAE-cellulose. After the rechromatography on CM-cellulose, 73 mg of TSH were obtained with a potency of 10 to 15 units per mg and a LH contamination of 0.02 times the standard S-1.

A typical separation of the subunits of human TSH is shown in Fig. 3. The subunits were recovered by freeze-drying the fractions indicated by the solid bars. Additional subunits were obtained by repetition of the dissociation and gel filtration of material obtained by combining the fractions from 720 ml to 775 ml and from 850 ml to 900 ml with the undissociated TSH (fractions 460 ml to 720 ml). To conserve yield, additional gel filtration to remove contamination of each subunit with the other (5) was not performed. The electrophoretic patterns of the fractions obtained after both chromatographic runs are shown in Fig. 2, top (Samples a to f). One hundred runs are shown in Fig. 2, top (Samples a to f). One hundred

FIG. 3. Gel filtration on Sephadex G-100 of 60 mg of human TSH. The material was dissociated overnight in 120 ml of 1 N propionic acid, freeze-dried, then dissolved in 2 to 3 ml of 0.126 M ammonium bicarbonate and applied to the column (300 X 2.5 cm). Elution was with 0.126 M ammonium bicarbonate at a flow rate of 25 ml per hour. The solid bars indicate the fractions pooled and freeze-dried. The yield of a was 14 mg and that of b 10 mg. The peaks containing the subunits are designated.

TABLE I

<table>
<thead>
<tr>
<th>Residue</th>
<th>hTSH-α</th>
<th>hLH-α</th>
<th>HCG-α</th>
<th>hTSH-β</th>
<th>hLH-β</th>
<th>HCG-β</th>
<th>kTSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>5.7 ± 0.0</td>
<td>6</td>
<td>6</td>
<td>7.9 ± 0.1</td>
<td>3.0</td>
<td>4</td>
<td>13.7</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.0 ± 0.0</td>
<td>3</td>
<td>3</td>
<td>3.0 ± 0.1</td>
<td>2.8</td>
<td>1</td>
<td>6.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.3 ± 0.0</td>
<td>3</td>
<td>3</td>
<td>5.3 ± 0.1</td>
<td>10.7</td>
<td>11</td>
<td>16.8</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>5.7 ± 0.0</td>
<td>5</td>
<td>6</td>
<td>10.5 ± 0.1</td>
<td>8.6</td>
<td>11</td>
<td>16.8</td>
</tr>
<tr>
<td>Threonine</td>
<td>7.3 ± 0.2</td>
<td>8</td>
<td>8</td>
<td>11.3 ± 0.2</td>
<td>7.9</td>
<td>9</td>
<td>17.9</td>
</tr>
<tr>
<td>Serine</td>
<td>6.8 ± 0.1</td>
<td>8</td>
<td>8</td>
<td>5.6 ± 0.2</td>
<td>7.3</td>
<td>11</td>
<td>13.6</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>8.9 ± 0.0</td>
<td>9</td>
<td>9</td>
<td>8.8 ± 0.3</td>
<td>0.4</td>
<td>8</td>
<td>20.2</td>
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<tr>
<td>Proline</td>
<td>7.1 ± 0.8</td>
<td>6</td>
<td>7</td>
<td>8.5 ± 0.5</td>
<td>17.6</td>
<td>24</td>
<td>16.6</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.5 ± 0.1</td>
<td>4</td>
<td>4</td>
<td>5.5 ± 0.5</td>
<td>9.0</td>
<td>8</td>
<td>10.5</td>
</tr>
<tr>
<td>Alanine</td>
<td>4.4 ± 0.0</td>
<td>4</td>
<td>5</td>
<td>7.0 ± 0.0</td>
<td>6.1</td>
<td>7</td>
<td>12.0</td>
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<tr>
<td>Half-cystine</td>
<td>8.9</td>
<td>10</td>
<td>10</td>
<td>11.8</td>
<td>12.8</td>
<td>10</td>
<td>17.5</td>
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<td>Valine</td>
<td>6.7 ± 0.0</td>
<td>7</td>
<td>7</td>
<td>4.6 ± 0.0</td>
<td>19.7</td>
<td>11</td>
<td>12.4</td>
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<tr>
<td>Methionine</td>
<td>2.6 ± 0.1</td>
<td>3</td>
<td>3</td>
<td>2.2 ± 0.0</td>
<td>2.0</td>
<td>1</td>
<td>4.9</td>
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<tr>
<td>Isoleucine</td>
<td>1.9 ± 0.3</td>
<td>1</td>
<td>1</td>
<td>9.0 ± 0.2</td>
<td>4.9</td>
<td>5</td>
<td>9.6</td>
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<tr>
<td>Leucine</td>
<td>4.5 ± 0.1</td>
<td>4</td>
<td>4</td>
<td>7.8 ± 0.8</td>
<td>9.6</td>
<td>13</td>
<td>12.5</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.6 ± 0.5</td>
<td>4</td>
<td>4</td>
<td>11.5 ± 0.2</td>
<td>2.5</td>
<td>3</td>
<td>13.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.9 ± 0.1</td>
<td>4</td>
<td>4</td>
<td>4.5 ± 0.0</td>
<td>9.4</td>
<td>2</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Approximate number of residues 89.7 89 92 125 129 139 215 215

