"BIG" HUMAN PLACENTAL LACTOGEN: DISULFIDE-LINKED PEPTIDE CHAINS*

by

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"Big" human placental lactogen has been purified by affinity chromatography. On SDS[1]-polyacrylamide disc gel electrophoresis, "big" hPL had a molecular weight of about 45,000. Following reduction with mercaptoethanol, a single band with a molecular weight of 23,000 was noted. These observations suggest that "big" hPL consists of two peptide chains linked by a disulfide bond.

In extracts of normal human placenta and in sera from pregnant women, there is a form of hPL[1] whose chromatographic behavior indicates it to be of larger molecular weight than the major form of the hormone (1). Similar observations have been made for pituitary growth hormone (2,3) and prolactin (4). The physiologic significance of these "big" hormones has not yet been determined although it has been speculated that they are precursors in the synthesis of the native hormones. Earlier studies indicated that "big" hPL did not arise as an artifact of extraction and purification; it was also not a simple aggregate since 75% was stable in strong denaturants like 8M urea (5). Since "big" hPL could be isolated in quantities sufficient to

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[1]Abbreviations: Sodium dodecyl sulfate, SDS; Human placental lactogen, hPL.

perform direct chemical analysis, studies on the structure of "big" hPL were initiated. This report provides evidence that "big" hPL is a covalently linked disulfide-dimer of peptide chains most likely identical to native hPL.

METHODS

Preparation of "big" hPL. Freshly delivered human placentas were obtained after spontaneous deliveries resulting in normal infants. The membranes were removed and the tissue minced with scissors. Homogenization on ice was carried out with a Polytron type 200D (Brinkman) at a setting of 7 for two-one minute periods. The homogenate was stirred at 4°C for 90 minutes before centrifugation at 500 x g for 10 minutes and at 60,000 x g for 15 minutes. The supernatant fluid was then subjected to gel filtration (x2) over a Sephadex G-100 column in 0.05M ammonium bicarbonate followed by ion exchange chromatography over DE-52 (Whatman) with the same buffer. The first Sephadex G-100 column was increased in size to 15 x 120 cm to handle the large protein load. Details of the purification procedure are presented elsewhere (5). The nomenclature adopted for the various molecular species observed refers to their elution properties on Sephadex G-100. The predominant form of hPL, molecular weight 22,000, is referred to as native or "little" hPL. "Big" hPL refers to the species which is less retarded than native hPL and has a molecular weight about twice as large; the latter elutes between hPL and the void volume. "Little derived from big" hPL refers to the species which are obtained from "big" hPL by denaturation or disulfide bond reduction and which elute at a position analogous to native hPL. These species may, in fact, be identical with each other and with native hPL, but until this is established, "little derived from big" hPL needs to be indicated separately.

Radioimmunoassay of hPL. The radioimmunoassay of hPL was performed by the double antibody method as described previously (5,6).

Affinity Chromatography. Affinity chromatography of partially purified "big" hPL after DE-52 chromatography was carried out according to the method of Hwang et al (7). Anti-hPL serum was prepared in rabbits with antigen that was purified by Sephadex chromatography. Specific anti-hPL antibodies were concentrated by passage of the antiserum over a column of hPL linked to Sepharose 4B by the cyanogen bromide method (8). The retained antibodies were eluted with 4M NaSCN in 0.01 M phosphate 0.15 M NaCl (pH 7.4) and then coupled to Sepharose 4B. "Big" hPL was passed over the column and was eluted with 4M NaSCN. Fractions containing immunoreactive hPL were dialyzed against 0.05 M ammonium bicarbonate and concentrated by lyophilization. Since this affinity step involved denaturing conditions, a final separation on Sephadex G-100 in 0.05 M ammonium bicarbonate was performed.

SDS-disc gel electrophoresis. Polyacrylamide disc gel electrophoresis in SDS was carried out according to the method of Weber et al (9). All proteins used to calibrate molecular weight were reduced by heating for 1 minute at 100°C in 1% mercaptoethanol - SDS.

