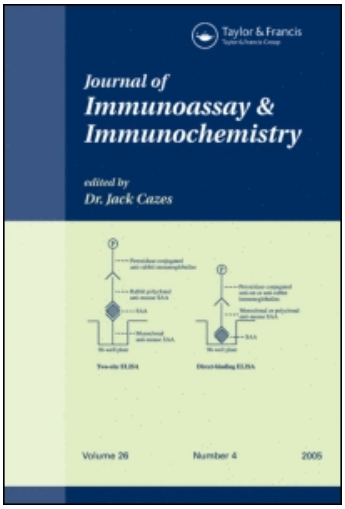

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Isolation of hCG and its Characterization by Radioimmunoassay, Enzyme-Immunoassay, and Radio-Receptor Assay

Pramod V. Prasad[a]; Shail K. Chaube[a]; T. G. Shrivastav[a]; G. L. Kumari[b]; K. Muralidhar[c]; S. Duraiswami[b]
[a] Department of Reproductive Biomedicine, National Institute of Health and Family Welfare, Munirka, New Delhi, India [b] Hormone Research Foundation, Nebsarai, New Delhi, India [c] Department of Zoology, University of Delhi, Delhi, India

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Isolation of hCG and its Characterization by Radioimmunoassay, Enzyme-Immunoassay, and Radio-Receptor Assay

Pramod V. Prasad, Shail K. Chaube, and T. G. Shrivastav
Department of Reproductive Biomedicine, National Institute of Health and Family Welfare, Munirka, New Delhi, India

G. L. Kumari and S. Duraiswami
Hormone Research Foundation, Nebsarai, New Delhi, India

K. Muralidhar
Department of Zoology, University of Delhi, Delhi, India

Abstract: The nature of human chorionic gonadotropin (hCG) molecules present during early pregnancy of Indian women is poorly understood. Therefore, a study has been undertaken to isolate hCG and characterize different forms of hCG from urine. The hCG molecules from urine of pregnant women (45–75 days post LMP) were adsorbed onto kaolin, eluted with ammonium hydroxide, and precipitated using acetone and then lyophilized. The lyophilized extract was subjected to Sephadex G-100 chromatography followed by ion-exchange fractionation. Three major fractions of protein (i.e., Peaks I, II, and III) associated with carbohydrate activity were obtained. Peaks II and III eventually resolved into a single peak I following repeated ion exchange chromatography, which suggested the presence of aggregates of molecules. Further purification on an affinity column resolved all three peak fractions into one unadsorbed and two adsorbed (A and B) fractions. These adsorbed fractions were characterized by radioreceptor assay (RRA), radioimmunoassay (RIA), and enzyme linked immunosorbent assay (ELISA). The activity was standardized against WHO reference preparation 75/589. Peaks I (A and B) were found to have maximum at about 75% of immunologically potent hCG, followed by peaks II (40%) and III (5%). The molecular sizes of peaks I, II, and III on a Sephadex G-200

Address correspondence to T. G. Shrivastav, Department of Reproductive Biomedicine, National Institute of Health and Family Welfare, Munirka, New Delhi 110067, India. E-mail: tgsrivastava@nihfw.org

column corresponded to 27,500D, 66,000D, and 84,000D, respectively. Relative mobilities of all adsorbed fractions in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed the presence of hCG-α (mol. wt. 19,539D) and hCG-β (28,870D) subunits. The presence of both subunits of hCG were also revealed by Western blot analysis. For the first time, we report the low molecular weight hCG molecule, of 27,500D by size exclusion chromatography, which has immunological and biological activity as measured by RIA, ELISA, and RRA.

Keywords: Pregnant Indian women, Low molecular weight hCG, Peak I hCG

INTRODUCTION

Several investigators have attempted to purify hCG from the urine of pregnant women and patients with trophoblastic diseases, since the discovery of hCG in 1927.[1] Extraction procedures,[2–5] their advantages, and limitations were discussed extensively in an earlier workshop.[6] Several other procedures have also been reported for the purification of hCG.[7–14] They purified hCG mainly by gel exclusion chromatography and fractionation on ion-exchange columns. Various molecular forms of hCG were reported and the emphasis was mainly on the isolation of the biologically active form of hCG.[8–11,13]

The information available on purification of hCG pertains to Western women; the molecular forms and sizes have not been studied in the Indian population. Therefore, in the present study, we made an attempt to isolate hCG from the urine of Indian women during early pregnancy. Summarizing the earlier extraction procedures,[6] we felt that kaolin-acetone extraction method would provide a better approach for retaining biologically and immunologically active hCG. The purification of hCG was achieved by size exclusion chromatography, ion exchange chromatography, and, finally, fractionation with a Concanavalin A-Sepharose-4B column. This procedure is similar to that which other investigators have used and, therefore, the molecular forms can be compared. For this purpose, studies have been undertaken to isolate hCG from pooled pregnancy urine and to characterize different molecular forms.

