Characterization of a Carboxyterminal Peptide Fragment of the Human Choriogonadotropin β-Subunit Excreted in the Urine of a Woman with Choriocarcinoma

SANIA AMR, ROBERT E. WEHMANN, STEVEN BIRKEN, ROBERT E. CANFIELD, and BRUCE C. NISULA, *Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205; Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore City Hospitals, Baltimore, Maryland 21224; Department of Medicine, College of Physicians & Surgeons of Columbia University, New York 10032*

ABSTRACT We have observed low-molecular weight carboxyterminal fragments of the human choriogonadotropin (hCG) β-subunit in the urines of several women with choriocarcinoma, and we have characterized one fragment in detail. Its apparent molecular weight by gel chromatography on Sephadex G-100 was 14,200. The fragment was not adsorbed to concanavalin A-Sepharose, indicating that it lacked the asparagine-linked carbohydrate groups of intact hCGβ. It was active in radioimmunoassays (RIA) using antisera either to the hCGβ carboxyterminal peptide (CTP) or to the desialylated hCGβ CTP (hCGβ as-CTP), indicating the presence of not only the hCGβ carboxyterminus but also desialylated *O*-serine-linked carbohydrate side chains on the fragment. It lacked luteinizing hormone/choriogonadotropin radioreceptor activity and hCGβ conformational immunoreactivity (SB6 RIA). On Sephadex G-100 gel chromatography, the elution profiles of this fragment and the hCGβ as-CTP(115–145) prepared by trypsin digestion of as-hCG were essentially indistinguishable (apparent molecular weights 14,200 and 14,000, respectively). The immunological characteristics of the fragment in both hCGβ CTP and hCGβ as-CTP RIA were indistinguishable from those of the hCGβ as-CTP(115–145) glycopeptide. Carboxyterminal fragments of hCGβ were evident in urine specimens obtained from 10 of 11 patients with choriocarcinoma but not in those obtained from normal subjects who were given an intravenous infusion of highly purified hCG. Of six pregnant women, only the one at term excreted carboxyterminal fragments of hCGβ and then only in trace amounts. We conclude that hCGβ carboxyterminal fragments, including one that is indistinguishable from the tryptic glycopeptide hCGβ as-CTP(115–145), can occur naturally in the urine of patients with choriocarcinoma.

INTRODUCTION

During pregnancy and in patients with neoplasms that produce human choriogonadotropin (hCG),[1] a variety of molecules related to hCG circulate in the blood or are excreted in the urine. Some of these forms of the hormone appear to be subunits of hCG (1–7), and some appear to be fragments, as they exhibit molecular sizes smaller than those of the hCG subunits (1, 5, 6, 8–11). When obtained from different sources, hCG itself has been found to have variable biological, physicochemical, and immunological properties; this microheterogeneity seems to be related, in part, to variations in its carbohydrate composition (10, 12–15).

Address reprint requests to Dr. B. C. Nisula, National Institutes of Health, Bethesda, MD 20205.

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[1] *Abbreviations used in this paper*: as-hCG, desialylated hCG; Con A, concanavalin A; hCG, human choriogonadotropin; hCGα, hCG alpha subunit; hCGβ, hCG beta subunit; hCGβ as-CTP(115–145), asialo form of the hCGβ glycopeptide with amino acid residues 115–145; hCGβ as-CTP(123–145), asialo form of the hCGβ glycopeptide with amino acid residues 123–145; hCGβ CTP, carboxyterminal peptide of hCGβ; hCGβ CTP(123–145), carboxyterminal glycopeptide of hCGβ with amino acid residues 123–145; LH, luteinizing hormone; RRA, radioreceptor assay.

Of the fragments of hCGβ that are excreted in the urine, one type retains the conformational immunological determinant that is recognized by antisera to the hCGβ subunit. Fragments of this type have been observed in the urine of pregnant women (5, 6, 8, 9), in crude commercial preparations of hCG from pregnancy (16), and in the urine of several patients with trophoblastic neoplasms (1) and nontrophoblastic tumors (1, 8, 11). We have observed that this type of fragment also appears in the urine after infusion of highly purified hCGβ subunit into normal subjects (17) and, therefore, it is a product of the metabolism of hCGβ. Since this latter type of fragment has been shown to exhibit reduced activity in radioimmunoassays (RIA) that use antiserum to the hCGβ carboxyterminal peptide (CTP) determinant (16, 17), we have termed it an hCGβ core fragment. This hCGβ core fragment is not a prominent product of degradation of the intact hCG molecule; only one of seven subjects who received an 8-d intravenous infusion of purified intact hCG excreted this fragment and then only in small quantities (18). Given the existence of a metabolic product of hCGβ that lacks carboxyterminal immunoreactivity, we sought to determine whether we could identify carboxyterminal fragments, presumably resulting from the cleavage of the hCGβ subunit, in any clinical situations. Indeed, in a number of cases of choriocarcinoma we have observed hCGβ carboxyterminal fragments in the urine. This report summarizes the characterization of the most abundant fragment, which had immunological and physicochemical properties indistinguishable from those of the hCGβ as-CTP(115–145) glycopeptide.

METHODS

Materials. The highly purified hCG (CR121) preparation was obtained from the Center for Population Research, National Institute of Child Health and Human Development. The method for purification and its biological and immunological characteristics were indistinguishable from those of hCG (CR119) (19). Asialo-hCG (as-hCG) was prepared by neuraminidase digestion of purified hCG (CR118) (20). Preparation of the hCGβ carboxyterminal peptides, hCGβ as-CTP(115–145), hCGβ as-CTP(123–145) and hCGβ CTP(123–145) has been described elsewhere (21). Concanavalin A, (Con A) covalently bound to Sepharose 4B (Con A Sepharose) was purchased from Pharmacia Fine Chemicals, Piscataway, NJ.

