Location of Major Antigenic Sites of the β Subunit of Human Chorionic Gonadotropin†

N. Swaminathan* and Glenn D. Braunstein

ABSTRACT: Purified β subunit of human chorionic gonadotropin (hCG) was partially reduced with β-mercaptoethanol, carboxymethylated, and digested with chymotrypsin. The peptides were isolated by high-voltage electrophoresis and paper chromatography. Five major disulfide-containing peptides were isolated, and their location in the parent molecule was established by amino acid composition and amino- and carboxy-terminal analyses. All of these peptides inhibited the binding of ^{125}I-labeled hCG by anti-β hCG serum. The inhibitory effect of these peptides was lost when their disulfide bonds were reduced and alkylated. Synthetic carboxy-terminal peptides were not inhibitory. Based on these data it is concluded that a major antigenic site of hCG resides in the region of residues 21–23 with a disulfide bond connecting cysteine-23 or -26 with the cysteines at positions 72 or 110.

Human chorionic gonadotropin[1] is a glycoprotein hormone secreted by the synctiotrophoblast of the placenta throughout normal pregnancy (Braunstein et al., 1976). The amino acid sequence (Bahl et al., 1972; Bellisario et al., 1973; Carlsen et al., 1973; Morgan et al., 1975) and the monosaccharide sequence of the carbohydrate units (Bahl, 1969b; Kennedy and Chaplin, 1976) of this hormone have been elucidated. Human chorionic gonadotropin, as well as the other human glycoprotein hormones, LH, FSH, and TSH, is composed of two dissimilar, noncovalently linked subunits designated α and β. The α subunits of LH, FSH, and TSH are identical and differ from hCG by a two-residue amino acid inversion and a three-residue amino acid deletion at the amino terminus (Pierce et al., 1971b; Bellisario et al., 1973; Morgan et al., 1975; Papkoff et al., 1973; Rathnam and Saxena, 1974; Shome and Parlow, 1973; Ward et al., 1973). The β subunit of each of the glycoprotein hormones is different and confers the immunologic and biologic specificity (Pierce et al., 1971a; Shome and Parlow, 1974; Ward et al., 1973; Binoux et al., 1974; Vaitukaitis et al., 1972).

Antisera generated against the intact hCG molecule generally do not discriminate between hCG and LH (Paul and Ross, 1964). However, antisera raised against the purified β subunit of hCG demonstrate a substantially lower cross-reaction with LH (Vaitukaitis et al., 1972). The reasons for the reduction in antigenic recognition of LH by antisera produced against the β subunit of hCG are not entirely clear. Eighty percent of the sequence of the first 115 amino-terminal residues of the β subunit of hCG are identical to those found in the β subunit of LH (Shome and Parlow, 1973, 1974; Closset et al., 1973; Sairam and Li, 1973). In addition, the β subunit of hCG has an additional 30 amino acid residues in the carboxyl-terminal portion of the molecule which are not shared by LH (Carlsen et al., 1973; Morgan et al., 1975). Therefore, antibodies raised against the β subunit of hCG may recognize this unique portion of the hCG β subunit molecule. Alternatively, the secondary or tertiary structure of the β subunit of hCG may be important for immunologic specificity, since S-carboxymethylation or S-amidomethylation of either hCG or its β subunit results in complete loss of antigenic recognition by anti-β hCG sera (Bahl et al., 1977). The present study was undertaken in order to better delineate the antigenic sites on the hCG molecule. The interaction of various peptides derived from the β subunit of hCG which contained partially reduced disulfide bonds and synthetic carboxyl-terminal β-hCG peptides with several antisera against the intact B subunit of hCG was studied.

Materials and Methods

Antisera. Antiserum (SB 6) raised against the intact β subunit of hCG was obtained from the Hormone Distribution Officer, NIAMDD and NICHD, National Institutes of Health, Bethesda, Md. This antiserum was used at a final dilution 1:200 000 for all inhibition studies. In addition, an anti-β hCG serum (SN-4) was raised in rabbits in our laboratory utilizing a highly purified preparation of the β subunit of hCG conjugated to hemocyanin as the immunogen (Swaminathan et al., 1978). β-hCG was conjugated to hemocyanin using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as the coupling agent (Goodfriend et al., 1964). This antiserum was used at a final dilution 1:1 000 000 in all inhibition studies. In order to increase the specificity to hCG of the latter antiserum, extensive immunoabsorption of the serum was carried out on a 1 × 10 cm column of LH LER-1724 covalently linked to Sepharose (Swaminathan, unpublished). This absorbed antiserum, designated SN-4ab, was used at a final concentration of 1:25 000 for all inhibition studies.

Preparation of Subunits of hCG. Human chorionic gonadotropin was purified from a crude commercial urinary preparation (Organon Inc., West Orange, N.J.) according to the method of Bahl (1969a). The purified hCG had a biologic activity of 17 000 international units/mg relative to the second international standard hCG, as determined by the rat ventral prostate weight bioassay of Greep as modified by McArthur (1952). The subunits were dissociated with 8 M urea and separated by DEAE-Sephadex chromatography as described by Swaminathan and Bahl (1970).