EXPERIMENTAL

Materials

Kaolin used in this study was from Fisher Scientific Company, New Jersey, USA. Celite was from Johns-Manville Products Corporation, Lompoc, California, USA. Bovine serum albumin, blue dextran, trizma base, urea, glycine, sodium dodecyl sulphate (SDS), N,N,N′,N′-tetramethyl

ethylenediamine (TEMED), Coomassie blue G-250, 4-chloro-1-napthol and visking tubing (No. 3) were all purchased from Sigma Chemical Company, St. Louis, USA. Sephadex G-25, G-100, G-200, DEAE-Sephadex A-25, DEAE-Sephadex A-50, and Concanavalin A-Sepharose-4-B were procured from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals and solvents used were of analytical grade and were procured locally in India.

Collection of Urine from Pregnant Women Volunteers

Pregnancy was established by PREG TEST card (Mitra International, Haryana, India), and corroborated by clinical symptoms. The urine was collected in thymol containing bottles, within 45 to 75 days of pregnancy, for 24 hours. A total of 33 liters urine was collected intermittently from five women and processed for hCG isolation.

Methods for Protein Estimation

Two methods of protein estimation, viz., Lowry's method[15] and Bradford assay[16] were adopted, depending upon the amount and sensitivity needed.

Isolation of hCG from Pregnant Urine

Extraction of Urinary hCG by Kaolin Adsorption and Acetone Precipitation

Urine samples collected within 48 hrs were pooled and processed together, following the method of Borth et al.[5] To 320 mL of chilled acetone (-20°C), the precooled hCG eluate (150 mL) from kaolin was added, while stirring. After 30 minutes in the deep freezer (-20°C), the precipitate was recovered by centrifugation and decantation. The precipitate was washed three times with 10 mL chilled acetone and ether (1 : 1, v/v), air dried, made in powder form by grinding, and then dessicated at -20°C until use. Protein content was measured by Lowry's method.[15] All steps of purification were carried out at 4°C unless otherwise stated.

Size Exclusion Chromatography

A crude hCG extract (about 100 mg) was reconstituted in 2 mL of 0.02 M sodium phosphate buffer, pH 7.2, and loaded onto the Sephadex G-100 column. Eluates from the column were monitored for protein content with the Bradford assay method[16] using BSA as a standard and for carbohydrate content by the phenol-sulfuric acid method[17] using sucrose as a standard.

Fractions containing maximum protein and carbohydrate content were pooled and dialysed at 4°C, extensively, against several changes of distilled water, followed by lyophilization.

Ion Exchange Chromatography

The above lyophilized material, in terms of 100 mg protein, was reconstituted in 100 mL of 0.02 M tris-phosphate buffer, pH 8.7, and applied onto a DEAE-Sephadex A-50 column. Isocratic elution was initiated with 0.02 M tris-phosphate buffer, pH 8.7, followed by discontinuous, stepwise gradient elution using sodium chloride. Fractions of 10 mL were collected and monitored for protein and carbohydrate content. The peak fractions I, II, and III were separately pooled. Repeated ion exchange chromatography of peaks I, II, and III and dialysis were also performed.

Affinity Chromatography on Concanavalin A-Sepharose-4B

The dialysed fractions were further desalted with a Sephadex G-25 column. A total of 1 mg of protein in starting buffer (0.02 M tris-HCl containing 1 mM $MnSO_4$, and 1 mM $CaCl_2$, pH 7.2) was loaded onto a Con-A-Sepharose-4B column and glycoproteins were eluted with 0.1 M followed by 0.2 M α-methyl-D-glucoside in starting buffer. Eluates were monitored for protein content and tested for immunological activity by RIA and ELISA.

Determination of Biological and Immunological Potencies of Purified Peak Fractions

Each peak fraction was assayed for biological activity by radio-receptor assay and immunological potency by RIA and ELISA.

Radio-Receptor Assay (RRA)

A total of 100 μg of hCG was solubilized in 1.0 mL of 0.1 M sodium phosphate buffer and stored at −80°C in aliquots, each containing 2.5 μg of hCG. It was iodinated by following the chloramine-T method.[18] Radioreceptor assay was performed by following the method of Sjodin et al.[19]

Radioimmunoassay (RIA)

A 100 μL amount each of hCG standards and purified peak fractions were taken in duplicate tubes and radioimmunoassay was performed as per a published protocol.[20]

ELISA of Purified Peak Fractions

To polystyrene tubes coated with secondary antibody (ARGG), as described elsewhere,[21] 100 μL of hCG-β antibody (1 : 2,000), followed by 100 μL of hCG standards (WHO reference preparation 75/589) or purified peak fractions, 100 μL of enzyme-conjugate, i.e., hCG-β penicillinase (1 : 800) and 300 μL of assay buffer (50 mM PBS containing 0.1% BSA plus 0.1% sodium azide) were added to each tube. In non-specific binding tubes, only 100 μL of enzyme conjugate and 500 μL of assay buffer were added. The contents of the tubes were mixed and incubated at 37°C for two hours. The contents were decanted and tubes were washed four times with tap water. The enzyme activity was monitored by adding 800 μL of substrate[22] in each tube. The tubes were incubated at 37°C for 30 minutes, after which 400 μL of stop solution (4 N HCl) was added to terminate the reaction. The absorbance was read at 620 nm.