Patients and subjects. Pregnant women and women with gestational trophoblastic neoplasia collected 24-h specimens of urine. Healthy young volunteers at the Clinical Center, National Institutes of Health, also collected 24-h specimens of urine during the final 2 d of an 8-d continuous intravenous infusion of purified hCG (CR121) (0.8 μg/min) as described previously (18).

Urine concentration procedure. Concentrates of 24-h urine collections were prepared by the kaolin-acetone procedure (22), and stored at −20°C.

RIA systems. RIA were performed using antisera generated to the hCGβ subunit (SB6), the hCGβ carboxyterminal peptide (hCGβ CTP) (R529), and the hCGβ asialo-carboxyterminal peptide (hCGβ as-CTP) (R141); each of these antisera has been characterized previously (21, 23–26). The radioligands were ^{125}I-hcG (CR121) for the hCGβ CTP and hCGβ (SB6) RIA, and ^{125}I-as-hcG for the hCGβ as-CTP RIA. Radioiodinations were performed by the chloramine-T method (27), and the radioligands displayed a specific radioactivity of 70–100 μCi/μg. Relative potencies of the RIA were expressed in terms of mass of hCG (CR121) (SB6 and R529 antisera) or as-hCG (R141 antiserum). The cross-reaction of hCG (CR121) in the as-hCG RIA was 0.1% by weight, while the cross-reaction of as-hCG in the hCGβ CTP RIA was 100% (16).

Luteinizing hormone/choriogonadotropin (LH/CG) radioreceptor assay (RRA). The LH/CG RRA was performed using rat testis homogenate (28). Binding potencies were expressed in terms of highly purified hCG (CR121).

Gel filtration. Urine concentrates, unconcentrated urine, and sera were fractionated on a column of Sephadex G-100 (72.8 × 1.6 cm). ^{125}I-hCG (10,000 cpm) and ^{125}I-hCGα (10,000 cpm) were added as markers to each sample that was applied to the column. Where indicated, samples were separately chromatographed with ^{125}I-as-hCG (10,000 cpm) to compare the elution position of the as-hCG with those of the moieties in the sample. The elution patterns, with respect to qualitative and quantitative characteristics, obtained with ^{125}I-hCG, ^{125}I-hCGα, ^{125}I-as-hCG, and the various hCGβ carboxyterminal glycopeptides were essentially identical whether these molecules were filtered in the presence or absence of the patients' specimens. Thus, interference, such as proteolytic degradation by the specimens per se during the analytical procedures, was not an appreciable problem. The volume of each aliquot applied to the column was adjusted to 2.0 ml with phosphate-buffered saline (PBS, 0.15 M NaCl, 0.01 M PO_4, pH 7.4) containing 0.1% bovine serum albumin (BSA). The elution was performed at 4°C by upward flow at 25 ml/h using PBS. Fractions of 2.3 ml were collected. The column was calibrated with globular proteins: bovine serum albumin, ovalbumin, chymotrypsinogen, and ribonuclease A.

The partition coefficient (K_{av}) for the eluted peaks was calculated by the formula (29, 30): $K_{av} = (V_e - V_0)/(V_t - V_0)$, where V_e is the elution volume of the substance, V_0 is the void volume (indicated by blue dextran), and V_t is the volume of the salt peak (indicated by free ^{125}I). Apparent molecular weights were determined by interpolation using a plot of K_{av} vs. the log of the molecular weight of the standard proteins.

Analysis of data. RIA data were analyzed by computer as described by Rodbard (31).

RESULTS

Gel filtration of the urine concentrate of a patient with choriocarcinoma. To examine the hCG-related forms in the kaolin-acetone concentrate of the urine of patient (M.K.), a woman with gestational trophoblastic neoplasia (choriocarcinoma), we fractionated the urine concentrate by Sephadex G-100 gel filtration and measured the activity in the eluted fractions in several hCG assay systems. When the fractions were analyzed by the hCGβ CTP RIA (Fig. 1, top panel),

