The Characterization of Alpha-Subunit of Glycoprotein Hormone Produced by Undifferentiated Carcinoma

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Abstract

The glycoprotein hormone α-subunit was extracted and purified from the urine of a patient with undifferentiated carcinoma producing isolated α-subunit. Its final specific immunoactivity was 0.92 (mg α-subunit/mg protein). The α-subunit exhibited virtually identical immunoantigenicity to hCG-α antiserum with standard hCG-α. The molecular weight of the α-subunit determined by gel chromatography on Sephadex G-100 was greater than that of standard hCG-α dissociated by urea in vitro. By SDS disc electrophoresis, however, the α-subunit moved faster than hCG-α separated by mercaptoethanol reduction. The amino acid composition of the α-subunit was quite similar to that of standard hCG-α. In the isoelectric focusing, the major components of the α-subunit from undifferentiated carcinoma and the α-subunit from urine of normal pregnant women (third trimester) were distributed over the range from pH 3.5 to 6.0, while standard hCG-α was distributed in the fractions ranging from pH 6.0 to 8.0. The result of a combination study in vitro indicated that both α-subunits from undifferentiated carcinoma and from urine of normal pregnant women did not actively combine with hCG-β.

These results suggest that the α-subunit secreted by undifferentiated carcinoma is virtually identical with standard hCG-α as the protein moiety but differs in regard to carbohydrate moiety, and also suggest that the excess of α-subunit, which is not associated with β-subunit, may have undergone some intracellular modification, and consequently, the electric charge of the freely secreted α-subunit changes and it no longer has the ability to combine with the β-subunit.

In addition to the eutopic synthesis and secretion of free subunits of glycoprotein hormones (Franchimont and Reuter, 1972; Laburthe et al., 1973; Vaitukaitis et al., 1976; Nishimura et al., 1979), ectopic production has been demonstrated in vivo (Weintraub and Rosen, 1973; Rosen and Weintraub, 1974) and in vitro (Tashjian et al., 1973; Chou et al., 1976; Lieblich et al., 1976). Pioneering studies on the characterization of ectopically produced α-subunits have been reported by the group of Weintraub (Weintraub et al., 1975), who extracted and purified α-subunit from in vivo material to permit the amino acid analyses.

In placenta, hCG is likely synthesized via the association of α- and β- subunits which are synthesized from two different messenger RNAs (Boime et al., 1978). Therefore, to know the characteristics of free α-subunit is worthwhile in investigating the biosynthesis of glycoprotein hormones.

In the present study, we extracted and purified α-subunit from urine of a patient with
undifferentiated carcinoma producing large amounts of isolated α-subunit, and compared its characteristics with standard hCG-α separated from hCG in vitro and α-subunit from urine of normal pregnant women.

Materials and Methods

Case report
A 32-year-old para 2 Japanese woman was admitted to Osaka National Hospital in September 1977 because of gradual enlargement of a left femoral tumor, which was resected in December 1977. A chest X-ray showed multiple round shadows eight months after the initial treatment. In July 1979, we were consulted concerning her amenorrhea of three months duration, and noted a positive pregnancy test. Further examination revealed the ectopic production of immunoreactive hCG-α. The lung lesions were identified with the femoral tumor by autopsy, and diagnosed histologically as undifferentiated carcinoma. The localization of hCG-α was detected by immunohistological study.

Extraction and purification of α-subunit from the urine of a patient with undifferentiated carcinoma

Extraction of urine
Urine of the patient was extracted by the standard kaolin-acetone method which had been developed as an extraction method for urinary hCG (Borth et al., 1961).

All steps of extraction and purification were carried out at 4°C. Eluates from the column were monitored for protein content by measuring absorbance at 280 nm with an Hitachi 124 spectrophotometer and assayed by homologous hCG-α radioimmunoassay (Ashitaka et al., 1974). Protein determinations for specific immunoactivity of α-subunit were made by the method by Lowry et al., (1951).

Chromatography on DEAE-cellulose
The lyophilized powder was dissolved in 50 ml of 0.005 M Tris-HCl buffer, pH 8.0. This solution was applied to a column (2 x 30 cm) on DEAE-cellulose (Brown, Lot No. MOK 8299) equilibrated with a 0.005 M Tris-HCl buffer, pH 8.0. The elution was performed by a linear gradient of NaCl concentration, from 0-0.3 M, in 500 ml of the same buffer. Five ml fractions were collected at a flow rate of 15 ml/hour with a ISCO fraction collector.