Molecular Weight Determination of Peak Fractions I, II, and III

Estimates of molecular size of peaks I, II, and III were determined by (i) gel exclusion chromatography on Sephadex G-200; and (ii) SDS-PAGE[23] using relevant protein molecular weight markers. The purity of the preparations was determined as per published protocol.[24]

Native PAGE and Western Blotting

In order to analyse the native state of molecule, native gel electrophoresis was performed as per published protocol.[25] A 7.5% slab gel was run at a constant voltage of 60 V. The gel was stained with Coomassie blue and then photographed using a gel documentation system. To characterize the various molecular forms of hCG, Western blotting was performed by following the method of Towbin et al.[26]

Analysis of Sialic Acid Content of hCG Fractions

Sialic acid content of pooled peaks I(A), I(B), II(A), II(B), III(A), and III(B) were analysed by following the thiobarbituric acid method.[27]

RESULTS

Extraction of hCG from Urine

From 33.0 liters of pooled urine, 5.11 g acetone precipitate (protein content = 2.89 g) was obtained.

Sephadex G-100 Chromatography

The elution profile exhibited a single peak of protein associated with carbohydrate activity (Fig. 1). Pooled fractions (11 to 18) of the peak region were dialysed extensively, followed by lyophilization.

Fractionation on DEAE-Sephadex A-50 Column

The elution profile of ion exchange chromatography exhibited three major peaks of protein, which have carbohydrate activity. These major peaks were designated peaks I, II, and III. Fractions 34 to 49 comprised peak I, fractions 82 to 92 gave rise to peak II, while peak III was composed of fractions 155 to 170 (Fig. 2).

Ion Exchange Chromatography of Peaks I, II, and III

All peak fractions were subjected to a second stage of ion exchange fractionation with a DEAE-Sephadex A-50 column. Peak I emerged as a single peak on 0.1 M NaCl in 0.02 M tris-phosphate buffer elution (Fig. 3). Peak II resolved into two peaks, these being eluted with 0.1 M NaCl and 0.2 M NaCl in buffer, resolving into I* and II* (Fig. 4). Peak III, on rechromatography, further separated into three major peaks (I*, II*, and III*) of protein having carbohydrate activity (Fig. 5). Peaks I*, II*, and III* eventually resolved into a single peak I following repeated chromatography on an ion exchange column.

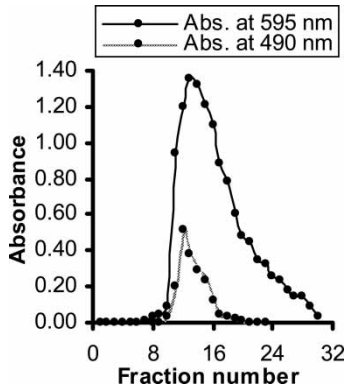

Figure 1. Elution profile of sephadex G-100 chromatography.

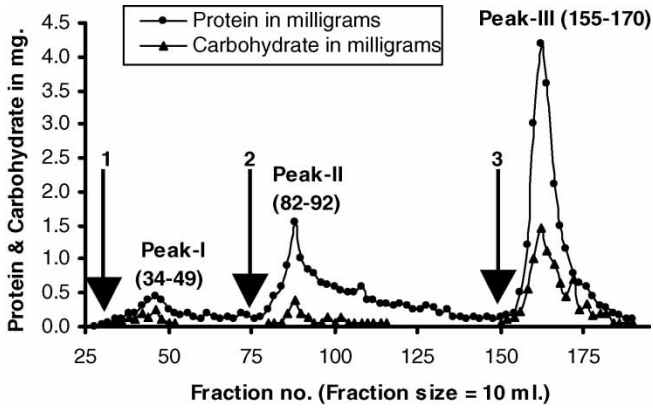

Figure 2. Elution profile of ion exchange chromatography of G-100 fraction. Isocratic elution using 0.02 M tris-phosphate buffer, pH 8.7, was performed upto fraction no. 30. Arrows nos. 1, 2 and 3 indicate fraction nos. where changes of buffer for discontinuous gradient elution (0.1 M NaCl, 0.2 M NaCl and 0.4 M NaCl in buffer, respectively) were made. Pooled fractions of peaks-I, II, and III are noted in parentheses.