Preparation of Chymotryptic Peptides from β-hCG. Six-

† From the Departments of Medicine, Cedars-Sinai Medical Center and the University of California at Los Angeles School of Medicine, Los Angeles, California 90048. *Received April 6, 1978.* Supported by United States Public Health Service Research Grants CA 19392, CA 18362, and RR 05468.

[1] Abbreviations used: hCG, human chorionic gonadotropin; LH, luteinizing hormone; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; DTE, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; Con-A Sepharose, concanavalin A-Sepharose; IA, iodoacetamide; DEAE, diethylaminoethyl.

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teen milligrams of the purified β subunit was desialyzed by incubating with 2 mL of 0.05 M HCl at 80 °C for 1 h. The solution was dialyzed against distilled water and lyophilized. The lyophilized material was dissolved in 2.5 mL of water. The pH was adjusted to 8.0 after addition of 40 μL of β-mercaptoethanol. Nitrogen was bubbled through the reaction mixture and incubated at 37 °C for 1 h. The reduced β subunit was precipitated with 5 vol of cold acetone. The precipitate was suspended in 2 mL of water, 100 mg of iodoacetic acid was added in small portions, and the pH was adjusted to 8.0 with triethylamine after each addition. The mixture was incubated at 37 °C for 1 h, dialyzed against 0.05 M ammonium bicarbonate (pH 8.0), and lyophilized. Amino acid analysis of an aliquot showed that about 70% of the disulfide bonds were reduced and alkylated in this process. The alkylated subunit was digested with 1% (w/w) of chymotrypsin overnight at 37 °C under N_2. The pH was maintained at 7.5 with additions of a 1% solution of triethylamine. Following the chymotryptic digestion, 0.1 M acetic acid was added to adjust the pH to 4.0, and the solution was lyophilized and reconstituted in 2 mL of 1% ammonium bicarbonate (pH 8.0). The solution was then loaded on a Sephadex G-50 column (0.9 × 50 cm), equilibrated, and developed with 1% ammonium bicarbonate (pH 8.0). Three-milliliter fractions of the eluate were collected, and the absorbance at 230 and 280 nm was recorded. The peak fractions were pooled and lyophilized.

Concanavalin A–Sepharose Chromatography. Peak fractions from the Sephadex G-50 column which demonstrated a positive reaction for carbohydrates by the method of DuBois et al. (1956) were chromatographed on a 1 × 10 cm column of concanavalin A–Sepharose. The column was washed with 50 mL of 10 mM sodium phosphate buffer (pH 6.8), and the adsorbed glycoproteins were eluted with 200 mM methyl α-D-glucopyranoside in 10 mM sodium phosphate buffer (pH 6.8) containing $MnCl_2$, $MgCl_2$, and $CaCl_2$ at a final concentration of 1 mM each. The breakthrough fraction, washings, and methyl α-glycoside eluate were concentrated by ultrafiltration under positive N_2 pressure using Amicon (UM-2) membranes.

Purification of Peptides. The peptides were checked for purity by high-voltage electrophoresis at pH 1.9, 3.5, and 6.5 and also by descending paper chromatography on Whatman 3MM paper with 1-butanol–pyridine–acetic acid–water (15:10:3:12, v/v) as solvent. Peptides were located by ninhydrin (Dreyer and Bynum, 1967) and/or *o*-phthalaldehyde (Mendez and Gavilnez, 1976). Purification of peptides on a large scale was performed by preparative high-voltage electrophoresis (at 40–50 V/cm, current 40–160 mA) in Varsol-cooled tanks in a Savant apparatus. Preparative descending paper chromatography (16–20 h) was also utilized for some of the peptides, depending upon the resolution obtained from high-voltage electrophoresis.

Characterization of Peptides. The position of the peptides in the parent β-hCG molecule was ascertained from their amino acid composition and amino- and carboxy-terminal analyses. Amino-terminal analysis was performed by the dansyl method of Hartley (1970) on polyamide sheets. Carboxy-terminal analysis was performed by digestion with carboxypeptidases A and B (Sigma), and the released amino acids were quantitated in a fluorometric amino acid analyzer using *o*-phthalaldehyde. Amino acid analysis was performed after hydrolysis of peptides with 6 N HCl for 24 to 72 h at 110 °C in vacuo, in a Durrum 500 amino acid analyzer using ninhydrin for detection. Cysteine was determined as cysteic acid after performic acid oxidation according to the method of Moore (1963). In some experiments, amino acids were determined as their isobutyl *N*-heptafluorobutyl esters by gas–liquid chromatography on OV-1 columns in a Hewlett-Packard 5710 A gas chromatograph fitted with an electronic integrator (Swaminathan, unpublished).