Chromatography on Sephadex G-100
The major peak from DEAE-cellulose chromatography was passed over a Sephadex G-100 column (2.8 x 96 cm) in 0.05 M sodium phosphate buffer, pH 7.4. Three ml fractions were collected at a flow rate of 10 ml/hour. All fractions under the peak of the absorbance and the immunoactivity of hCG-α were pooled, dialyzed extensively against distilled water and lyophilized.

Extraction and partial purification of α-subunit from urine of normal pregnant women
α-subunit was extracted from 50 l of urine of normal pregnant women (third trimester) using the kaolin-acetone method. Extracted material was subjected to gel chromatography on Sephadex G-100 in the same conditions as described for the purification of α-subunit from urine of the patient.

Elution profile of α-subunit from undifferentiated carcinoma on Sephadex G-100
125I-labeled α-subunit from undifferentiated carcinoma was gel-filtered on a 1.5 x 90 cm Sephadex G-100 column. The column was calibrated with blue dextran, 125I-hCG and 125I-hCG-α. A ISCO fraction collector was used to collect 2.5 ml fractions at 4°C at a flow rate of 12 ml/hour.

SDS disc electrophoresis
Sodium dodecyl sulfate gel electrophoresis was performed in 7.5% polyacrylamide gel as described by Weber and Osborn (1969). The samples were dissolved in 0.02 M phosphate buffer, pH 7.2, containing 1% SDS, 2% 2-mercaptoethanol, 0.05% bromophenol blue and heated at 100°C for two minutes before the electrophoretic run. For the determination of molecular weight by electrophoresis the standard molecular marker (BDH, monomer of lysozyme=14,300) was applied under the same conditions.

Amino acid analysis of α-subunit from undifferentiated carcinoma
Approximately 1 mg of the α-subunit was hydrolyzed with 6 N HCl in a sealed tube at 110°C for 12 hours. After the removal of acid, the hydrolysate was analyzed by amino acid analyzer (JLC-6AH, JEOL). Tryptophan was not determined.

Isoelectric focusing
A column apparatus (Kato Co. Ltd., Japan) of 110 ml capacity was used for this study. Approximately 2 μg of samples were introduced into the sucrose gradient from 0 to 50%. The mean concentration of ampholine (LKB) was 2%. The column was refrigerated by circulating cold ethanol using a refrigerated circulating bath (Neslab, Model RTE 5). Isoelectric focusing was carried out for 12 hours at 400 volts, followed by 40 hours at 750 volts by a power supply (MS). Fractions (1.5 ml) were then collected with a microfractionator (Gilson), and the pH of these fractions was immediately measured.

Combination of alpha- and beta-subunits
The ability of α-subunit from undifferentiated carcinoma to combine hCG-β subunit was determined by hCG-RIA. The recombination of standard hCG-α and hCG-β, separated by urea in vitro, was also
studied as a control. The methods of combination study and calculation of the per cent of theoretical combination at time course were performed as described by Weintraub et al. (1977).

Fifteen μg of the α subunit and standard hCG-α were individually incubated with 23 μg of hCG-β according to molecular weight. The incubation was carried out at 4°C and 27°C in 100 μl of 0.01 M sodium phosphate, pH 7.4, in plastic tubes (5 × 20 mm) sealed with parafilm. Five μl aliquots were removed at zero time and varying time, diluted in 2 ml of cold 0.05 M sodium phosphate, pH 7.4, containing 0.05% BSA and stored at −80°C until assay.

Results

Extraction and Purification

Table 1 shows the results of extraction and purification of α-subunit from urine of the patient with undifferentiated carcinoma. Although the kaolin-acetone standard method had been developed as an effective extraction method for urinary hCG, α-subunit could be also extracted from urine virtually by the same procedure. As the starting material was urine, 0.92 of the final specific immunoactivity could be attained.

Chromatography on DEAE-cellulose. (Fig. 1) Most of the immunoactivity was adsorbed with a DEAE-cellulose column and eluted as a broad large peak at conductivity ranging from 2 to 7 mmhos.

Gel chromatography on Sephadex G-100. (Fig. 2) Although two major peaks were observed by absorbance at 280 nm, the immunoactivity of the α-subunit was determined only in the preceding peak. This fraction containing high immunoactivity of α-subunit was collected, dialyzed against distilled water and lyophilized.