Affinity Chromatography with Concanavalin A Sepharose 4B

It is clear, from Fig. 6, that each peak fraction was resolved into three protein peaks in terms of protein content, i.e., one unadsorbed and two adsorbed (A and B) components. The adsorbed components (A and B) of peaks I, II, and

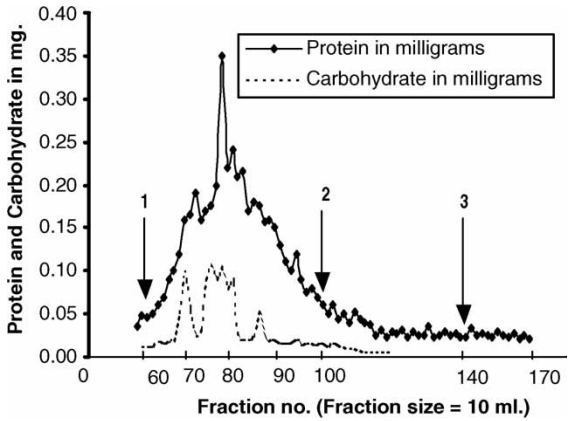

Figure 3. Ion exchange chromatography of peak I on DEAE-Sephadex A-50 column. Isocratic elution was performed up to fraction no. 60. Arrows nos. 1, 2, and 3 indicate fraction nos. where changes of buffer for discontinuous gradient elution were made. Elution with 0.2 M and 0.4 M NaCl in buffer did not result in any peak fraction.

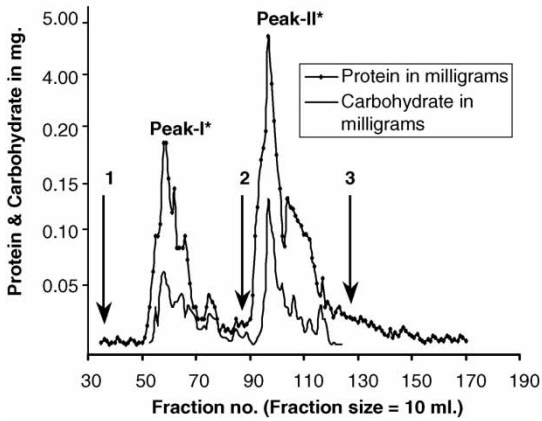

Figure 4. Ion exchange chromatography of peak II on DEAE-Sephadex A-50 column. Isocratic elution was performed upto fraction no. 35. Arrows nos. 1, 2 and 3 indicate fraction nos. where changes of buffer for discontinuous gradient elution were made. Elution with 0.4 M NaCl in buffer did not result in any peak fraction.

III were eluted with 0.1 M and 0.2 M α-methyl-D-glucoside in tris-phosphate buffer, respectively. Peak I(A) comprised fractions 7 to 10, while peak I(B) contained fractions 13 to 15. Peak II(A) comprised fractions 7 to 11, while peak II(B) included fractions 13 to 15. Similarly, peak III(A) contained fractions 7 to 10, whereas III(B) was also composed of fractions 13 to 15.

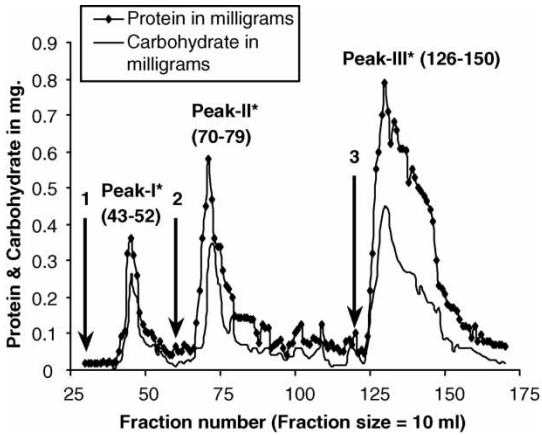

Figure 5. Ion exchange chromatography of peak III on DEAE-Sephadex A-50 column. Isocratic elution was performed upto fraction no. 30. Arrows nos. 1, 2 and 3 indicate fraction nos. where changes of buffer for discontinuous gradient elution were made. Pooled fractions 43–52, 70–79, and 126–150 constituted Peak I*, Peak-II*, and Peak-III*, respectively.

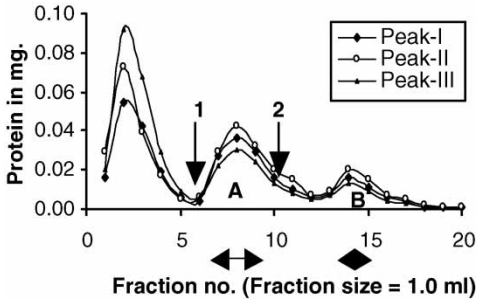

Figure 6. Concanavalin A-Sepharose 4-B column chromatography of pooled peaks I, II, and III. Arrows nos. 1 and 2 indicate fraction nos. from where elution with 0.1 M and 0.2 M α-methyl D-glucoside was initiated, respectively. Double headed arrows indicate pooled fractions of components A and B.

