PURIFICATION OF A LACTOGENIC HORMONE IN SHEEP PLACENTA

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SUMMARY

An ovine placental lactogenic hormone (OPL) was purified from placentas. Principal steps of purification were : saline extraction at pH 9.5, acid precipitation at pH 4.5, ammonium sulfate precipitation, DEAE Sephadex chromatography and gel filtration on Sephadex G75 S.F. This hormone is purified about 1000 fold. It is not contaminated either by prolactin or by growth hormone. Its activity estimated by radioreceptor assay and biological tests was equivalent to 15 IU/mg of NIH ovine prolactin standard. Apparent molecular weight of OPL was about 20,000-22,000 and its pI was about 7.2 . Isohormones and prohormone phenomenons have been observed. The purified OPL showed no immunological cross-reaction with ovine prolactin, human and ovine growth hormone and human placental lactogen. Mammotrophic and lactogenic activities have been discussed.

INTRODUCTION

A placental lactogenic activity has been already found in Rodents and Primates (1). Human placental lactogen (HPL) has been highly purified and its primary sequence established (2). In Ruminants, the presence of a placental lactogenic activity was suggested in 1961 by DENAMUR and MARTINET (3) and was recently proven by FORSYTH (4,5) by means of cocultures of placenta and mammary gland. The present paper describes the isolation of this hormone together with some of its biophysical, biochemical and immunological properties.

MATERIAL AND METHODS

A) Radioreceptor assay (6,7) : Membrane proteins (200 µg/sample obtained from rabbit lactating mammary gland (after centrifugation at 90,000g, 90 mn) were incubated for 16 h at 4°C with 10^5 cpm of iodinated prolactin (80 µCi/µg) (8) and increasing NIH S7 prolactin concentrations (24 IU/mg) for the standard curve. Estimation of placental lactogenic activity was obtained by incubating samples of the placental extract in the place of the unlabelled prolactin.

B) Purification of OPL :

Placentas were obtained from sheeps at 100-120 days of pregnancy (9). Foetal cotyledons were thoroughly blotted and frozen at - 15°C. All steps of purification were carried out at 4°C.

Extraction : About 500 gm of cotyledons were thawed and homogenized in 0.01 M phosphate buffer pH 7.6 , 0.3 M KCl (Ultra-Turrax and Potter Elvehjem), filtered on cheese-cloth, stirred at pH 9.5 for 4 h and centrifuged. The pellet was washed with the same buffer and centrifuged again.

Precipitations : The pooled supernatants were adjusted to pH 4.5 and left overnight. After centrifugation (12,000 g, 30 mn) the resulting supernatant was adjusted to pH 7 and brought to 50 % saturation with $(NH_4)_2 SO_4$. The precipitate was removed, dissolved in 10 mM sodium borate buffer pH 8.6 and dialyzed against the same buffer for 16 h.

DEAE Sephadex chromatography : The dialyzed product was applied on a DEAE Sephadex A25 (Pharmacia) column (8 x 20 cm) equilibrated with the previous buffer and eluted by 0.1 M KCl in sodium borate buffer.

Gel filtration on Sephadex G75 : After lyophilization, OPL enriched fractions were applied on a Sephadex G75 S.F. (Pharmacia) preparative column (4 x 100 cm) equilibrated with 25 mM ammonium bicarbonate buffer. The more active and homogeneous material was run again on a Sephadex G75 S.F. column (1.5 x 90 cm).

C) Analytical techniques :

Polyacrylamide gel electrophoresis was routinely performed according to the method of ORNSTEIN (10) and DAVIS (11) at pH 9.6.

SDS-polyacrylamide gel electrophoresis was employed as used by WEBER and OSBORN (12).

Gel filtration was performed on an Ultrogel Ac-A 5-4 (LKB) column (1.5 x 90 cm) equilibrated with 25 mM ammonium bicarbonate buffer, 0.4 M KCl.

Isoelectric focusing : LKB 8102 Ampholine electrofocusing equipment (440 ml) was used. Carrier Ampholine with a pH range of 5-8 was used at a final concentration of 1 %. A partially purified active material (peak B, fig. 3) was used. Electrofocusing was run for 48 h at 800 V.