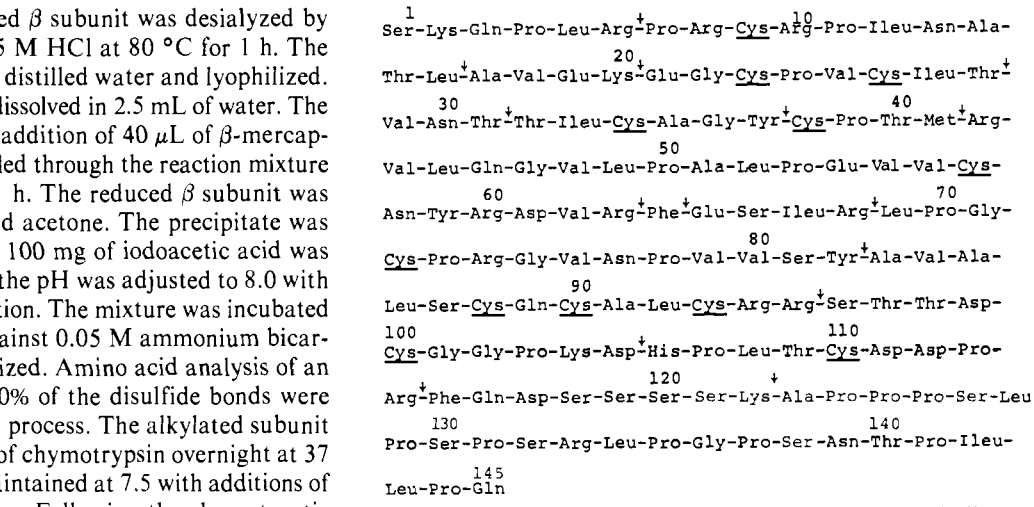

FIGURE 1: Sites of cleavage on β-hCG during the chymotryptic digestion.

Synthetic Peptides. Dr. V. C. Stevens of Ohio State University kindly provided generous quantities of four synthetic peptides containing hCG β residues 105–145, 111–145, 118–145, and 113–145. Purified β-LH (AF-290-B) was kindly supplied by Dr. A. F. Parlow, Harbor General Hospital, Los Angeles.

Hormone Standards. The highly purified urinary hormone preparations of hCG (CR 117), α-hCG (CR 117 α), β-hCG (CR 117 β), pituitary hLH (LER 907 and LER 960), and α- and β-LH, and antisera against the α and the β subunits of hCG (Sa-6 and Sb-6, respectively) were kindly provided by the Hormone Distribution Officer, MIAMDD and NICHD, National Institutes of Health, Bethesda, Md.

Inhibition Studies. The effect of the various peptides derived from the β subunit of hCG, the synthetic peptides, intact hCG, the α and β subunits of hCG, LH (LER-960 and LER-907) and β-LH (AFP-290-B), on the inhibition of binding [^{125}I]-hCG by antiserum raised against the β subunit of hCG (Sb-6, SN-4, and SN-4ab) was studied in the hCG radioimmunoassay (Vaitukaitis et al., 1972; Braunstein et al., 1976). The reaction mixture contained in a total volume of 1 mL: 10 mM EDTA (pH 7.4) 600 μL of 2.5% normal rabbit serum in phosphate-buffered saline (pH 7.4), containing the peptide or hormone to be studied, 220 pg of [^{125}I]hCG, and 200 μL of suitably diluted anti-β-hCG serum. The hCG bound to antibody was separated from free hCG with sheep anti-rabbit globulin. The methods of Rodbard (1974) were utilized to analyze the radioimmunoassay data. A comparison of the inhibition of the binding of [^{125}I]hCG by the various anti-β-hCG sera was carried out by determination of the mean concentration of each peptide or hormone that resulted in a 50% displacement of the labeled hCG from the antibody.

Results

Purification and Characterization of Peptides. Approximately three to four of the six disulfide bonds of the β subunit of hCG were reduced by treatment with β-mercaptoethanol and iodoacetic acid. Figure 1 illustrates the sites of cleavage of the β subunit molecule following extensive digestion with chymotrypsin. These cleavage sites were determined by amino acid and N- and C-terminal analysis (vide infra). In addition

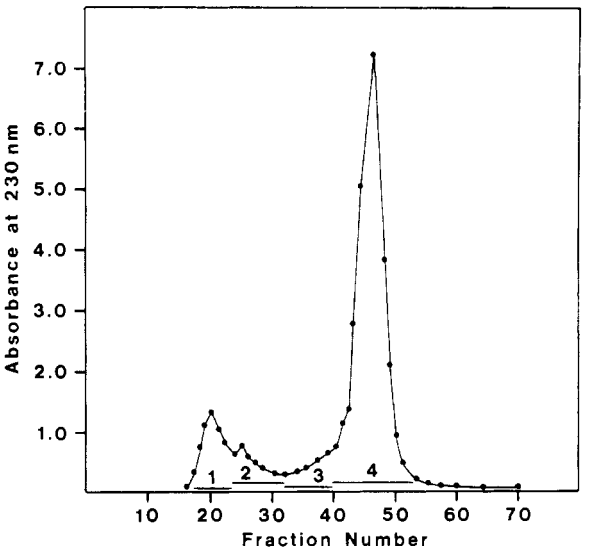

FIGURE 2: Elution profile of chymotryptic peptides on a Sephadex G-50 column; the four major fractions were pooled as shown and concentrated.

to the cleavage sites expected of chymotrypsin, several trypsin like cleavages were also noted, presumably due to tryptic activity in the chymotrypsin preparation. The major peptides present in the chymotrypsin digest were separated on Sephadex G-50 (Figure 2). The eluate was divided into four major fractions (designated fractions 1-4, in Figure 2) and lyophilized. Concanavalin A-Sepharose chromatography was carried out on the major carbohydrate-containing fraction (Fraction 1). Both the concanavalin A adsorbed portion (F) and unadsorbed portion (D) of fraction 1, as well as fractions 2-4, were subjected to high-voltage electrophoresis and paper chromatography. The purification scheme of the peptides is shown in Figure 3 and the amino acid analysis of the major peptides is given in Table I.