Table 1. Extraction and purification of α-subunit from urine of patient with undifferentiated carcinoma

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Protein (mg)</th>
<th>Total Alpha (mg)</th>
<th>Specific Activity (mg alpha/mg protein)</th>
<th>Recovery (%)</th>
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</thead>
<tbody>
<tr>
<td>URINE</td>
<td>518</td>
<td>72</td>
<td>0.14</td>
<td>100</td>
</tr>
<tr>
<td>KAOLINE-ACETONE</td>
<td>123</td>
<td>34</td>
<td>0.28</td>
<td>47</td>
</tr>
<tr>
<td>DEAE-C</td>
<td>31</td>
<td>18</td>
<td>0.58</td>
<td>25</td>
</tr>
<tr>
<td>SEPHADEX G-100</td>
<td>12</td>
<td>11</td>
<td>0.92</td>
<td>15</td>
</tr>
</tbody>
</table>
Fig. 3. Partial purification of $\alpha$-subunit from urine of normal pregnant women by gel chromatography on Sephadex G-100 in the same conditions as described for the purification of $\alpha$-subunit from urine of the patient.

Fig. 3 shows the results of gel chromatography of the extracts from urine of normal pregnant women on Sephadex G-100. Although three major peaks were observed by absorbance at 280 nm, the immunoactivity was determined in both second and third peaks. The immunoactivity of the second peak was ascertained to be due to the cross-reactivity of large amounts of hCG. The fractions under the peak of hCG-$\alpha$ immunoactivity were collected, dialyzed against distilled water and lyophilized. The specific immunoactivity of this fraction was 0.26 (mg $\alpha$-subunit/mg protein). This partially purified $\alpha$-subunit was subjected to isoelectric focusing and a combination study.

**Elution Profiles on Sephadex G-100**

Fig. 4 depicts the elution profiles of $^{125}$I-$\alpha$-subunit from undifferentiated carcinoma, $^{135}$I-hCG and $^{125}$I-hCG-$\alpha$ on a Sephadex G-100 column (1.5×90 cm). Ve/Vo of $\alpha$-subunit=1.48, hCG=1.27, hCG-$\alpha$=1.71.

**Immunoaditivity in hCG-$\alpha$ radioimmunoassay**

Fig. 5 shows the dose response line of a purified $\alpha$-subunit from undifferentiated carcinoma in the homologous hCG-$\alpha$ RIA. The $\alpha$-subunit exhibited virtually identical immunoadrogenicity against hCG-$\alpha$ antiserum with that of standard hCG-$\alpha$.

**SDS disc electrophoresis**

Fig. 6 shows the results obtained following SDS disc electrophoresis of a purified $\alpha$-subunit from undifferentiated carcinoma and hCG (KOBE) after mercaptoethanol reduction. hCG was separated into two different migrating bands (Fig. 6, gel 2), whereas $\alpha$-subunit from undifferentiated carcinoma appeared as a single band (Fig. 6, gel 1). Molecular weights estimated from their mobilities showed that the faster and slower moving bands of hCG were about 19,000 and 28,000.
respectively, and that of the α-subunit was about 18,000 (Fig. 7).

Amino acid analysis

The results of amino acid compositions of an α-subunit from undifferentiated carcinoma and the average of various glycoprotein hormone α-subunits by literature analyses quoted from Weintraub et al. (1975) are listed in Table 2. There is considerable similarity in their amino acid compositions. The aspartic acid, serine, glutamic acid and glycine content in the α-subunit was slightly higher than the literature analyses of standard α-subunits, but the half-cystine and methionine content was lower conversely.

Isoelectric focusing

Fig. 8 depicts the isoelectric focusing pat-
terns of α-subunit from undifferentiated carcinoma (panel A), α-subunit from urine of normal pregnant women (panel B) and standard hCG-α (panel C). The major components of tumor derived α-subunit and α-subunit from urine of normal pregnant women were distributed over the pH range from 3.5 to 6.0, whereas standard hCG-α was distributed in the fractions ranging from pH 6.0 to 8.0.

Discussion

An α-subunit could be extracted and purified from urine of the patient with undifferentiated carcinoma. This tumor was ascertained immunohistochemically to be an isolated α-subunit producing tumor, and both immunoreactive hCG and free hCG-β were undetectable in urine and serum of the patient.