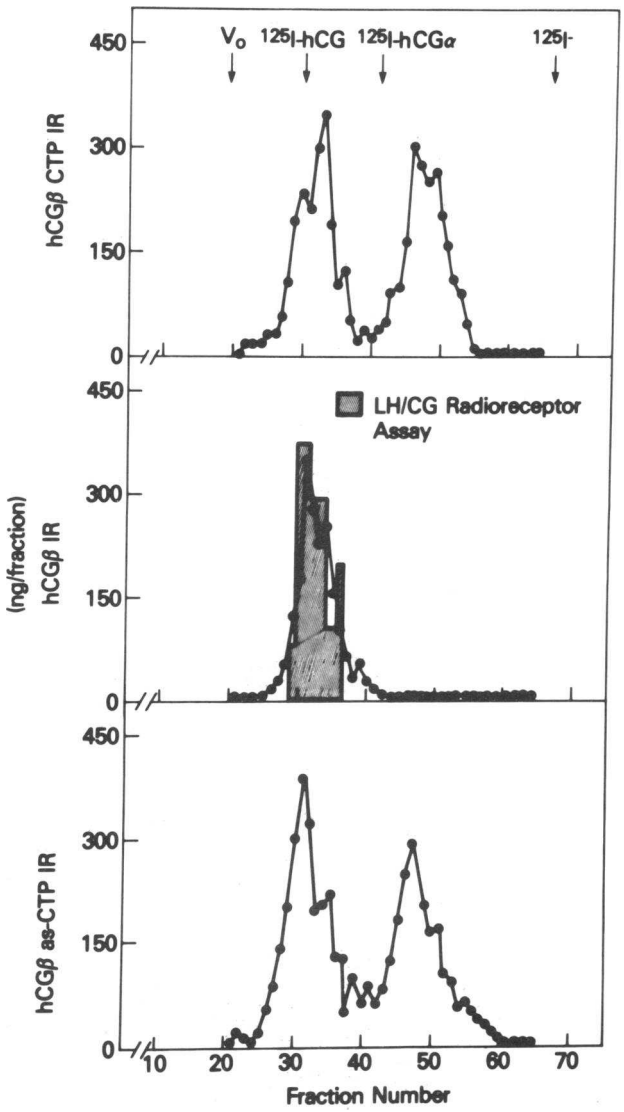

FIGURE 1 Sephadex G-100 gel filtration of a urine concentrate of a woman (M.K.) with choriocarcinoma. 1.5 ml of the urine concentrate was applied to a column (72.8 × 1.6 cm) of Sephadex G-100, and eluted by upward flow (25 ml/h, 4°C) with PBS. Fractions of 2.3 ml were collected and aliquots were assayed in various systems. Immunoreactivity (IR) is shown as follows: top panel—hCGβ CTP RIA; middle panel—hCGβ RIA; bottom panel—hCGβ as-CTP RIA. LH/CG radioreceptor activity is shown as the hatched area in the middle panel.

immunoreactive material was apparent not only in fractions 25–37 corresponding to the elution position of the LH/CG radioreceptor activity and the hCGβ immunoreactivity (SB6) (Fig. 1, middle panel), but also in fractions 42–56 corresponding to an apparent molecular size far less than that of hCGβ or even hCGα. The material in fractions 42–56 was not free hCGβ, since it exhibited no hCGβ conformational immunoreactivity (SB6) (Fig. 1, middle panel), and free hCGβ elutes between the positions of hCG and hCGα; thus, fractions 42–56 seemed to contain an hCGβ carboxyterminal fragment(s). In addition, these fractions were quite active in the hCGβ as-CTP RIA (Fig. 1, bottom panel). However, when assessed in the LH/CG RRA for receptor binding activity, the fractions 42–56, which contain the carboxyterminal fragment(s), showed no ability to displace labeled hCG from testis membranes (Fig. 1, middle panel).

The hCGβ conformational immunoreactivity (SB6) (Fig. 1, middle panel) eluted in a single peak that was coincident with the activity in the LH/CG RRA and with the first peak with hCGβ CTP and the hCGβ as-CTP immunoreactivity. Note that this peak of hCGβ conformational immunoreactivity was shifted to the right of the position of the cochromatographed purified ^{125}I-hCG; thus, most of the hCG in this specimen displayed a smaller apparent molecular size than authentic hCG. The slightly greater elution volume of this form of hCG in conjunction with its activity in the hCGβ as-CTP RIA indicated a sizeable degree of desialylation of the hCG in fractions 25–37 (Fig. 1, bottom panel). However, while the detection of immunoreactivity with the hCGβ as-CTP RIA permits one to deduce that there is desialylation of the *O*-serine-linked carbohydrate chains in the CTP region of hCGβ, it provides no information about the extent of sialylation of the *N*-asparagine-linked carbohydrate chains in the aminoterminal region of the hCG-related molecules. That this form of desialylated hCG exhibited LH/CG receptor activity is compatible with previous observations that desialylated hCG can bind to testis membrane receptors at least as avidly as does intact hCG (32).

Con A binding properties of the hCGβ carboxyterminal fragments. Because hCG, its subunits, and the hCGβ core fragment are glycoproteins that bind to the lectin Con A (17, 33), we investigated whether the carboxyterminal fragments in our patient's urine concentrate would also bind to Con A. An aliquot (1.5 ml) of urine concentrate was incubated with 1.0 ml of Con A Sepharose at room temperature for 1 h and gently mixed every 10–15 min. The supernatant was collected, and 1.5 ml of PBS was added to the gel, mixed, and centrifuged. The second supernatant was added to the first, and an aliquot was applied to the Sephadex G-100 column (Fig. 2). Clearly, the fragments were not absorbed by the Con A Sepharose, while nearly all of the hCGβ CTP immunoreactivity in the region with the receptor binding activity had been adsorbed by Con A. Note that there was a major peak at fraction 44 and a minor peak at fraction 53 of hCGβ CTP immunoreactivity (Fig. 2), indicating the presence of

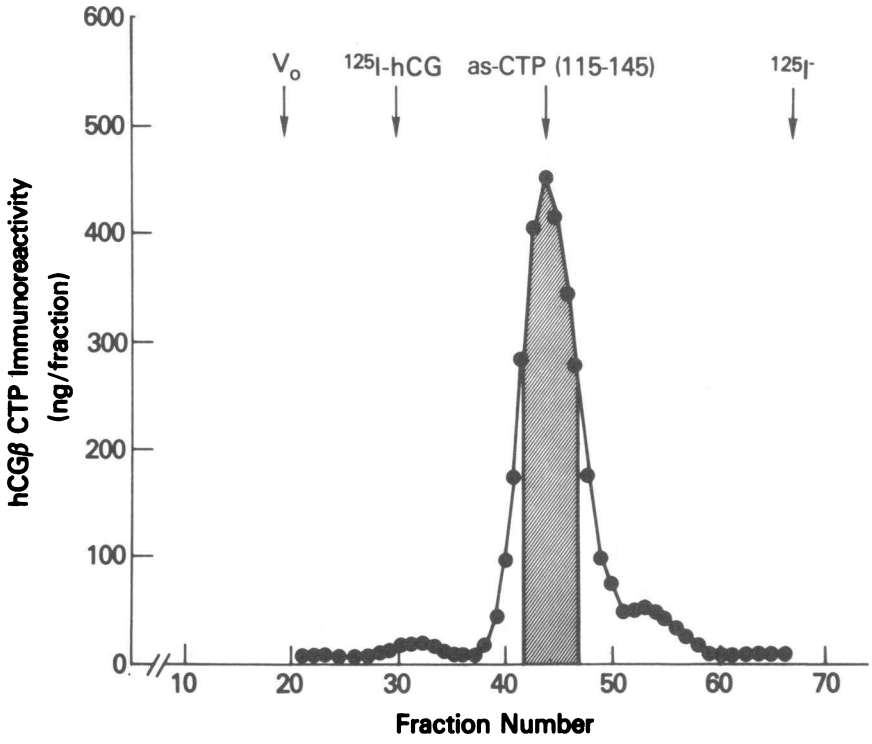

FIGURE 2 Sephadex G-100 gel filtration of the Con A Sepharose nonadsorbed fraction of the urinary concentrate of a woman (M.K.) with choriocarcinoma. 1 ml of Con A Sepharose gel was incubated with 1.5 ml of the urine concentrate, washed with 1.5 ml of PBS-BSA, and a 1.0 ml aliquot of the combined supernatants was applied to the Sephadex G-100 column with ^{125}I-hCG as an internal marker. The elution position of the hCGβ as-CTP(115–145) was determined from a separate run. Aliquots of the fractions were analyzed in the hCGβ CTP RIA. Fractions 42–47 indicated by the shaded area were pooled for further studies.