Peptide D1 was isolated from fraction 1 of the Sephadex G-50 column by concanavalin A-Sepharose chromatography followed by high-voltage electrophoresis. This peptide demonstrated a single spot on high-voltage electrophoresis (R_{Arg}, 0.1). A small aliquot was reduced with excess DTE and alkylated with iodoacetamide. Three peptides were isolated by paper chromatography. From the amino acid composition and amino-terminal analysis (Table II) these peptides were found to correspond to regions 21-28 (D_1E_1), 69-74 (D_1E_2), and 106-122 (D_1E_3), respectively. This indicates that peptide D_1 is composed of these three peptides connected by two disulfide bonds.

Peptide D_2 was isolated from fraction 2 of the Sephadex column by high-voltage electrophoresis (R_{Arg}, 0.02). This peptide remained close to the origin during high-voltage electrophoresis and demonstrated a single spot on paper chromatography. Reduction and alkylation followed by paper chromatography resulted in two peptides (D_2E_1 and D_2E_2). Amino acid composition and amino-terminal analysis (Table II) indicated that they were derived from region 21-37 (D_2E_1) and 109-145 (D_2E_2), respectively.

Peptide D_3 was isolated from fraction 3 of the Sephadex G-50 column by high-voltage electrophoresis (R_{Arg}, 0.22). This peptide demonstrated a single spot on high-voltage electrophoresis (pH 1.9, 4.7) and also on paper chromatography. Reduction of an aliquot with DTE and alkylation with iodoacetamide resulted in three peptides (D_3E_1, D_3E_2, and D_3E_3) which were separated by paper chromatography. Analysis of the peptides (Table III) showed that they were derived from regions 17-28 (D_3E_1), 65-82 (D_3E_2), and 96-122 (D_3E_3), respectively. Therefore, peptide D_3 is composed of three peptides connected by two disulfide bridges.

Peptide F was isolated from fraction 1 of the Sephadex G-50 column by concanavalin A-Sepharose chromatography. The peptide was adsorbed onto the column and was eluted with methyl α-glucoside. After removal of methyl α-glucoside by ultrafiltration, two peptides (F_1 and F_2) were isolated by high-voltage electrophoresis (R_{Arg} 0.2 and 0.7). Both of these