Determination of Biological and Immunological Potencies of Purified Peak Fractions

Fractions obtained after affinity chromatography were analysed by RRA, RIA, and ELISA. The standard profiles of RRA, RIA and ELISA are shown in Figs 7, 8 and 9, respectively. The activities obtained by RRA were less, compared to RIA and ELISA (Table-1). In terms of activity, peaks I(A), I(B) and II(A) seemed to be more potent than II(B), III(A) and III(B). However, their potency was lesser than the WHO standard. The analysis of hCG directly in these fractions using ELISA with IRP (75/589) indicated that only 75% hCG of peak I(A), 74% of peak I(B) and 40% of peak II(A)

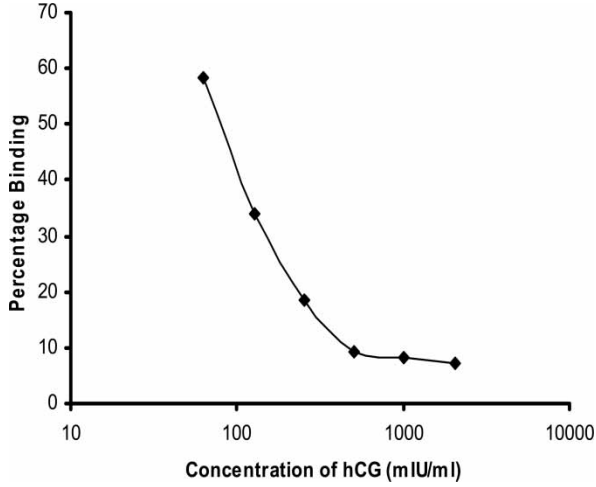

Figure 7. Standard profile of radioreceptor assay.

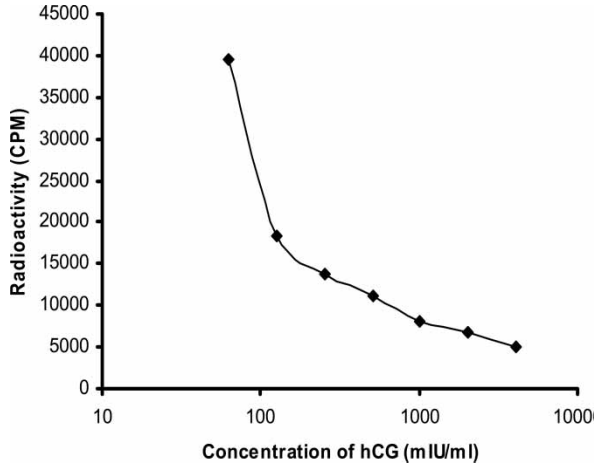

Figure 8. Standard profile of radioimmunoassay.

and 37.68% of peak II(B) were immunologically potent. Peak III (A) and (B) were less potent and thus have less immunological and biological activity.

Molecular Weight Determination of Peak Fractions by Sephadex G-200 Chromatography

Figure 10 represents the elution patterns of standard protein molecular weight (MW) markers and protein fractions. By plotting V_e/V_o against log MW,

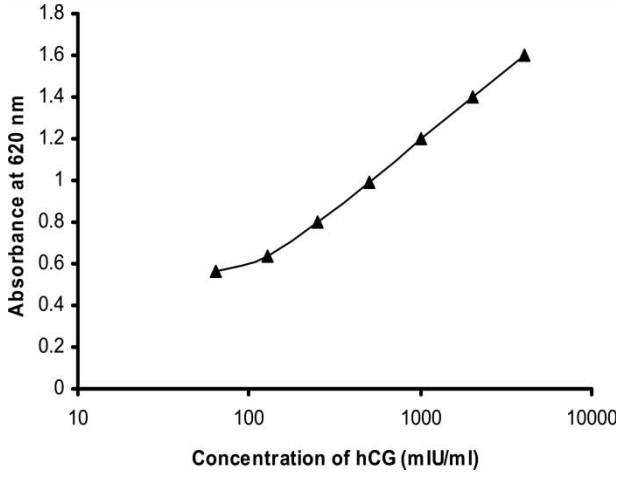

Figure 9. Standard profile of ELISA.