Ectopic syntheses of subunits of glycoprotein hormones have been demonstrated in many nontrophoblastic tumors and tumor-
derived cell lines (Weintraub and Rosen, 1973; Tashjian et al., 1973; Rosen and Weintraub, 1974; et al., 1976). Weintraub et al. (1975) extracted and highly purified α-subunit (A.L.-α, 1.84 of specific activity) from gastric carcinoïd tumor tissue, and analyzed its amino acid composition. From the present results of the amino acid analysis of α-subunit from undifferentiated carcinoma, there was considerable similarity between tumor derived α-subunit and standard α-subunit compositions from the literature analyses. However, Weintraub et al. (1975) reported that the amino acid composition of A.L.-α showed certain differences from standard, especially high glycine content. Benveniste et al. (1979) also pointed out the size heterogeneity of JEG-α (choriocarcinoma cell origin) and suggested the possibility that minor heterogeneity existed in its protein moiety. On the other hand, Ruddon et al. (1980) have demonstrated that subunits secreted by JAG cells (choriocarcinoma cell origin) are heterogenous and appear to be a mixture of glycoproteins with simple high-mannose and complex sialic acid-containing oligosaccharides. In the present study, since α-subunit from undifferentiated carcinoma exhibited similar properties to standard hCG-α in regard to the parallel dose response and dose inhibition in homologous hCG-α radioimmunoassay system, it was considered that little difference affecting the immunoactivity may exist between tumor derived α-subunit and standard hCG-α. This problem should be elucidated by the study of the amino acid sequence of the α-subunit.

It has been demonstrated that the various ectopically produced α-subunits have apparent larger molecular weights in gel exclusion chromatography. Also in the present study, the molecular weight of an α-subunit from an undifferentiated carcinoma determined by gel chromatography on Sephadex G-100 was larger than that of standard hCG-α. By SDS disc electrophoresis, however, the molecular weight of α-subunit from undifferentiated carcinoma was smaller than that of hCG-α separated by mercaptoethanol reduction. Weintraub et al. (1975) have already reported such a discrepancy in molecular weight estimation of α-subunit (A.L.-α) between gel chromatography and by SDS disc electrophoresis, and suggested this is attributable to the possibility that the α-subunit might be binding more SDS than the standard α-subunit.

In the results of the isoelectric fusing, the major components of α-subunit from undifferentiated carcinoma were observed in the fractions ranging from pH 3.5 to 6.0. This isoelectric focusing pattern was similar to that of partially purified α-subunit from the urine of normal pregnant women, but quite different from that of standard hCG-α which ranged from pH 6.0 to 8.0. Benveniste et al. (1979) showed that the great majority of JEG-α was dissolved in a peak pI of 4.8 which was virtually absent in standard hCG-α. The electric charge of glycoprotein was considered to be affected by various factors, such as the differences in amino acid and carbohydrate compositions and conformation. It is probable that the tumor derived from the α-subunit differs from standard hCG-α in regard to carbohydrate moiety, since the protein moiety of the α-subunit is considered to be virtually identical with that of standard hCG-α.

The results of the combination studies involving α- and β-subunits indicate that α-subunit from undifferentiated carcinoma does not actively combine with standard hCG-β. Although similar findings (Benveniste et al., 1979; Weintraub et al., 1977) have been shown in the various α-subunits, only A.L.-α showed a small but significant degree of combination in both RIA and RRA. By the preliminary study, the partially purified α-subunit from urine of normal pregnant women was not significantly active in combining with standard hCG-β (data not shown). Therefore, the nature of hCG-α obtained by the dissociation of hCG may be dissimilar to that of the urinary α-subunit, and freely secreted α-subunit might no longer have the ability to combine with hCG-β. We suppose that A.L.-α...
has the ability to combine with the \( \beta \)-subunit because it originates from tumor tissue.

HCG may be synthesize via the association of \( \alpha \)- and \( \beta \)-subunits which are synthesized from two different messenger RNAs (Boime et al., 1978), and hCG-\( \beta \) may be a rate-limiting factor of hCG production in the placenta (Franchimont and Reuter, 1972; Chatterjee and Munro, 1977). Hence we speculate that the excess of \( \alpha \)-subunit, which has not associated with \( \beta \)-subunit, may have undergone some intracellular modification of its chemical structure, and consequently, the freely secreted \( \alpha \)-subunit electric charge changes and has no longer the activity to combine with the \( \beta \)-subunit.

We are now also studying the chemical properties and the combination of free \( \alpha \)-subunit in placental tissue.

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References