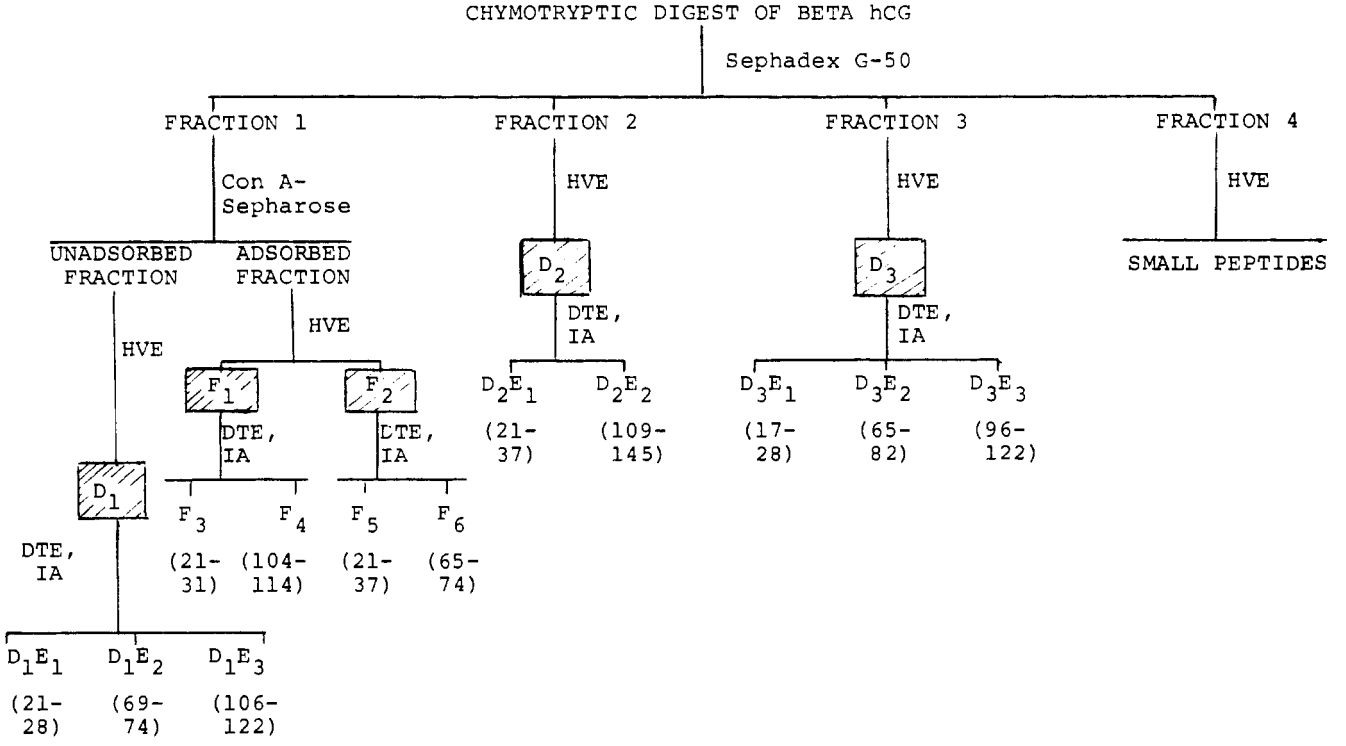

FIGURE 3: Scheme of separation of peptides. Peptides shown in shaded boxes contain intact disulfide bonds.

TABLE I: Amino Acid Composition[a] of Peptides from the Chymotryptic Digest of the β Subunit of hCG.

	D_1	D_2	D_3	F_1	F_2
Asp	3.0 (3)[b]	4.6 (5)	6.1 (6)	3.0 (3)	1.0 (1)
Thr	1.9 (2)	4.7 (5)	4.1 (4)	3.1 (3)	2.9 (3)
Ser	2.9 (4)	6.8 (8)	6.3 (7)		1.0 (1)
Glu	2.0 (2)	2.9 (3)	3.9 (4)	1.0 (1)	1.9 (2)
Pro	4.6 (5)	12.2 (11)	6.5 (7)	2.6 (3)	2.9 (3)
Gly	1.8 (2)	3.0 (3)	5.1 (5)	1.0 (1)	3.0 (3)
Ala		2.0 (2)	1.0 (1)		0.9 (1)
Val	0.9 (1)	1.7 (2)	4.6 (5)	1.9 (2)	1.0 (2)
Ile	0.9 (1)	2.6 (3)	1.8 (2)	1.0 (1)	2.9 (3)
Leu	2.0 (2)	2.9 (2)	2.0 (2)	0.9 (1)	1.0 (1)
Tyr		1.0 (1)	1.0 (1)		0.9 (1)
Phe	1.1 (1)	1.0 (1)	1.0 (1)		
His	1.0 (1)	1.0 (1)	0.9 (1)	0.9 (1)	
Lys	1.0 (1)	1.1 (1)	2.8 (3)		2.1 (2)
Arg	1.9 (2)	1.6 (2)	2.9 (3)	1.0 (1)	4.0 (4)
Cys	3.6 (4)	1.7 (2)	4.0 (4)	1.6 (2)	1.9 (2)
CMC		1.6 (2)	1.0 (1)	0.9 (1)	2.0 (2)
yield (μmol)	0.068	0.34	0.26	0.21	0.25
total res	31	54	57	20	31

[a] Results expressed as residues per mole of peptide. [b] Numbers in parentheses indicate expected integral values.

TABLE II: Amino Acid Composition[a] of the Component Peptides D_1 and D_2.

	D_1E_1	D_1E_2	D_1E_3	D_2E_1	D_2E_2
Asp			2.8 (3)[b]	0.8 (1)	3.6 (4)
Thr	0.6 (1)		0.8 (1)	2.6 (3)	1.8 (2)
Ser			3.6 (4)		7.5 (8)
Glu	1.0 (1)		1.0 (1)	1.0 (1)	1.8 (2)
Pro	0.9 (1)	1.8 (2)	1.9 (2)	0.8 (1)	9.5 (10)
Gly	1.1 (1)	1.0 (1)		2.1 (2)	0.9 (1)
Ala				0.9 (1)	0.8 (1)
Val	0.9 (1)			1.8 (2)	
Ile	0.8 (1)			1.8 (2)	0.8 (1)
Leu		0.6 (1)	0.8 (1)		1.7 (3)
Tyr				0.8 (1)	
Phe			1.0 (1)		1.0 (1)
Lys			1.1 (1)		0.9 (1)
His			0.9 (1)		
Arg		0.9 (1)	1.0 (1)		1.8 (2)
CMC	1.9 (2)	0.8 (1)	0.8 (1)	2.6 (3)	0.9 (1)
N terminal[c]	Glu	Leu	His	Glu	Thr
C terminal[d]	Thr		Lys	Tyr	

[a] Results expressed as residues per mole of peptide. [b] Numbers in parentheses indicate expected integral values. [c] Identified as the dansyl derivatives. [d] Carboxypeptidase A or B was used for digestion. No amino acid was released from D_1E_2 or D_2E_2.

peptides contained intact disulfide bonds. The component peptides were identified by reduction with DTE and alkylation with iodoacetamide and separated by paper chromatography. Amino acid analysis and amino-terminal analysis (Table III) revealed that F_1 is composed of two peptides, 21-31 (F_3) and 106-114 (F_4), and peptide F_2 also contained two peptides with the linear amino acid sequence of 21-37 (F_5) and 65-74 (F_6) connected by a disulfide bond.