RESULTS

1) Isolation : Table I summarizes the various purification steps described. Lactogenic activity was not adsorbed by DEAE Sephadex columns which retained nucleic acids and hemoglobin. During gel filtration on Sephadex G75, OPL was eluted after the residual hemoglobin (fig. 1). At this step, in addition of

Table I - Main results of the OPL purification steps

Steps of purification	Proteins (mg)	Total OPL activity(2) (mg)	Recovery of total activity %	Specific activity(3)	Purification
Crude extract	54,000(1)	31	100	0.57	1
Precipitation at pH 4.5	14,750	33.5	108	2.3	4
$(NH_4)_2 SO_4$ precipitation	2,275	20.6	61.5	9	15.6
DEAE Sephadex	210	12.25	59	58	100
1st Sephadex G75	30	5.5	45	183	320
2nd Sephadex G75	5	3	55	600	1056

(1) approximately 10 % of wet tissue weight
(2) NIH ovine prolactin mg equivalents (PS7 24IU/mg)
(3) ovine prolactin mg equivalents/protein gm

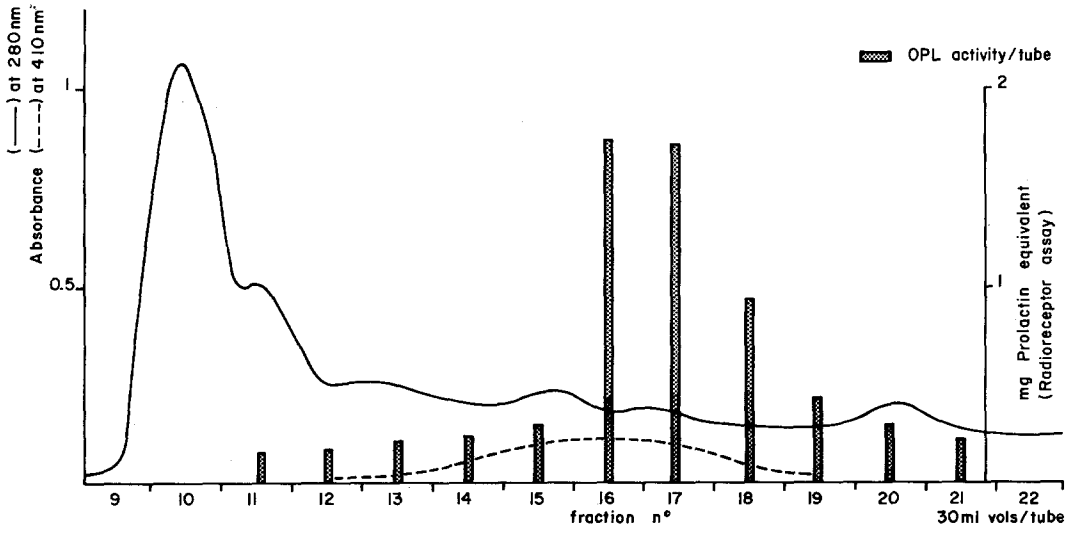

Fig. 1 - Purification of material from DEAE Sephadex chromatography by gel filtration through a Sephadex G75 S.F. column.

radioreceptor assay for activity, OPL enriched fractions were tested by disc electrophoresis for homogeneity before any further purification. The lactogenic activity was confirmed in vitro by lactose and casein synthesis assays (13,14).

When compared to the crude extract, OPL was purified about 1000 fold (table I). It was not contaminated by prolactin measured by radioimmunoassay (8). Its activity was equivalent to about 15 IU/mg of the NIH ovine prolactin standard.

When no DEAE Sephadex chromatography step was included in the purification schedule, the material in the main peak (peak B, fig. 3) from the Sephadex G100 column was found heterogeneous by disc electrophoresis (fig. 5). Moreover a small lactogenic activity was present in the material excluded from the gel.