RESULTS

Affinity chromatography of "big" hPL. Affinity chromatography of "big" hPL was performed in order to obtain sufficiently pure hormone to estimate

its molecular weight by the SDS-polyacrylamide technique. This was possible because the antisera raised against hPL reacted identically with both "big" and "little" hPL (5). Prior to affinity chromatography, the partially purified material had a single peak of immunologic activity in the position of "big" hPL (Fig. 1, upper panel).

When the affinity column was eluted with 4M NaSCN, the recovery of "big" hPL was between 70 and 80%. We had previously observed that 8M urea converted about 25% of "big" hPL to a species the size of the native hormone. Since 4M NaSCN is an effective denaturant (10), the material recovered from affinity chromatography was rerun over Sephadex G-100. In agreement with the results obtained with 8M urea, a portion of the "big" hPL was converted to hPL by the denaturing conditions (Fig. 1, lower panel). The fractions comprising stable "big" hPL were combined, concentrated by lyophilization, and used for subsequent polyacrylamide electrophoresis.

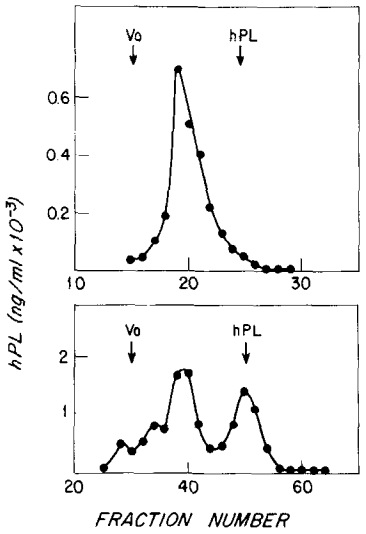

Fig. 1. Sephadex G-100 chromatography of DE-52 purified-(upper panel) and affinity chromatography purified-(lower panel) "big" hPL. Affinity chromatography was performed on "big" hPL which had been purified over two Sephadex G-100 columns and DE-52 ion exchange chromatography. The position of Vo was determined with blue dextran and of hPL with ^{125}I-hPL ("b" hPL).

Exposure of hPL to reducing agents in the presence of urea or guanidine results in a reduction of both disulfide bonds with the loss of most of the immunologic activity (11). Under non-denaturing conditions (i.e. in the absence of denaturants and room temperature), only a single disulfide bond is reduced (12) and immunologic activity is retained (Table I). The immunologic activity of "big" hPL is also retained after reduction under non-denaturing conditions (Table I).

The position of "big" hPL on Sephadex G-100 following reduction under non-denaturing conditions (2% mercaptoethanol, 0.2% human albumin, in 0.05 ammonium carbonate buffer at 24°C for 16 hours) is shown in Fig. 2. All of the activity was recovered in the area of "little" hPL, with none remaining in the position of "big" hormone. The 60% recovery of hPL by radioimmunoassay from this column was consistent with the recovery experienced in other purification procedures where carrier protein was absent.

Table I

Effect of reduction on the immunologic activity of hPL and "big" hPL.

Experimental Conditions	Concentration by Radioimmunoassay	Immunologic Activity Retained
	(ng/ml ± S.E.M.)	(% of Control)
hPL - Control	745 ± 20	
hPL - Reduced 24°C	785 ± 6	105
"big" hPL - Control	46.7 ± 0.9	
"big" hPL - Reduced 24°C	42.4 ± 2.3	91

Reduction of protein was performed in 0.05 M ammonium carbonate, and 2% mercaptoethanol for 16 hours at 24°C.

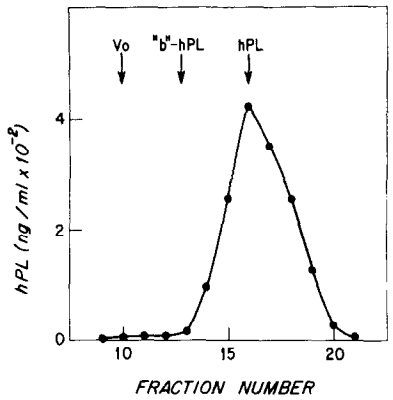

Fig. 2. Sephadex G-100 chromatography of "big" hPL after reduction under nondenaturing conditions. The position of "big" hPL was determined by passage of unreduced "big" hPL through the column.