Table 1. Biological and immunological potencies of purified fractions of hCG

Protein fraction	Total protein (mg)	Amount of protein used for assay in ELISA (ng)	Amount of hCG detected by ELISA (ng)	Percentage of immunologically potent hCG as detected by ELISA (%)	ELISA (IU/mg) (using in house antibody)	RIA (IU/mg) (using NIH antibody)	RRA (IU/mg)
Peak I(A)	35	340	255	75	10,970	12,617	9,441
Peak I(B)	30	500	371	74	9,880	11,000	9,400
Peak II(A)	73	500	200	40	3,812	6,400	3,503
Peak II(B)	42	2,900	310	37.68	2,886	4,200	3,720
Peak III(A)	293	2,500	135	5.41	502	2,400	90
Peak III(B)	116	2,500	130	5.21	446	2,689	90

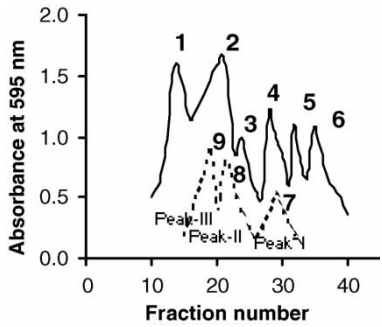

Figure 10. Determination of molecular weight of purified hCG peaks by gel filtration on Sephadex G-200 column. A 1.5 mL mixture of each of the following proteins (1)Blue Dextran [MW = 2,000,000D]; (2)BSA [MW = 66,000D]; (3)Peroxidase [MW = 44,000D]; (4)Carbonic anhydrase [MW = 29,000D]; (5)Trypsinogen [MW = 24,000D]; (6) α-Lactalbumin (MW = 14,200D) was applied to a column of Sephadex G-200 previously equilibriated with 0.02 M sodium phosphate buffer, pH 7.2, at 4°C. Peaks 7, 8, and 9 represent elution profile of 100 μg of peak I, 200 μg of peak II, and 328 μg of peak III, respectively, in 0.2 mL buffer chromatographed separately on the same column under identical conditions.

relative molecular sizes of protein fractions were calculated (Fig. 11). Peak I corresponds to 27,500D, peak II to 66,000D, and peak III to 84,000D. Table 2 shows the K_{av} values for all the proteins tested.

SDS-PAGE

Relative mobilities of peaks I(A), I(B), II(A), II(B), III(A), and III(B) with marker proteins are shown in Fig. 12. The relative mobilities, of proteins in

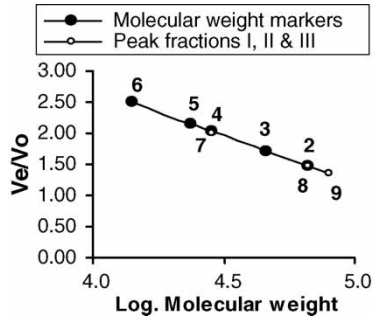

Figure 11. Calibration plot of Ve/Vo and Log molecular weight of standard proteins. Hollow points 7, 8, and 9 indicate log molecular weights of Peaks I, II, and III, respectively. See the legend of Figure 10.

Table 2. Molecular weight determination of peak fractions by Gel Filtration chromatography on Sephadex G-200

Standard proteins & peak fractions	Molecular weight of standard proteins	Average mol. wt. $K_{av} = (V_t - V_e)$ $(V_t - V_o)$	Estimated molecular weight of peak fractions
Bovine serum albumin (BSA)	66000 D	0.72	—
Peroxidase	44000 D	0.60	—
Carbonic anhydrase	29000 D	0.44	—
Trypsinogen	24000 D	0.28	—
α-lactalbumin	14200 D	0.16	—
Peak-I	—	0.40	27500 D
Peak-II	—	0.72	66000 D
Peak-III	—	0.80	84000 D

Where, V_t = Total vol. of the column; V_e = Mean elution volume; V_o = Void volume.

comparison to standard marker proteins, were calculated and plotted against log MW (Fig. 13). The MWs of the test samples were calculated from the plot and were found to be 19,539D (for hCG-α) and 28,870D (for hCG-β), which correspond to hCG obtained from Sigma. These were due to dissociation of hCG into subunits. This experiment clearly suggests that peaks II and III are aggregates of the native hCG molecule.

Native PAGE

Electrophoretograms of native-PAGE for all peak fractions are shown in Fig. 14. All peak fractions appeared as single bands (native state) and have

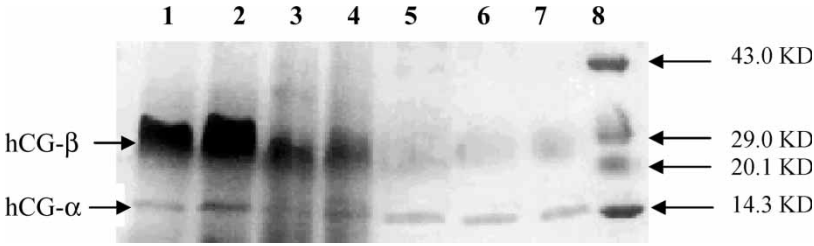

Figure 12. SDS-PAGE of purified peaks I, II, and III. Lanes: 1. Peak IA; 2. Peak IB; 3. Peak IIA; 4. Peak IIB; 5. Peak IIIA; 6. Peak IIIB; 7. Sigma hCG; 8. protein molecular weight marker.