2) Biochemical characteristics : as shown in the fig. 4, only one band was found when the more purified preparation of OPL was analyzed by disc electrophoresis. OPL and OGH migrated

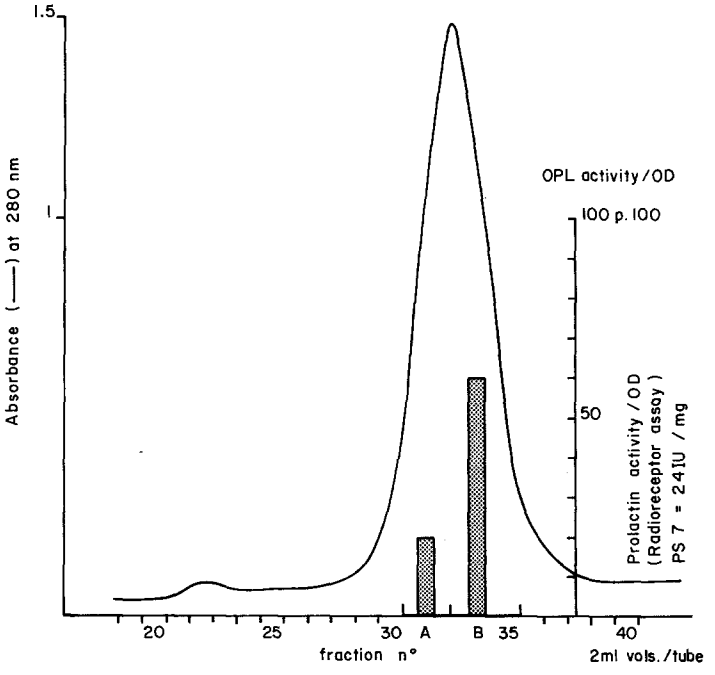

Fig. 2 - Purification of material from gel filtration Sephadex G75 S.F. by gel filtration through a Sephadex G75 S.F. column.

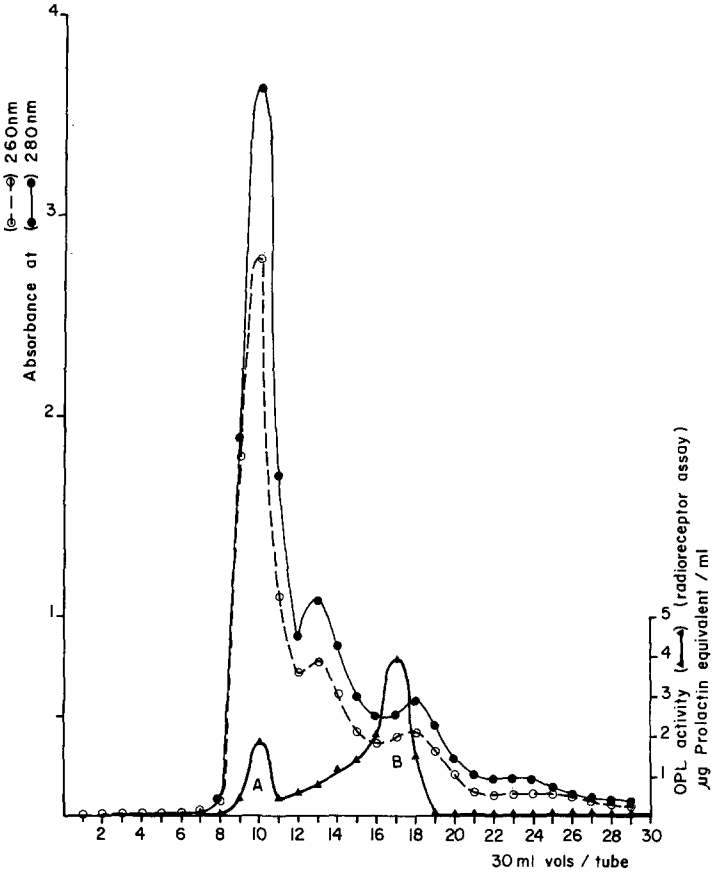

Fig. 3 - Purification of material from $(NH_4)_2 SO_4$ precipitation by gel filtration through a Sephadex G100 column equilibrated with 50 mM ammonium bicarbonate buffer.

with an identical mobility and more slowly than ovine prolactin and HPL. When a large quantity of OPL was applied to the gels (peak I, fig. 5), an isohormone pattern was observed as found with BGH, ovine prolactin and HPL (fig. 4) (15).