The calculations are based on the average number of micromoles per residue of several stable amino acids, with the number of these residues determined by examination of molar ratios and the assumption of a molecular weight of 14,000 for each subunit. With the exception of cysteine, all values are the average of two determinations. Half-cystine was determined after oxidation of the subunits with performic acid.

a Obtained from the sequence reported by Papkoff et al. (14).

b Obtained from sequences reported by Bahl et al. (12).

c Data of Stoeckl Hartroft et al. (20), recalculated on the basis of a subunit molecular weight of 14,000.
Table II
Carbohydrate compositions of human TSH subunits as compared to subunits of human LH and HCG

The values are expressed as residues per 14,000 molecular weight; a 10% correction for moisture has been included. Each value represents the average for two determinations.

<table>
<thead>
<tr>
<th>Residue</th>
<th>bTSH-α</th>
<th>bLH-α</th>
<th>HCG-α</th>
<th>hTSH-β</th>
<th>hLH-β</th>
<th>HCG-β</th>
<th>hTSH α+β</th>
<th>hTSH</th>
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</thead>
<tbody>
<tr>
<td>Fucose</td>
<td>0.2</td>
<td>0.5</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>1.5</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Mannose</td>
<td>4.1</td>
<td>4.3</td>
<td>4.7</td>
<td>2.0</td>
<td>2.0</td>
<td>4.9</td>
<td>6.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Galactose</td>
<td>1.3</td>
<td>2.2</td>
<td>1.3</td>
<td>0.4</td>
<td>0.9</td>
<td>7.6</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>5.2</td>
<td>7.2</td>
<td>7.4</td>
<td>2.1</td>
<td>3.3</td>
<td>7.6</td>
<td>7.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>1.5</td>
<td>1.1</td>
<td>0.2</td>
<td>0.7</td>
<td>0.5</td>
<td>2.0</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>1.4</td>
<td>0.2</td>
<td>1.8</td>
<td>0.3</td>
<td>0.0</td>
<td>5.5</td>
<td>1.7</td>
<td>1.7</td>
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<tr>
<td>Total residues</td>
<td>13.7</td>
<td>15.5</td>
<td>15.8</td>
<td>6.0</td>
<td>7.3</td>
<td>29.1</td>
<td>19.7</td>
<td>19.3</td>
</tr>
</tbody>
</table>

α Values for HCG-β are based on a molecular weight of 16,500 estimated from its amino acid sequence (12) and carbohydrate composition (30).

β The values for bLH-α and bLH-β are those reported by Stockell-Hartree et al. (29).

d Analyses performed at the same time as those for the subunits. Values in this column taken from Pierce et al. (4).

e Sialic acid was cleaved from these subunits during their isolation (29).

Recombination Experiments—The ability of the human TSH subunits to recombine with complementary subunits of bovine LH, bovine TSH, and HCG is shown by disc gel electrophoresis in Fig. 2, bottom. Loss of bands characteristic of the individual subunits with the generation of new bands is indicative of recombination. Samples a to d show that, by electrophoretic criterion, each human TSH subunit recombines with its complementary bovine chain. Samples i to l show that human TSH-α will recombine with bovine LH-β and HCG-β and that human TSH-β recombines with bovine LH-α and HCG-α. Control patterns of the free bovine LH and HCG subunits are shown in Samples e to k, while those of the TSH subunits are given in Fig. 2, top (Samples g to k).

Each recombination involving a TSH-β subunit was tested for biological activity. Typical results are shown in Fig. 4. In these experiments, which included several bioassays, the best recovery of biological activity involving a human TSH-β subunit was obtained with recombinants formed between the human TSH-β subunit and bovine TSH-α or HCG-α. Thus, human recombinants usually gave a recovery of approximately 25% of the activity of intact human TSH while the recombinant between bovine TSH-α and human TSH-β yielded 50%. In all cases activity significantly beyond the summation of that of the individual subunits was regenerated.

