Purification of ovine placental lactogen (oPL) using high-performance liquid chromatography

Evidence for two forms of oPL

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After initial purification of ovine placental lactogen (oPL) using the procedures described previously [(1976) Endocrinology 98, 65–75], the oPL preparation was further purified by high-performance liquid chromatography (HPLC) using an anionic exchange column (Bio-Sil TSK DEAE-2-SW). Two forms of oPL with different relative mobilities on HPLC were isolated and designated oPL-I and oPL-II. Subsequent analysis by polyacrylamide gel electrophoresis containing SDS revealed that oPL-I and oPL-II are nearly homogeneous (greater than 90% pure) and are identical in apparent M_r (approx. 22 000–23 000). Like human growth hormone (hGH), oPL-I and oPL-II are equally active in the radioreceptor assays for growth hormone-like activity (RRA-GH) and for prolactin-like activity (RRA-RRL). In the radioimmunoassay of oPL, both oPL-I and oPL-II are immunologically similar. Analysis of amino acid composition revealed that oPL-I and oPL-II consist of 199 and 196 residues, respectively, and have almost identical residues except that oPL-I has a higher content of glycine. Furthermore, both oPLs have a general similarity in amino acid composition to oGH and oPRL except for a lower content of methionine and leucine but with a higher content of lysine. Our studies demonstrated the presence of two similar forms of oPL. Whether these two similar forms of oPL share identical primary structure remains to be determined.

HPLC *Ovine placental lactogen*

1. INTRODUCTION

Ovine placental lactogen (oPL) is a polypeptide hormone which has been previously isolated [1–4]. It has been demonstrated that oPL has potent somatotropic and mammotropic activity in various bioassays [1–10]. Furthermore, it has been shown that oPL inhibits glycogenolysis in fetal rat liver [11]. We have also shown that oPL binds specifically to growth hormone receptor in ovine maternal and fetal tissues but not to lactogenic hormone receptors [12]. Thus, these studies suggested that oPL may play a physiological role in mammogenesis, in amino acid and protein metabolism during pregnancy. It may also play an important role in the maintenance of normal pregnancy and the support of fetal growth during gestation. On the other hand, little information is available on the chemistry of oPL. The only studies, performed by Hurley et al. [13], showed that oPL is similar to ovine growth hormone (oGH) and ovine prolactin (oPRL) in amino acid composition. The main obstacle to studies on the primary sequence of oPL is that it is very difficult to obtain a sufficient quantity of oPL pure enough for chemical characterization. This paper describes the use of high-performance liquid chromatography (HPLC) as a final procedure during the purification of oPL. By employing this method, one could obtain a nearly homogeneous oPL preparation.

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2. MATERIALS AND METHODS

2.1. *Hormone preparations*

All hormone preparations listed below, with the exception of oPL, were kindly supplied by the NIAMDD of the National Institutes of Health (Bethesda, MD): human growth hormone (NIADDK-hGH-I-1), ovine growth hormone (NIAMDD-oGH-12 and NIADDK-oGH-I-3), ovine prolactin (NIADDK-oPRL-17 and NIAMDD-oPRL-I-1), and human placental lactogen (hPL).

2.2. *Purification of oPL*

oPL was prepared according to [1], with slight modifications. 2–4 mg oPL (>1500-fold purification) were purified from 2–3 kg mid-term ovine placenta. The oPL preparation was further purified by HPLC using an anionic exchange column (Bio-Sil TSK DEAE-2-SW, Bio-Rad). A linear gradient of 0–25% of 0.5 M NaCl in 0.025 M Tris-HCl, pH 8.0, over 200 min was employed. Fractions of 0.5 ml were collected and directly assayed for growth hormone-like activity using radioreceptor assay for GH (RRA-GH) [16]. The fractions containing GH-like activity were appropriately pooled, desalted on a Sephadex G-15 column and finally lyophilized.

2.3. *Characterization of oPL*

2.3.1. Polyacrylamide gel electrophoresis

The purified oPL preparations were analyzed by polyacrylamide gel electrophoresis containing SDS using a linear gradient of 10–15% polyacrylamide gel [14,15]. The protein standard markers (low M_r) were purchased from Pharmacia.