two discernable carboxyterminal fragments, neither of which adsorbed to the Con A Sepharose. The fragment of larger apparent molecular size was present in sufficient quantity for more detailed characterization, and a pool of fractions 42–47 was prepared (Fig. 2, shaded area) for subsequent studies.

Apparent molecular weight of the hCGβ carboxyterminal fragment. Anomalous behavior of hCG and other glycoproteins on Sephadex G-100 gel filtration results in rather wide discrepancies between actual molecular weight and apparent molecular weight judged by comparison with standard globular proteins (29, 30). Due to the oligosaccharide side chains, the estimates of molecular weights of glycoproteins based on calibration curves with standard proteins are erroneously high. For example, we have found that hCG, whose actual molecular weight is 36,700 (by chemical composition), exhibits an apparent molecular weight of 68,600 on our Sephadex G-100 column (Table I). Accordingly, in addition to calibrating our Sephadex G-100 column with standard globular proteins, we used a series of known hCG-related glycopeptides as markers with which to compare the moieties in the urine of patient M.K. In addition to hCG and as-hCG, we chose the tryptic glycopeptides, hCGβ CTP(123–145), hCGβ as-CTP (115–145), and hCGβ as-CTP(123–145) (Table I).

The hCGβ carboxyterminal fragment exhibited an apparent molecular weight of 14,200 (Table I); this was not significantly different from that of the hCGβ as-CTP(115–145) (14,000, Table I), but was significantly greater than that of the hCGβ as-CTP(123–145) (10,700, Table I).

Note further in Table I that the peak corresponding to the radioreceptor activity, which contained a desialylated form of hCG (Fig. 1), displayed nearly the same apparent molecular weight as the as-hCG molecule (59,000). Thus, the urine of this patient with choriocarcinoma contained not only an as-hCGβ fragment but also a form of hCG quite similar, if not identical to as-hCG derived from neuraminidase digestion of native hCG.

Immunological characterization of the hCGβ carboxyterminal fragment. The apparent molecular size

TABLE I

Apparent Molecular Weights of Several Glycopeptides Derived from Purified hCG and the hCG-related Moieties in the Urine of a Patient with Choriocarcinoma

Material	Calculated molecular weight*	Apparent molecular weight‡
Markers		
hCG	36,700	68,600 (66,900–70,300)§
as-hCG	31,800	59,000 (57,700–60,300)
hCGβ CTP (123–145)	5,400	17,300 (16,200–18,500)
hCGβ as-CTP (115–145)	4,788	14,000 (13,000–15,100)
hCGβ as-CTP (123–145)	3,521	10,700 (10,200–11,300)‖
M.K. urine		
Receptor activity peak	—	59,600 (58,100–61,100)
hCGβ fragment peak	—	14,200 (13,300–15,100)

* Molecular weight is calculated based on chemical composition; where carbohydrate heterogeneity is known to pertain, the value is rounded off.

‡ Sephadex G-100 column was calibrated with bovine serum albumin, ovalbumin, chymotrypsinogen, and ribonuclease A.

§ Values in parentheses indicate 95% confidence limits.

‖ Value obtained by extrapolation beyond range of standards.

of the most abundant fragment and its behavior on Con A Sepharose prompted further examination of the hypothesis that it indeed consisted of some part of the 30 amino acid carboxyterminal glycopeptide unique to hCGβ (34–36). Since the hCGβ CTP determinant RIA is dependent upon the presence of the tetrapeptide in positions 142–145 (21, 25), this region was probably intact in the fragment. Further, recognition by the hCGβ as-CTP RIA depends upon the presence of at least some portion of the peptide sequence in positions 123–133, and in addition, a certain amount of carbohydrate since the synthetic peptide hCGβ(115–145), devoid of carbohydrate, does not cross-react in this RIA.

These features led us to perform a precise comparison of the immunological behavior of the hCGβ carboxyterminal fragment with that of the glycopeptide derived from trypsin digestion of as-hCGβ that consists of amino acid residues 115–145 (hCGβ as-CTP[115–145]). We used the pooled fractions 42–47 shown in Fig. 2 (shaded area). The hCGβ carboxyterminal fragment pool was devoid of the hCG-related species, hCG, hCGβ, hCGα, as-hCG, and hCGβ core fragment, as these were separated by the Con A Sepharose adsorption procedure and gel filtration as described. The slope of the dose-inhibition curve generated in the hCGβ as-CTP RIA with the carboxyterminal fragment pool (-0.93 ± 0.05 [SD]) was not significantly different from that of the purified hCGβ as-CTP(115–145) (-1.01 ± 0.08) (Fig. 3). Further, with respect to the hCGβ CTP RIA, which is directed to an entirely different immunological determinant, the slopes of the dose inhibition curves of the two substances (-1.35 ± 0.12 and -1.15 ± 0.07, respectively) again were not significantly different (Fig. 4). Not only was there similarity in slopes in the separate RIA, but the immunological potency of the hCGβ carboxyterminal fragment pool in the hCGβ as-CTP RIA was not significantly different from that in the hCGβ CTP RIA (7.1 ± 0.5 vs 6.5 ± 0.6 nM, $P > 0.05$). These results indicate essentially identical immunological behaviors between the hCGβ carboxyterminal fragment and the hCGβ as-CTP(115–145) glycopeptide.