Fraction 4 of the Sephadex G-50 column contained several small peptides without any intact disulfide bonds. These were separated by high-voltage electrophoresis and did not inhibit the binding of hCG by the anti-β subunit serum (data not shown). Figure 4 summarizes the amino acid sequences of the component peptides.

Immunochemical Studies. Peptides D_1, D_2, D_3, F_1, and F_2 were homogeneous, as tested by paper electrophoresis and paper chromatography. All of these peptides contained intact disulfide bonds connecting the various component peptides. They were studied for their effectiveness in inhibiting the binding of [^{125}I]hCG by anti-β-hCG antisera in the radioimmunoassay. All of these peptdies inhibited the binding, but there were substantial differences in the mass required for 50% displacement of the tracer from the antiserum. Table II enumerates the results of the inhibition studies with the peptides, as well as with the intact α β subunits of hCG, the intact hCG, LH, and the β subunit of LH. The β subunit of hCG and peptides F_1 and F_2 were the most inhibitory in this system. When the peptides containing intact disulfide bonds were reduced with DTE and alkylated with iodoacetamide, the derivatives were no longer inhibitory (Table IV). The four synthetic carboxy-terminal peptides did not inhibit the assay at concentrations as high as 10 μg/mL.

Discussion

The major objective of this study was to determine what antigenic properties or sites of the β-hCG molecule were required to elicit an antigenic response and which conferred specificity to the antiserum. The studies of Bahl et al. (1976) have shown that the antigenic determinants are probably related to conformational structure rather than the linear se-

TABLE III: Amino Acid Composition[a] of the Component Peptides of D_3 and F_1 and F_2.

	D_3E_1	D_3E_2	D_3E_3	F_3	F_4	F_5	F_6
Asp		0.8 (1)[b]	4.5 (5)	0.8 (1)	1.8 (2)	1.0 (1)	
Thr	0.7 (1)		2.6 (3)	1.8 (2)	1.0 (1)	2.8 (3)	
Ser		1.6 (2)	4.8 (5)				0.9 (1)
Glu	3.0 (3)	0.9 (1)	0.6 (1)	1.0 (1)		1.0 (1)	1.0 (1)
Pro	0.9 (1)	2.6 (3)	2.4 (3)	0.8 (1)	1.9 (2)	0.9 (1)	1.8 (2)
Gly		2.1 (2)	1.8 (2)	1.1 (1)		1.8 (2)	0.6 (1)
Ala	1.0 (1)					0.9 (1)	
Val	1.6 (2)	2.4 (3)		1.6 (2)		1.6 (2)	
Ile	0.9 (1)	0.6 (1)	0.8 (1)	0.8 (1)		1.7 (2)	0.8 (1)
Leu		0.9 (1)			0.9 (1)		0.8 (1)
Tyr		0.8 (1)				0.7 (1)	
Phe			1.0 (1)				
Lys	0.9 (1)		1.8 (2)				
His			0.9 (1)		0.9 (1)		
Arg		1.8 (2)	1.0 (1)		1.0 (1)		1.9 (2)
CMC	1.8 (2)	0.9 (1)	1.8 (2)	1.9 (2)	1.0 (1)	2.8 (3)	0.9 (1)
N terminal[c]	Ala	Glu	Ser	Glu	His	Glu	Glu
C terminal[d]	Thr	Tyr	Lys	Thr		Tyr	

[a] Results expressed as residues per mole of peptide. [b] Numbers in parentheses indicate expected integral values. [c] Identified as the dansyl derivative. [d] Carboxypeptidase A or B was used for digestion. No amino acid was released from F_4 or F_6.

TABLE IV: Inhibition Studies Results.

peptide	ng[f] required for 50% inhib		
	SN-4[a]	SN-4ab[b]	Sb-6[c]
D_1	5.79	770.07	12.19
D_2	0.34	1.24	0.34
D_3	15.47	297.45	23.44
F_1	0.04	1.60	0.04
F_2	0.08	1.92	0.08
reduced, alkylated peptide[e]	>10 000.00	>10 000.00	>10 000.00
synthetic COOH-term. peptide[d]	>10 000.00	>10 000.00	>10 000.00
α-hCG	64.00	430.00	77.00
β-hCG	0.0018	1.60	0.0024
hCG	0.29	2.70	0.33
LH (LER-960)	5.40	900.00	5.40
LH (LER-907)	30.00	10 000.00	32.00
β-LH	9.50	25.00	9.50

[a] Antiserum against the β subunit of hCG conjugated with hemocyanin. [b] SN-4 after absorption with hLH immunoabsorbent. [c] Antiserum against β-hCG supplied by NIH. [d] Synthetic peptides representing residues at 105–145, 111–145, 118–145, and 113–145 of the β subunit of hCG. [e] Reduced, alkylated D_1, D_2, D_3, F_1, and F_2. [f] The peptides were quantitated by amino acid analysis.

quence alone. They found that the completely reduced β subunit of hCG no longer inhibited the binding of labeled hCG with antiserum against the β subunit. Antisera raised against the synthetic or native peptides representing the carboxy-terminal portion of the β subunit were highly specific for hCG (Louvet et al., 1974; Chen et al., 1976; Stevens, 1976; Matsuura et al., 1978) with a probable major antigenic site in the region of residues 133–137. Thus, it is possible that some of the immunologic specificity noted with antisera generated against β-hCG could be the result of a population of antibodies directed against this carboxy-terminal portion of the molecule.