Antisera raised against human TSH and its subunits, human LH, and the subunits of HCG were tested for precipitating antibodies by double diffusion against human TSH, bovine TSH, and LH, and their subunits, human LH, HCG-α, and HCG-β. The results of these experiments are summarized in Table III. Anti-human TSH and anti-human LH react similarly against the antigens containing an α chain but do not cross-react with β chains from heterologous hormones. Anti-human TSH-α and anti-HCG-α react identically with the series of antigens. The anti-human TSH-β cross-reacts strongly with intact human TSH, human TSH-β, bovine TSH but only weakly with bovine TSH-β, indicating that when bovine TSH-β is bound to the α chain its similarities to human TSH-β are manifested to a greater degree. Anti-HCG-β was highly specific, cross-reacting only with itself. No cross-reactivity was found with bovine LH or its subunits against the antisera to human fractions. A difference between human and bovine hormones was found in that more antibodies against α determinants appeared to be present in the antisera raised against intact human TSH.
TABLE III

Relative cross-reactivity between antisera to human TSH, its subunits, human LH, and HCG-α and β to various glycoprotein hormones and their subunits

Based on relative intensities of precipitation lines observed during double diffusion. Antigen concentration was 0.05% unless otherwise noted. +, ++, and +++ indicate relative strength of precipitation seen without staining. * Indicates no line visible even after staining. ** Indicates a line visible following dilute diffusion. Antigen concentration was 0.05% unless otherwise noted. +, ++, and +++ indicate relative strength of precipitation.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Anti- hTSH</th>
<th>Anti- LH-α</th>
<th>Anti- LH-β</th>
<th>Anti- TSH-α</th>
<th>Anti- TSH-β</th>
<th>Anti- HCG-α</th>
<th>Anti- HCG-β</th>
</tr>
</thead>
<tbody>
<tr>
<td>hTSH</td>
<td>++</td>
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<td>++</td>
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<td>+</td>
<td>+</td>
<td>++</td>
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<td>+</td>
<td>++</td>
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</tr>
</tbody>
</table>

* In contrast to most antisera prepared thus far against intact bovine TSH and LH, these antisera contain a considerable population of antibodies against α determinants.

† This LH fraction is analogous to Fraction II (Fig. 1A), which was shown by radioimmunoassay to be one-tenth as immunoreactive as intact human TSH in radioimmunoassay against anti-human TSH-β; Fraction I was only one-three hundredth as immunoreactive (see “Discussion”).

‡ Not determined.

In amino acid composition, human TSH-α differs from bovine TSH-α (5) particularly in its lower content of lysine (6 versus 10 residues) and alanine and a higher content of serine and valine. The experimentally determined composition is almost identical with that of other human LH-α or HCG-α (Table I). Brief reports of the sequences of the latter two α chains have recently appeared (12-14); the sequences are the same except that HCG-α has three additional amino acids, NH2-Ala-Pro-Asx, at its NH2 terminus. The NH2-terminal residue of human LH-α is valine; this chain is 7 residues shorter than that of the maximum chain length found in its bovine or ovine counterparts. The summation of the composition of the human TSH-α (Table I) together with the finding of NH2-terminal valine indicate that human TSH-α is similarly shortened. The deviations of the experimentally determined composition of TSH-α from the composition of HCG-α or LH-α probably result from contamination with some TSH-β inasmuch, in order to conserve yield, as a second gel filtration (5) was not performed. The amino acid composition of the human TSH-β subunit differs considerably from human LH-β or HCG-β with particularly large differences in tyrosine and proline contents. Major differences from bovine TSH-β are higher contents of leucine and isoleucine. The carbohydrates of the human TSH subunits are qualitatively the same as for bovine TSH, with the addition of sialic acid. A previous study indicates sialic acid to be unnecessary for the activity of human TSH in the chick assay (15). The majority of the neutral and amino sugar residues are, as with bovine TSH, on the α chain together with most of the sialic acid. The neutral and amino sugars are distributed between the 2 subunits much as in human LH-α and β; comparison of sialic acid distribution cannot be made, as in the one report concerning the carbohydrates of human LH subunits (24), a considerable proportion of sialic acid was cleaved because of the low pH necessary for dissociation. HCG-α also has a similar carbohydrate composition (12, 30) to TSH-α; the contrast between the total carbohydrates of TSH and LH versus HCG is because of differences on the β chains.