Gel filtration of the serum of patient M.K. To determine whether hCGβ carboxyterminal fragments were apparent in the circulation, we examined serum that was obtained from patient M.K. on the same day as the urine that contained the hCGβ carboxyterminal fragments. Gel filtration of an aliquot of the serum on the same Sephadex G-100 column showed neither hCGβ CTP nor hCGβ as-CTP immunoreactivity in the position corresponding to the hCGβ carboxyterminal fragments that were present in the urine (Fig. 5). A single peak of the hCGβ CTP immunoreactivity that coincided with hCGβ immunoreactivity was apparent in the position of authentic hCG (Fig. 5); hCGβ as-CTP immunoreactivity was also detected in this region of the chromatograph.

Gel filtration of urine of subjects given an hCG infusion. To examine whether the hCGβ carboxyterminal fragment was a natural product of the metabolism of authentic hCG, we examined the elution patterns of urine obtained from normal volunteers who were given an infusion of highly purified hCG (Fig. 6, top panel). Gel filtration of the kaolin-acetone urine concentrate revealed no hCGβ CTP immunoreactivity

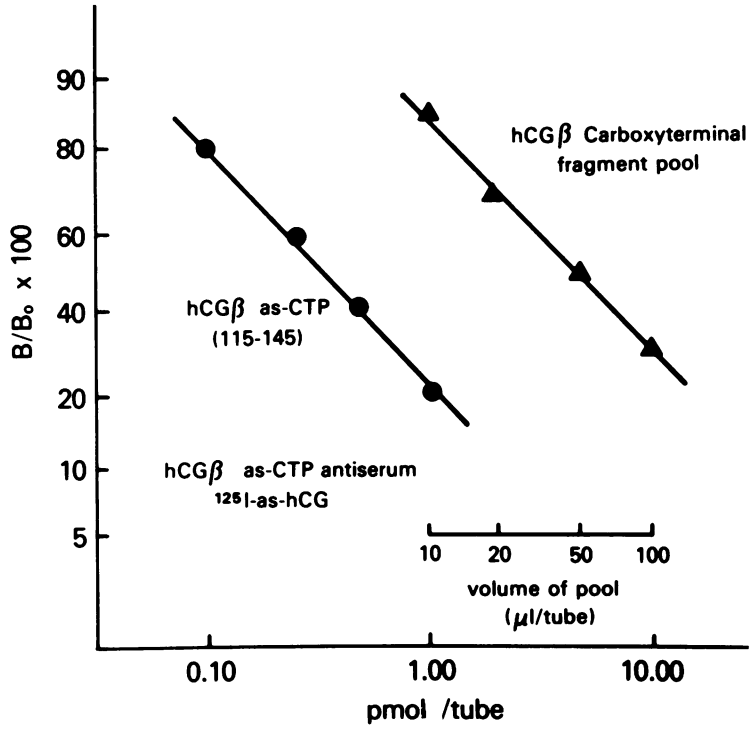

FIGURE 3 Dose-inhibition curves of the hCGβ carboxyterminal fragment pool prepared as in Fig. 2, and the glycopeptide, hCGβ as-CTP(115–145) in the hCGβ as-CTP RIA.

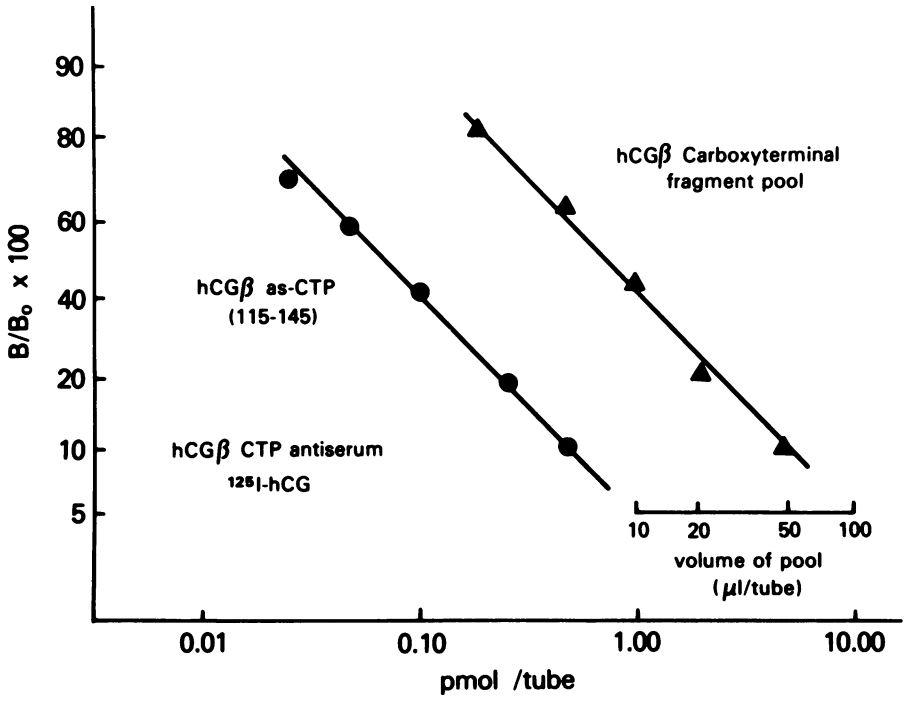

FIGURE 4 Dose-inhibition curves of the hCGβ carboxyterminal fragment pool prepared as in Fig. 2, and the glycopeptide, hCGβ as-CTP(115–145) in the hCGβ CTP RIA.