The present studies indicate that the antigenic determinants reside in the secondary, and possibly the tertiary, structure of the β subunit of hCG and that the linear amino acid sequence of the β subunit is not sufficient to account for the immunologic specificity of antisera generated against the intact β subunit.

Prior studies (Carlsen et al., 1973; Morgan et al., 1975) have established the linear amino acid sequence of β-hCG, but the positions of the disulfide bridges have not yet been determined due to the difficulty in obtaining specific cleavages resulting in peptides that maintain intact disulfide bonds. Under the conditions employed in the present study, the β-mercaptoethanol reduced three to four of the six disulfide bonds present in the β subunit of hCG. Among the five peptides isolated, three of them contained one disulfide bond and two of them contained two disulfide bonds. The bonds that survived reduction and alkylation are the ones located between positions 23, 26, 72, and 110. Although it is not clear which residues are connected to each other by these disulfide bonds, it is apparent that at least two of the disulfide bridges in the β subunit of hCG connect residues 23, 26, 72, and 110.

The reduction appeared to be almost stereospecific and this is perhaps due the absence of denaturing agents like urea or guanidine hydrochloride during reduction and alkylation. Lack of complete unfolding of the β subunit might have led to the reduction of exposed disulfide bonds leaving the rest intact. Complete reduction of all the disulfide bonds has been reported in the presence of 8 M urea with a high ratio of β-mercaptoethanol to sulfhydryl groups (Bahl, 1969b).

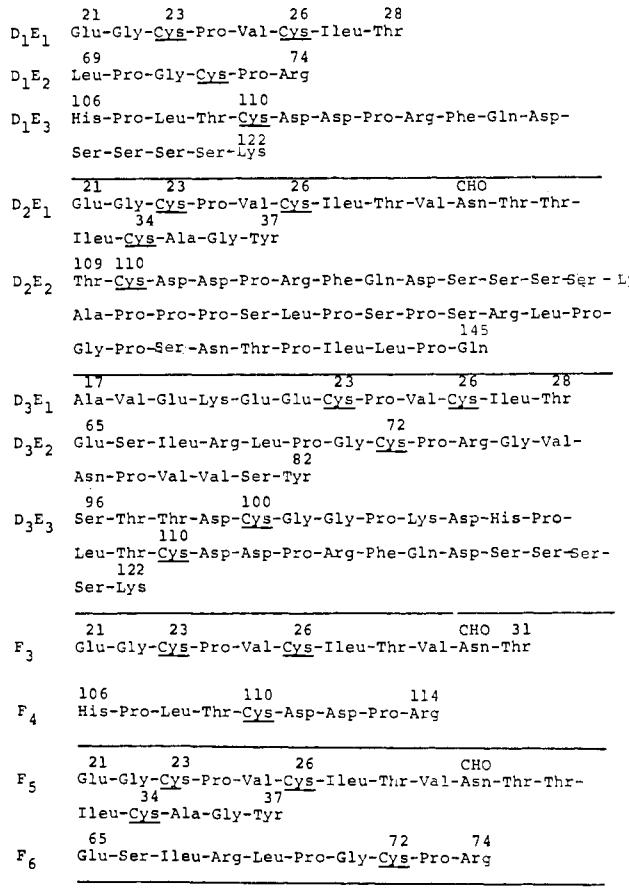

FIGURE 4: Structure of the isolated peptides. Structures shown are based on the proposed sequence of β-hCG by Morgan et al. (1975).

Based on the analysis of peptides obtained from partial acid hydrolysis, Ward et al. (1973) proposed a tentative scheme for the arrangement of disulfide bridges in the β subunit of ovine LH. In this arrangement, cysteine residues at positions 23 and 26 were linked with cysteines, at positions 72 and 110, respectively. It appears likely that the structural similarity of LH and hCG found in their linear sequence is conserved in their secondary structures as well.