When the material of the peak B (fig. 3) was studied by isoelectric focusing, the lactogenic activity was only located in peak I corresponding to an apparent isoelectric point of 7.2 . Disc electrophoresis analyses (fig. 5) of the different fractions obtained after electrofocusing experiments unambiguously showed that the major band present in the peak I had the same mobility as purified OPL (fig. 4).

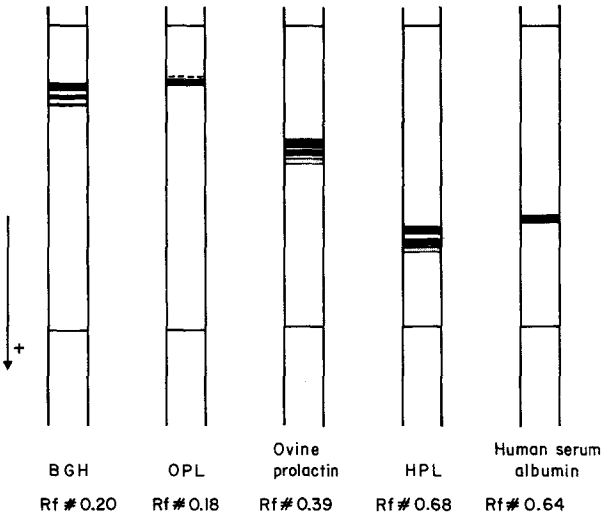

Fig. 4 - Comparative disc electrophoretic patterns at pH 9.6 of OPL, HPL, BGH and ovine prolactin.

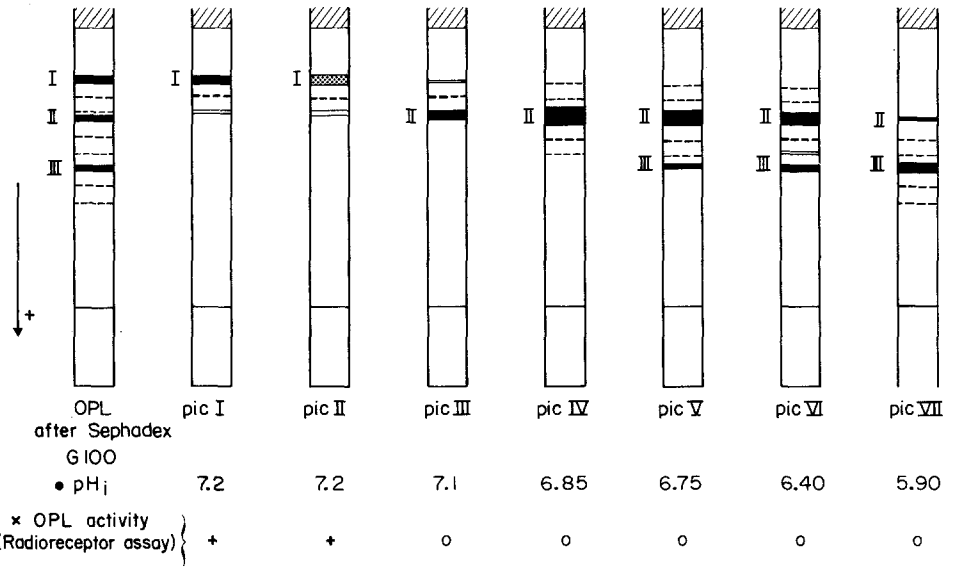

Fig. 5 - Analytical disc electrophoresis of fractions from isoelectric focusing.

Apparent molecular weight of OPL was about 19,000-22,000 determined by Ultrogel filtration and was about 20,000-22,000 by SDS disc electrophoresis (fig. 6).