Polyacrylamide disc gel electrophoresis of "big" hPL. "Big" hPL purified by affinity chromatography showed a single major band on polyacrylamide electrophoresis with SDS, with no protein being present in the area of native hPL. The mobility of "big" hPL relative to several reduced standard proteins is shown in Fig. 3. Its apparent molecular weight is 45,000 which is an approximation because an accurate determination by this method requires complete reduction and unfolding (13). Following reduction with mercaptoethanol in SDS, the "big" hPL band was completely shifted to a position corresponding to "little' hPL. "Little derived from "big" hPL which was recovered following affinity chromatography was also run on SDS-polyacrylamide electrophoresis; its position was the same as that of reduced "big" hPL.

DISCUSSION

"Big" hPL is found in extracts of normal human placenta and the sera of pregnant women; it appears to be analogous to "big" growth hormone (2,3) and "big" prolactin (4). Previous experiments indicate that it is not produced by the methods of extraction but is a "physiologically" occurring form of the hormone (5).

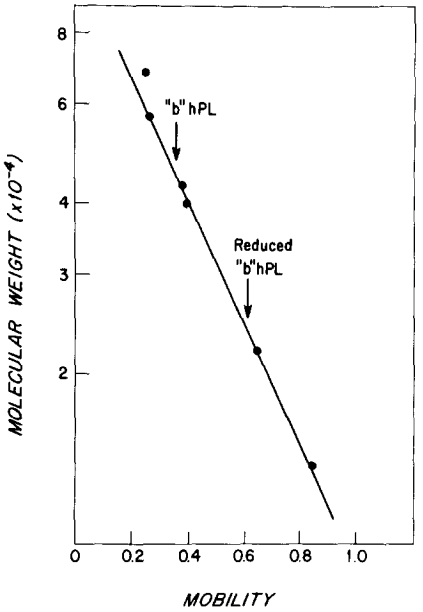

Fig. 3. SDS-polyacrylamide disc gel electrophoresis of unreduced and reduced "big" hPL. Reduction of "big" hPL and the standard proteins ribonuclease, hPL, aldolase, ovalbumin, pyruvate kinase, and human serum albumin, was carried out by boiling in 1% mercaptoethanol-SDS for 1 minute.

Some structural information about "big" hPL can be inferred from its previously described properties. It coelutes with hPL from an anion exchange resin at pH 7.8 and gives identical displacement curves in the radioimmunoassay. Furthermore, both "big" and "little" hPL give parallel dose response curves in the binding assay with mid-pregnant rabbit mammary gland membranes[2] (14) and in the induction of N-acetyllactosamine synthetase activity[2] (15). Taken together, these findings indicate a high degree of similarity between hPL and "big" hPL.

The present results indicate that "big" hPL is composed of two peptide chains. These are likely to be identical subunits since reduction leads to a single band at approximately half the original molecular weight, but it

2. Manuscript in preparation.

remains to be determined that these subunits are identical to native hPL. Since one of the two disulfide bonds can be reduced without unfolding the native hPL molecule, it is likely that the same one is involved in this interpeptide bond. Since the location of the disulfide bonds is similar in hPL and growth hormone (16), the same interpeptide bond may be involved in producing other "big" hormones of the growth hormone-prolactin family.

"Big" hPL is not analogous, therefore, to prohormones such as proinsulin or proparathyroid hormone. Rather, it is a disulfide-linked dimer of peptide chains most likely identical to monomeric hPL. Biosynthetic studies are necessary to define its physiologic role in the placenta and indicate why it is synthesized and released into the circulation. The methods of purification described and the relatively large amounts of "big" hPL present in placenta should provide sufficient material for such structural and physiologic analyses.

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