While the peptides with intact disulfide bonds inhibit the binding of β-hCG with the antiserum, the reduced peptides do not displace the [^{125}I]hCG from the anti-β-hCG sera, indicating that the disulfide bonds must remain intact as a prerequisite for antigenic recognition. Of interest, there was a marked difference in reactivities of the various peptides despite their derivation from similar regions in the molecule. The decreased reactivities of peptides D_1 and D_3 compared to D_2 are perhaps due to the presence of the glucosamine-bound carbohydrate moiety and the presence of the carboxy-terminal region in peptide D_2. Peptides F_1 and F_2 are the most inhibitory, although they contain only one difuslfide bond compared to those found in D_1 or D_3. It appears, therefore, that the core antigenic region must contain the residues 21-31 of the linear amino acid sequence, the carbohydrate moiety, and at least one disulfide bond connecting the cysteine residue at position 23 or 26 with a corresponding residue at position 72 or 110. The carbohydrate moiety by itself is not antigenic, as removal of the carbohydrate does not seem to abolish immunologic reactivity of β-hCG (Moyle et al., 1975). However, it is likely that the presence of the bulky carbohydrate moiety helps to retain the conformation necessary to immunologic reactivity of the β subunit of hCG. It is quite possible that there may be other antigenic sites in the regions of disulfide bonds other than those connecting residues 23, 26, 72, and 110.

It is of interest to note that anti-β-hCG serum on immunoabsorption with hLH becomes more specific to hCG and shows little or no reactivity with LH. Peptides D_2, F_1, and F_2 inhibit the binding of hCG by the absorbed antiserum to a greater degree than do D_1 and D_3. It appears, therefore, that in spite of structural similarities some of the antigenic sites on the β subunit are not exposed in intact LH, while they are available in intact hCG. The recognition of β-LH by the hLH immunoabsorbed antiserum supports this thesis.

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Unusual Organization of DNA Sequences in the Chicken†

Francine C. Eden* and John P. Hendrick

ABSTRACT: Single-copy and repeated sequences in the chicken genome were identified by measuring the reassociation kinetics of short fragments of DNA by hydroxylapatite chromatography. Eighty-seven percent of the DNA is single copy and 13% is repetitive. The repetitive DNA can be divided into two classes based on repetition frequency; about one-third and two-thirds of the sequences are repeated 15- and 1500-fold, respectively. The arrangement of single-copy and repeated sequences was determined by measuring the fraction of labeled DNA fragments bound to hydroxylapatite by repetitive duplexes as a function of the DNA fragment length. DNA sequence organization was also studied by electron microscopy of reassociated DNA. The results indicate that 40% of the chicken genome is arranged in an alternating pattern of single-copy and repetitive sequences, with single-copy regions about 4500 nucleotides in length. A smaller fraction of the genome has this same alternating arrangement but contains even longer single-copy regions. Almost one half of the DNA consists of very long single-copy regions, uninterrupted by repeated sequences for distances of at least 17 500 nucleotides. To measure the lengths of repeated sequences directly, long fragments of chicken DNA were reassociated until the repeated sequences had formed duplexes, but the single copy sequences had not yet done so. Single strands were digested away using S1 nuclease, and the lengths of repetitive duplexes were measured by gel-filtration chromatography. Most chicken repeated sequences are at least 2000 nucleotides long. These characteristics place the chicken genome in a category intermediate between the extremes of sequence organization previously described.

Exploration of DNA sequence organization in a number of eukaryotic genomes has revealed a regular alternating arrangement of repetitive and single-copy sequence elements. A short-period interspersion pattern, in which single-copy sequences 1000-1500 nucleotides in length are flanked by repetitive sequences 300 nucleotides in length, characterizes the genomes of a number of animals (Davidson et al., 1973; Graham et al., 1974; Angerer et al., 1975; Firtel and Hindle, 1975; Schmid and Deininger, 1975; Efstratiadis et al., 1976; Crain et al., 1976b; Smith and Boal, 1978) and plants (Zimmerman and Goldberg, 1977; Walbot and Dure, 1976). This pattern of sequence organization is represented widely in both vertebrate and invertebrate phyla (Davidson et al., 1975).

A few clear exceptions to this general pattern have been discovered. For example, the genomes of three insects, *Drosophila melanogaster* (Manning et al., 1975; Crain et al., 1976a), *Chironomus tentans* (Wells et al., 1976), and the honeybee *Apis mellifera* (Crain et al., 1976a) and the mold *Achlya* (Hudspeth et al., 1977) are characterized by long-period interspersion, in which single-copy regions are uninterrupted by repeated sequences for distances of many thousands of nucleotides. In the genome of *Drosophila melanogaster,* the repetitive sequences average 3500 nucleotides in length, and the average single-copy length exceeds 13 000 nucleotides (Manning et al., 1975). Although most of these examples of long-period interspersion occur in insects, the genomes of two other insect species, the silkmoth *Antheraea pernyi* and the housefly *Musca domestica,* contain short-period interspersion as the predominant pattern (Efstratiadis et al., 1976; Crain et al., 1976b). The occurrence of both long- and short-period interspersion even within the same insect order (*Drosophila* and *Musca* are both Diptera) suggests that patterns of DNA sequence arrangement may undergo rapid evolutionary change.

The occurrence of these contrasting patterns of DNA sequence organization has provided the impetus for a detailed investigation of other animal genomes. Many specific issues can be addressed by extending our present knowledge of DNA sequence organization. The broad phylogenetic occurrence of short-period interspersion and its striking similarity in diverse animal species suggest that this pattern arose shortly after the inception of metazoan life and confers some evolutionary advantage. However, relatively few individual species have been

† From the Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received July 27, 1978.*