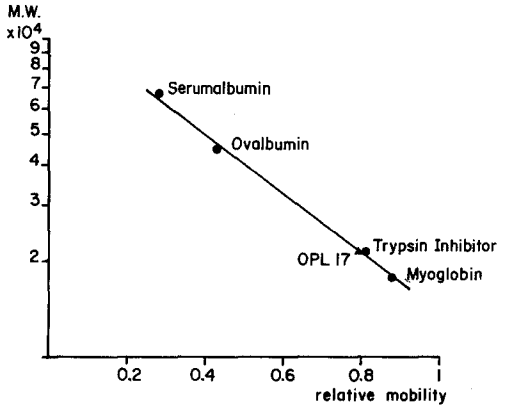

Fig. 6 - Molecular weight estimation by electrophoresis in sodium dodecyl sulfate - polyacrylamide gels. Plot of protein migration distance as a function of molecular weight.

3) Immunological properties are summarized on table II. These properties of OPL were studied by immunoprecipitation in tubes and in agar plates and, in addition, by radioimmunoassay for prolactin (8). At the different OPL concentrations studied (20 µg to 200 µg) OPL did not react with antiserum against ovine prolactin, HGH, HPL, OGH and BGH. Only few OPL lots cross-reacted with antiserum against OGH suggesting an OGH contamination of these preparations.

DISCUSSION

Because OPL placental concentrations are particularly high on days 100-120 of pregnancy (9) this period has been chosen for taking placentas.

OPL seems stable at pH 4.5, lactogenic activity being completly retained after 16 h under these conditions (table I). The presence of a lactogenic activity in the material excluded from Sephadex G100 column (peak A, fig. 3) might suggest the intracellular presence of a prohormone as described for different lactogenic hormones (HGH, HPL, prolactin) (16).

The apparent isoelectric point value of 7.2 determined by electrofocusing agrees with the results of electrophoresis and DEAE or CM Sephadex chromatography. The apparent pI value of OPL is very close to the pI of OGH and very different from

Table II - Immunological properties of OPL

	Anti-OPL antiserum	Anti-ovine prolactin antiserum	Anti-HPL antiserum	Anti-HGH antiserum	Anti-OGH antiserum
OPL A (placenta at day 100 of pregnancy)	+	-	-	-	-
OPL B (placenta at term)	+	-	-	-	+
OPL 17 (placenta at day 100 of pregnancy)	+	-	-	-	-

Results of immunoprecipitation in tubes and in double immunodiffusion (method of Ouchterlony) between some preparations of purified OPL (lots A, B, 17) and antiserum against pituitary or placental hormones.

ovine prolactin, HGH or HPL. The apparent molecular weight of OPL (20,000-22,000) is close of the monomeric forms of growth hormones and lactogenic hormones (prolactin and HPL). But only structural studies will be able to prove the presence of identical sequences in these different molecules.

No immunological cross-reaction is seen between OPL and ovine prolactin, HPL, HGH, BGH, OGH. The cross-reaction of some of the OPL lots with OGH may be explained by the high levels of the latter hormone in sheep foetuses (about 100 ng/ml serum) (17) whereas it is known that in the blood of the pregnant sheep OPL levels raise to high values (about 500 ng/ml) (6,7) but OGH levels remain low (about 1 ng/ml) (17,18). Since OPL and OGH have nearly the same pI and molecular weight, the high initial contaminations of the former hormone with OGH might to a certain extent persist through the various purification steps. However, as opposed to the results of a recent communication (19) most of the lots obtained in the present work appeared to be completly devoid of OGH.

OPL lactogenic activities, as measured against equivalent NIH ovine prolactin by heterologous (rabbit) or homologous

(sheep) radioreceptor assays, are identical. OPL activities measured by radioreceptor assay and by biological assays (lactose and casein syntheses) are also equivalent (13,14). In vitro, in cultures of mammary tissues explanted from pseudopregnant rabbits, OPL induces a lactogenesis which may be demonstrated either histologically or by induction of lactose and casein syntheses (13,14).

In the sheep, the appearance of lactose in mammary gland (lactogenesis) (20) and the important increase of OPL concentrations (6,7,9) take place on about days 95-100 of pregnancy suggesting that OPL is directly involved in lactogenesis. Moreover, hypophysectomy (3) or injections of 2-bromo-α-ergocryptine during pregnancy in the sheep (unpublished results), show that some mammary growth and lactose synthesis are possible in the absence of prolactin. Hence the name of ovine placental lactogen (OPL) seems justified.

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