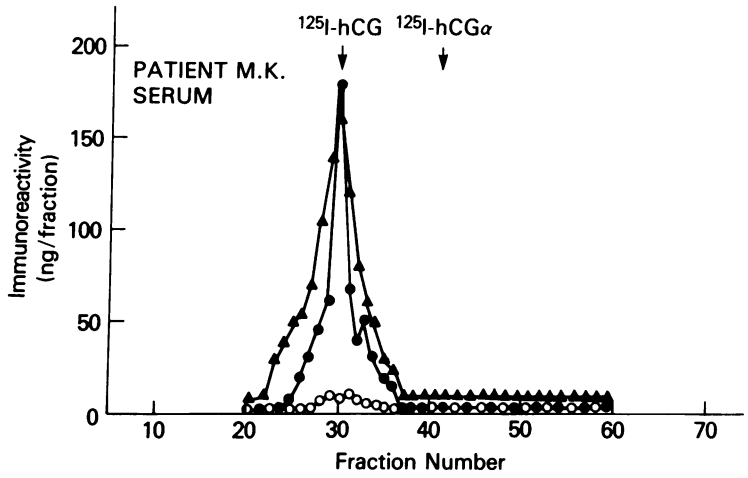

FIGURE 5 Sephadex G-100 gel filtration of the serum of patient (M.K.) with choriocarcinoma. An aliquot of serum was applied to column and eluted as described in Fig. 1. Aliquots of the fractions were analyzed in the RIA indicated. hCGβ IR (●); hCGβ CTP IR (▲); hCGβ as-CTP IR (○).

in the position corresponding to that of the fragment. The hCGβ CTP immunoreactivity coeluted with authentic ^{125}I-hCG in a single peak. There was no hCGβ as-CTP immunoreactivity detected in the chromatograph. In contrast, Sephadex G-100 gel filtration of the kaolin-acetone concentrate of the urine of a second patient (M.R.) with choriocarcinoma (Fig. 6, middle panel) and a second specimen collected on a different day from patient M.K. (Fig. 6, bottom panel) revealed substantial quantities of hCGβ carboxyterminal fragments; both the molecular forms of hCG and the fragments in these two patients exhibited hCGβ as-CTP immunoreactivity (Fig. 6, middle and bottom panels). In the case of patient M.K., the elution pattern obtained with this specimen was quite similar to that obtained with the earlier specimen (Fig. 1).

Gel filtration of pregnancy urine. We have also looked for carboxyterminal fragments in pregnancy urine. Urine specimens were collected from six women at different stages of pregnancy, and aliquots of these specimens were fractionated on Sephadex G-100. hCGβ CTP immunoreactivity was observed only in the position corresponding to authentic hCG (Fig. 7) in five of the women (10–34-wk gestation). Kaolin-acetone concentrates of this urine gave a chromatographic pattern similar to that shown in Fig. 7. One pregnant woman, who was in her 41st wk of gestation, excreted hCGβ CTP fragments in trace amounts (<2% of the total hCGβ CTP immunoreactivity). Parenthetically, the specimens shown in Fig. 7 contained the form of free alpha subunit that has been reported to be excreted in pregnancy (5, 6, 8, 9), and the 13,000-apparent molecular weight hCGβ core fragment that cross-reacts in RIA using antiserum to the hCGβ subunit (data not shown).

Prevalence of hCGβ carboxyterminal fragments in choriocarcinoma. To obtain a preliminary assessment of the prevalence of the excretion of hCGβ carboxyterminal fragments in gestational trophoblastic neoplasia, additional samples obtained from nine other patients with choriocarcinoma were fractionated on Sephadex G-100. To assess for the presence of the low-molecular weight forms with hCGβ CTP immunoreactivity, we combined the fractions eluted after the ^{125}I-hCGα marker and performed the hCGβ CTP RIA on the pool (Table II). In eight of nine patients, evidence of urinary carboxyterminal fragments was found (Table II). The dose-inhibition curves obtained with the forms in the pooled fractions, were parallel to that obtained with hCG in the hCGβ CTP RIA.

DISCUSSION

We have observed low-molecular weight carboxyterminal fragments of the hCGβ subunit in the urines of women with choriocarcinoma and we have characterized one fragment in detail. Its apparent molecular weight by Sephadex G-100 gel filtration was 14,200, indistinguishable from that of the hCGβ as-CTP(115–145), but clearly greater than that of the hCGβ as-CTP(123–145). Unlike hCG and its subunits, the fragment did not bind appreciably to Con A. Consideration of the binding specificity characteristics of Con A (37, 38) leads to the prediction that the binding of hCG,