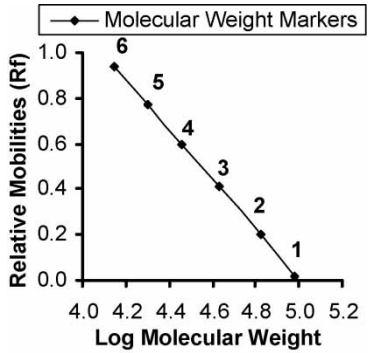

Figure 13. Calibration plot of Rf values and Log molecular weight of standard proteins. Protein MW markers used were: (1) Phosphorylase b; (2) BSA; (3) Ovalbumin; (4) Carbonic anhydrase; (5) Soyabean Trypsin inhibitor; (6) Lysozyme.

shown almost the same migration. The broadness of band in each lane, maximum being with Peaks IIA and IIB, might be due to the heterogeneous nature of hCG.

Western Blot Analysis

Figure 15 clearly shows the presence of hCG-α subunits in peaks I(A), II(A), and III(A) upon immunodetection using the anti-hCG-α antibody. The same fractions revealed the presence of β-subunits on treatment with anti-hCG-β antibody (Fig. 16).

Sialic Acid Content of hCG fractions

Table 3 depicts the sialic acid content of each pooled fraction, in which carbohydrate content (in terms of sucrose) was also measured. Peaks I(A and B),

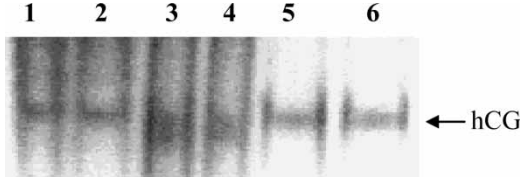

Figure 14. Native PAGE of purified peaks I, II, and III. Lanes: 1. Peak IA; 2. Peak IB; 3. Peak IIA; 4. Peak IIB; 5. Peak IIIA; 6. Peak IIIB.

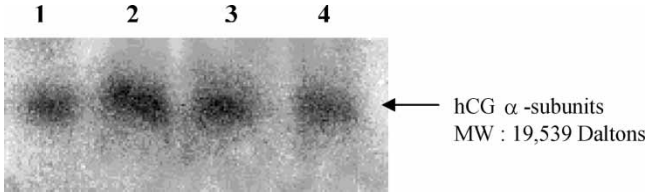

Figure 15. Western blot analysis of hCG peak fractions using anti-hCG-α antibody. Lanes: 1. Sigma hCG; 2. Peak IA; 3. Peak IIA; 4. Peak IIIA.

II(A and B) and III(A and B) had approximately 30% sialic acid in total carbohydrate content.

DISCUSSION

In general, the methods used for the isolation of hCG include gel exclusion and ion exchange chromatography, which were similar to those used by other investigators.[7,9,14,28–32] For the extraction of hCG from pregnancy urine, a kaolin-acetone method[5] was adopted. The crude residue was purified with a preliminary Sephadex G-100 column, followed by DEAE-Sephadex A-50 chromatography with discontinuous gradient elution to permit maximum resolution of the protein containing components. The elution pattern of the present study shows three peak protein fractions having carbohydrate activity, namely peaks I, II, and III. The pattern obtained is similar in few respects, but certainly not identical, with the available reports.[9,10,33,34,44]

Previous estimations of MWs of the highly purified native hCG preparations from pregnancy urine, as reported by various authors, differ quite widely according to the method used. The MW of 59,000 (by gel exclusion on Sephadex G-150),[33] greater than 67,000 (by gel exclusion on Sephadex G-100),[34] 65,000 (using Sephadex G-100 column),[35] and 58,000 (by gel exclusion on Sephacryl S-200)[36] have been reported. Using a sedimentation equilibrium method, Bahl[33] reported a MW of 47,000,

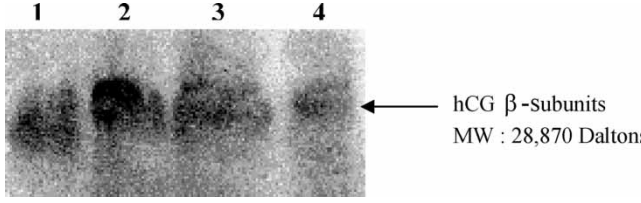

Figure 16. Western blot analysis of hCG peak fractions using anti-hCG-β antibody. Lanes: 1. Sigma hCG; 2. Peak IA; 3. Peak IIA; 4. Peak IIIA.