Incubation of either human TSH-α or β with the corresponding α or β subunit of bovine TSH or LH showed recombination by the electrophoretic criterion (Fig. 2), and TSH assays of the recombinants, in which α or β subunit was present, showed an increase in biological activity over the separate chains. This ability of either of the human TSH subunits to recombine with a bovine partner demonstrates that the differences in sequence (as reflected in the compositional differences) are not sufficient to prevent subunit-subunit interactions. The results are in agreement with earlier studies on interspecies hybrids of human LH-α plus bovine LH-β (36) and HCG subunits with bovine TSH subunits (10). The human TSH-α preparations, however, may have been damaged by oxidation or other modification.

DISCUSSION

The complete removal of human LH from TSH has remained a problem ever since the first major purification of human TSH was described by Condliffe (32). Chromatography on DEAE-cellulose, to date, has been the only feasible means found to separate human LH and TSH (16, 32, 33). The lesser amount of TSH obtained, which was approximately 20% of that obtained for LH, also demonstrates that the distribution coefficients of human TSH and LH do not favor easy separation. Although complete separation of LH from TSH was achieved by counterfiltration (19); this approach has not been successful with human TSH because human LH does not dissociate as readily as the bovine hormone (29, 34). Preliminary experiments also indicated that the distribution coefficients of human TSH and LH do not favor easy separation. One reason for the partially overlapping emergence of human LH and TSH on anion exchangers is that both contain sialic acid (in contrast to its absence in the bovine hormones). Similar problems have been encountered in other purifications of human TSH, e.g. References 32 and 33. An appreciable amount of LH, however, can be obtained almost completely free of TSH by anion exchange chromatography; material from Fraction I (Fig. 1A) had little cross-reactivity against the antisera to intact human TSH and radioimmunoassay* against an anti-human TSH-β showed it to be 300 times less immunoreactive than human TSH. Fraction II was 10 times less immunoreactive. The material removed from the TSH by the final chromatography on CM-cellulose is more anionic than TSH (see Fig. 2, top, Sample e); it probably contains some FSH not removed by the initial chromatography on CM-cellulose. FSH has an appreciably higher content of sialic acid than TSH and LH (35).
during collection of the pituitaries or subsequent preparative procedure because the recovery of activity from the human \( \alpha/\beta \) or human-\( \alpha \) bovine-\( \beta \) recombinants was less than when a bovine \( \alpha \) subunit was used. A difference in response because the assay system is heterologous both for human and bovine material appears unlikely because HCG-\( \alpha \) plus human TSH-\( \beta \) also gave a greater response than the human \( \alpha/\beta \) recombinant.

The results with the human TSH subunits extend further the concept that first became apparent from studies on bovine TSH and LH (4), namely, that within species the \( \alpha \) subunits are identical or nearly so in their protein portions and that both the biological and immunochemical specificities of the pituitary glycoprotein hormones and of the placental chorionic gonadotropin reside in the \( \beta \) subunits. The immunochemical data show that the major cross-reactivity of the antisera to the human \( \beta \) preparation is with its homologous antigen and homologous intact TSH, with bovine TSH cross-reacting to a lesser extent. Radioimmunoassay with antisera raised against the human TSH-\( \beta \) described in this work shows an inhibition plot, obtained by use of intact human TSH, which is almost parallel to that given by \( \beta \) subunit. Specificity of the TSH-\( \beta \) antisera for TSH versus LH and HCG was again demonstrated. Thus the obvious choice for specific radioimmunoassays of human TSH is an antisera to the \( \beta \), hormone-specific, subunit. Vaitukaitis et al. (8) have also shown by radioimmunoassay that a parallel inhibition plot is given by intact human LH against an anti-human LH-\( \beta \) serum. The 2 human \( \alpha \) subunits, TSH-\( \alpha \) and HCG-\( \alpha \), cross-reacted identically against both anti-human TSH-\( \alpha \) and anti-HCG-\( \alpha \), thus giving further evidence of the near identity of these 2 subunits. Bovine TSH-\( \alpha \) did not cross-react against the anti-human \( \alpha \) antisera. It is interesting that despite the relatively few changes between human and bovine \( \alpha \) chains (recent sequence studies in other laboratories show that approximately 70% of the sequence of human and ovine (or bovine) LH-\( \alpha \) are identical and that about another 21% of the positions represent a single base change in the genetic code), these subunits do not cross-react (or cross-react weakly) with heterologous \( \alpha \) antisera. Similar observations have been made recently by Vaitukaitis et al. (8) in radioimmunoassays which show the same lack of cross-reactivity between anti-human LH-\( \alpha \) and ovine, bovine, and rat LH-\( \alpha \). It thus appears as stated by these authors, and now shown for TSH as well, that the identical portions of the \( \alpha \) subunits may be inaccessible for antigen antibody reaction. Alternatively, the major anti-\( \gamma \) genetic determinants may be in the nonidentical portions of the \( \alpha \) structure.

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