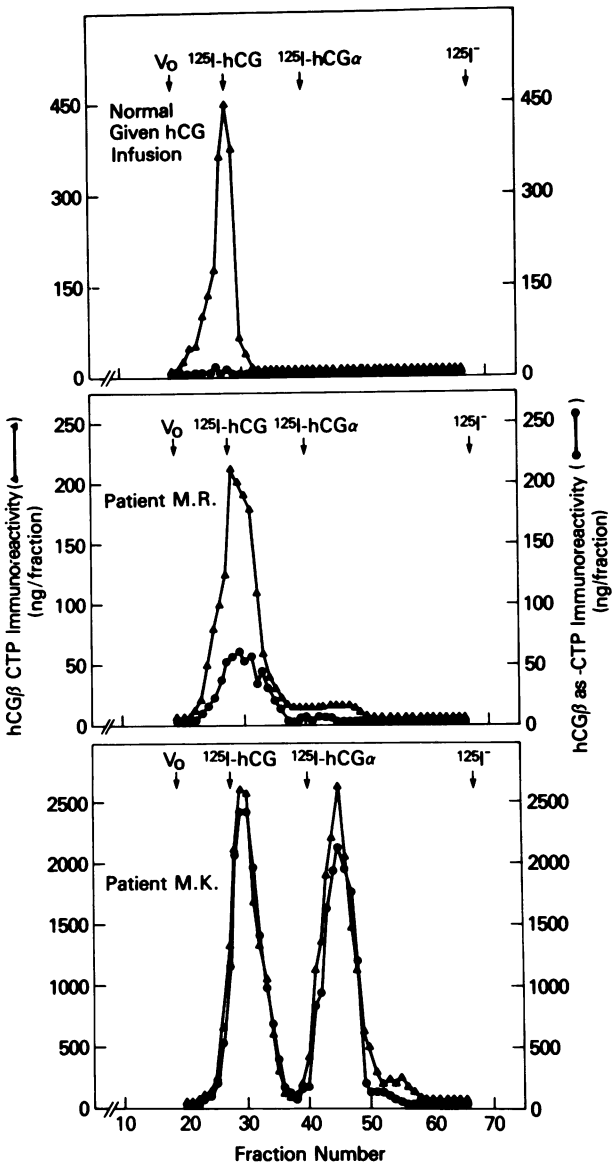

FIGURE 6 Sephadex G-100 gel filtration of urine concentrates obtained from a healthy young woman during an intravenous infusion of highly purified hCG (top panel), and from two women with choriocarcinoma (patient M.R., middle panel, and patient M.K., bottom panel). The samples were applied to the Sephadex G-100 column and eluted as described in Fig. 1. Aliquots of the fractions were analyzed in the hCGβ CTP RIA (left vertical scale) and in the hCGβ as-CTP RIA (right vertical scale).

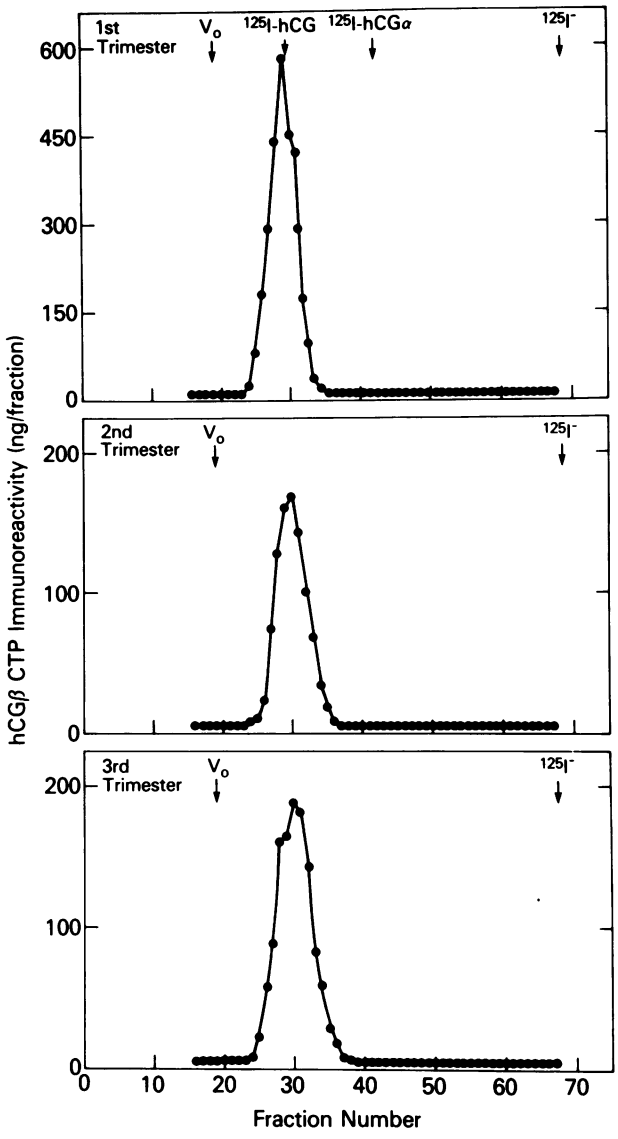

FIGURE 7 Sephadex G-100 gel filtration of urine specimens obtained from three women at different times during normal pregnancy. An aliquot of the urine was applied to the Sephadex G-100 column and eluted as described in Fig. 1. Aliquots of the fractions were analyzed by hCGβ CTP RIA.

as well as that of as-hCG, is attributable to an interaction between Con A and the *N*-asparagine-linked carbohydrate chains of hCG that are located at positions 13 and 30 in hCGβ, and at positions 52 and 78 in hCGα (25, 39), rather than an interaction with the *O*-serine-linked carbohydrate chains of the hCGβ CTP (40). Indeed, we have found that the tryptic glycopeptide hCGβ as-CTP(115–145) does not adsorb to Con A. An ectopic form of hCGβ that contains an unusual *N*-asparagine-linked carbohydrate chain structure also displays reduced binding to Con A; and its binding increases after treatment with *N*-acetylhexosaminidase (41). However, any form of hCGβ with hCGβ CTP immunoreactivity that contains both

TABLE II
Detection of Low-Molecular Weight hCGβ GTP Immunoreactivity in Urine Concentrates of Patients with Choriocarcinoma

Patient no.	hCGβ CTP immunoreactivity*	
	hCG pool‡	Low-molecular weight pool§
	ng/ml	
1	49	56
2	59	38
3	700	130
4	1,150	1,027
5	1,300	416
6	400	91
7	1,800	35
8	7,250	214
9	8	<3
10‖	624	645
11‖	52	14

* Concentrations of hCGβ CTP immunoreactivity are expressed in terms of the hCG (CR121) standard.
‡ Fractions containing the ^{125}I-hCG marker were pooled for hCGβ CTP RIA.
§ Fractions eluting after the ^{125}I-hCGα marker were pooled for hCGβ CTP RIA.
‖ Patients 10 and 11 are patients M.K. (Fig. 6, bottom panel) and M.R. (Fig. 6, middle panel), respectively.

N-asparagine and *O*-serine-linked carbohydrate chains would exhibit a Stokes' radius far greater than that observed for the carboxyterminal fragment and the hCGβ as-CTP(115–145) in the present study. Viewed in this context, our data concerning the molecular size and Con A bindability of the carboxyterminal fragment argue strongly against *N*-asparagine-linked carbohydrate being a component of its structure.