Table 3. Estimation of sialic acid in relation to total carbohydrate content of each pooled peak

Peak fractions	Total protein (mg)	Total carbohydrate (in mg/mg protein) (%)	Total Sialic acid (in mg/mg protein) (%)	Percentage of Sialic acid/mg carbohydrate/ mg protein (%)
Peak I(A)	35	0.284 (28.4)	0.086 (8.6)	30.2
Peak I(B)	30	0.280 (28.0)	0.085 (8.5)	30.3
Peak II(A)	73	0.299 (29.9)	0.090 (9.0)	30.1
Peak II(B)	42	0.296 (29.6)	0.091 (9.1)	30.7
Peak III(A)	293	0.278 (27.8)	0.084 (8.4)	30.2
Peak III(B)	116	0.291 (29.1)	0.085 (8.5)	29.3

while Bell, Canfield, and Sciarra[10] reported a MW of ~46,000. In the present study, we found that the MWs of peaks I, II, & III, as determined by gel exclusion chromatography on Sephadex G-200, are 27,500, 66,000, and 84,000 Daltons, respectively. Peak I seemed to be a low molecular weight hCG, while peak II had a similar value as described earlier. van Hell et al.[8] reported a MW of 62,000D and explained that there could be the existence of a dimer. In the present study, peaks II & III could also be considered as dimer and trimer of hCG, since re-chromatography of peaks II and III on an anion-exchanger have eventually resolved them into single peak I. This is further supported by SDS-PAGE, followed by Western blotting; it appears that all peak fractions have dissociated into hCG-α and hCG-β subunits. Had other proteins been associated with them, more than two protein bands would have been visible in the SDS-PAGE data. Moreover, immunoassays (ELISA and RIA) and bioassays (RRA) have further strengthened the possibility of the presence of a dimer and a trimer of hCG in peaks II and III, respectively. These dimers and trimers might be turning the epitopic region partially accessible/or unaccessible for binding to antibody in ELISA and RIA; while making the receptor binding region least accessible in RRA. Final resolution of peaks II and III into I facilitated minimal hindrance in accessibility to the epitope and/or receptor binding region. Then, only peaks I (A & B) could exhibit maximum potency in RIA, ELISA, and RRA. Peak I contained maximum immunologically potent hCG (75%), followed by peaks II (40%) and III (5%).

When SDS-PAGE of peak fractions was performed using protein MW markers, the results indicated that peak-I(A), which has been termed as 'native hCG' dissociated into two components and both of them migrated with different MW characteristics. The slower migrating component coincided with β-subunit, and MW estimate is in the order of 28,870

Daltons. The faster component has a migration coincident with an α-subunit, and its MW is estimated to be in the order of 19,539 Daltons. Native PAGE has confirmed the presence of the hCG molecule in its native state among all peak fractions. Moreover, the heterogeneous nature of hCG cannot be ruled out, as it is evident from the single broad band in each lane, the maximum being with Peaks IIA and IIB.

Peak-I seemed to have low molecular weight hCG molecules, compared to peaks II and III. Following affinity chromatography, each peak fraction was resolved into two components, A and B. Since anion exchanger discriminates among hCG molecules on the basis of sialic acid content,[10,33] sialic acid was measured in each fraction. Each fraction contained about 30% sialic acid. The evidence for variation in sialic acid content and its relationship to the heterogeneity of the hCG molecule was discussed earlier.[9,10,37] The resultant heterogeneity in electrical charge leads to starch gel or SDS-PAGE pattern that exhibits either broad bands or multiple bands. Since glycoproteins that possess sialic acid generally exhibit heterogeneity in electrical charge, it is not surprising to find this property with hCG. However, it makes it difficult to define the purity of a material since the usual criteria for protein purity, such as crystallization, salting out curves, and sharp bands after gel electrophoresis, are not met by this glycoprotein hormone.[38]

Immunological potencies, as verified by RIA and ELISA, differed due to the antibodies used in the assay system. The former utilized antibody developed against native hCG (from NIH, USA) and latter method employed hCG-β antibody developed in-house. The discontinuous buffer system used in purification certainly has high resolving power, even though the highest potencies obtained are 9,441 IU/mg by RRA, 10,970IU/mg by ELISA, and 12,617 IU/mg by RIA for peak I(A), which are similar to the potencies reported by other investigators.[2,3,8–10,29,33,35,39,40]

The presence of two bands following SDS-PAGE in each of the peak fractions has revealed that hCG preparations, namely peaks-I(A), I(B), II(A), II(B), III(A), and III(B) that were obtained by the procedure described in the present study were highly purified. Although possessing common physicochemical properties, as revealed by native and SDS-PAGE, Western blotting, the peak fractions, however, had different immunological and biological activities. It is also possible that there may be some differences in their carbohydrate and/or amino acid composition, as reported earlier.[10,28,37,41–43]

In summary, the present study demonstrates that two different forms of hCG, viz., peaks II (dimer) and III (trimer) were finally resolved into peak I. Peak I, which had low molecular weight hCG (27,500 D), could still be measured by RRA, RIA, and ELISA, thus showing the existence of a low molecular weight hCG in Indian pregnant women. This type of low molecular weight hCG, which is biologically and immunologically active, has never been reported so far.

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