The immunological, as well as the physicochemical properties of the hCGβ carboxyterminal fragment were indistinguishable from those of the hCGβ as-CTP(115–145) glycopeptide. The slopes of the dose-inhibition curves obtained with the fragment in RIA directed to two separate determinants of the hCGβ CTP were indistinguishable from those of hCGβ as-CTP(115–145). Complete cross-reactivity in the hCGβ CTP RIA was observed; this is consistent with the presence of the hCGβ CTP amino acid sequence including residues 142–145 in the fragment's structure (21, 25). The activity of the fragment in the hCGβ as-CTP RIA indicates that *O*-serine-linked carbohydrate chains were present in the CTP region, but that their terminal sialic acid residues were absent, since both native (sialylated) hCG and synthetic (carbohydrate-lacking) hCGβ CTP show negligible cross-reactivity in this RIA. On the other hand, neither the hCGβ carboxyterminal fragment nor the hCGβ as-CTP(115–145) glycopeptide exhibited an interaction with the LH/CG receptor in testis membranes, and both substances lacked hCGβ conformational immunoreactivity (SB6 RIA). These data strongly suggest that the fragment that we have characterized is very closely related, if not identical to the hCGβ as-CTP(115–145) glycopeptide and, therefore, that it can be a naturally occurring hCG fragment in the urine of patients with choriocarcinoma.

It is of interest that the hCGβ carboxyterminal fragments characterized in our patients with choriocarcinoma were associated with an abnormal form of hCG. We have deduced that this form of hCG contains desialylated *O*-serine-linked carbohydrate chains from its cross-reactivity in the hCGβ as-CTP RIA. While the degree of desialylation of its *N*-asparagine-linked carbohydrate chains cannot be deduced from this observation, it is noteworthy that this form of as-hCG did cochromatograph with the as-hCG molecule, and like as-hCG (32, 33), it adsorbed to Con A and interacted with the LH/CG receptor. Studies of choriocarcinoma cell lines cultured in vitro have provided evidence that the secreted forms of hCG can vary considerably in their degree of sialylation (42). Indeed, a patient with choriocarcinoma has been reported whose urinary hCG contained no measurable sialic acid (43). The extent to which peripheral desialylation of forms of hCG secreted by choriocarcinoma contributes to heterogeneity in the sialic acid content of plasma and urinary forms remains unknown.

The association of the fragment with a form of as-hCG suggests the possibility that the fragment originates as a metabolic product of this form of as-hCG. Since desialylation renders some proteins more susceptible to the actions of proteolytic enzymes (44), desialylated hCG is probably more susceptible to attack by proteolytic enzymes than is intact hCG. The fact that the forms of hCG urine after infusion of purified hCG showed negligible hCGβ as-CTP immunoreactivity is compatible with this interpretation. Given the possibility that proteolytic cleavage can occur at any of several sites in the body, we have considered several mechanisms that could account for the presence of the hCGβ carboxyterminal fragments in our patients' urine. One possible mechanism would involve cleavage of synthesized hCG in the malignant trophoblastic cells and subsequent direct secretion of the fragment into the circulation. Alternatively, the fragment could be produced by peripheral degradation of hCG with subsequent renal filtration and urinary excretion. However, the apparent molar concentration of the hCGβ carboxyterminal fragment in urine was approximately equal to the molar concentration

of hCG; if renal clearance of the circulating fragment were the applicable mechanism, the renal clearance rate of the fragment would have to be several orders of magnitude greater than the renal clearance rate of the circulating hCG to account for the fragment being undetectable in the serum. Another perhaps more reasonable mechanism for production of the hCGβ carboxyterminal fragment would involve enzymatic cleavage within the renal compartment itself.

Our data obtained on a small number of patients with gestational trophoblastic neoplasia give indication that hCGβ carboxyterminal fragments are commonly excreted in the urine of women with choriocarcinoma, but infrequently in the urine of pregnant women, and not at all during the infusion of purified hCG. This suggests that these abnormal forms of hCG may be useful as tumor markers. For instance, demonstration of substantial amounts of such fragments in urine could help differentiate recurrent trophoblastic disease from a normal pregnancy. Also, future studies might reveal that measurements of such fragments have clinical utility in selecting among therapeutic regimens or that chemotherapy modifies the production of these fragments in predictable ways.

It is noteworthy that Sephadex G-100 gel filtration, a labor-intensive and technically demanding procedure, would not necessarily be required to screen all urines for the hCGβ carboxyterminal fragments. We have found that the fragments can be separated from the majority of hCG-related molecules based on their resistance to adsorption by Con A. Thus, a Con A Sepharose batch extraction step, followed by hCGβ CTP RIA of the nonadsorbed fraction offers a simple approach to selecting those samples likely to contain carboxyterminal fragments. Such samples could then be filtered on Sephadex G-100 to document the levels of hCGβ carboxyterminal fragments.

Most studies of the prevalence of hCG and related peptides as markers in various types of nongestational, nongonadal neoplasms have relied upon the measurement of serum hCG. It is generally agreed that hCG can be detected in the serum of only 10–15% of these cancer patients using the serum hCGβ RIA. It is possible that improved methods of detection of abnormal forms of hCG will allow recognition of a higher prevalence of production of hCG-related molecules as markers in neoplastic conditions. For example, recently Papapetrou et al. (8) have described patients in whom serum hCGβ immunoreactivity was not detectable, but in whom the urinary hCGβ immunoreactivity was significantly increased due to the presence of an hCGβ core type fragment. Our observation that hCGβ carboxyterminal fragments are excreted in the urine of patients with choriocarcinoma raises the question of their prevalence in nontrophoblastic malignancies. Further studies will be necessary to establish just how prevalent and useful these fragments of hCG will be as cancer markers.

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