2.3.2. Amino acid composition analysis

After hydrolysis of oPL for 24, 48 and 72 h in 6 N HCl at 110°C under reduced pressure, the amino acid composition was determined on a Beckman 121M microcolumn amino acid analyzer. The half-cystine content was determined as cysteic acid following oxidation with performic acid [16]; tryptophan was determined by the method of Edelhoch [17].

2.3.3. Radioreceptor assays

The binding activities of the oPL preparations were examined in the radioreceptor assay for growth hormone (RRA-GH) [18] and prolactin (RRA-PRL) [19].

2.3.4. Radioimmunoassay (RIA)

The double-antibody RIA procedure was similar to that which we described previously for oPL [20], with slight modifications using ^{125}I-iodinated oPL-I or ^{125}I-iodinated oPL-II as tracers.

All iodinations were performed by a slight modification of the lactoperoxidase method of Thorell and Johansson [21].

3. RESULTS

Fig.1 shows the elution profile of partially purified oPL preparations on HPLC using anionic exchange column. It is apparent that two major peaks of growth hormone-like (GH-like) activity corresponding to the two major protein peaks (monitored by absorbance at 280 nm) were observed. These two peaks of GH-like activity were designated oPL-I and oPL-II in ascending order.

Analysis of the oPL preparations by polyacrylamide gel electrophoresis (10–15% gel) containing SDS, as shown in fig.2, revealed that the oPL preparation without the HPLC purification step (lanes B,C) is approx. 65–75% pure as shown by the staining intensity of the protein band. After further purification by HPLC, the oPL preparations (oPL-I, oPL-II) were nearly homogeneous as shown in lanes D and E. The apparent M_r values of oPL-I and oPL-II were calculated to be approx. 22000–23000, which are identical to that of the hPL standard. However,

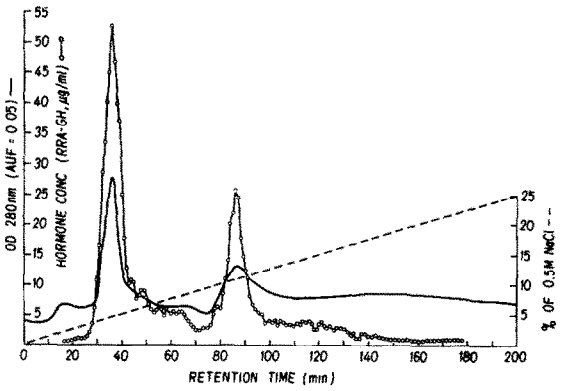

Fig.1. HPLC of oPL preparation using anionic exchange column (Bio-Sil TSK DEAE-2-SW, Bio-Rad).

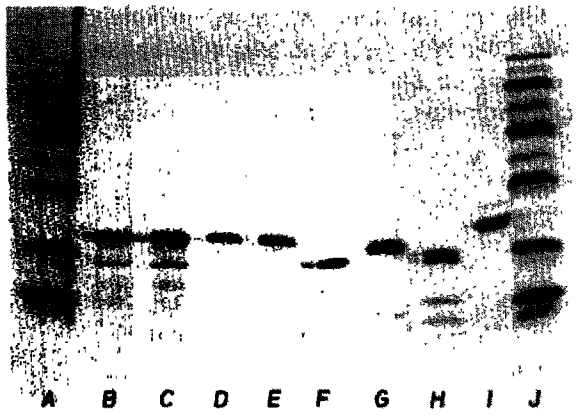

Fig.2. Polyacrylamide gel electrophoresis (10–15% gradient gel) containing SDS. Lanes: A,J, Pharmacia low-M_r standard; B, oPL without purification on HPLC, 10 μg; C, oPL without purification on HPLC, 20 μg; D, oPL-I, 10 μg; E, oPL-II, 20 μg; F, hGH NIADDK-hGH-I-1, 10 μg; G, hPL NIAMDD, 20 μg; H, oGH NIAMDD-oGH-12, 20 μg; I, oPRL NIADDK-oPRL-17, 20 μg.

there was no apparent difference in M_r between oPL-I and oPL-II on analysis by this method.

The amino acid compositions of oPL-I and oPL-II are compared to those of oPL isolated by Hurley et al. [13], oGH, oPRL and hPL as shown in table 1. It is apparent that oPL-I and oPL-II

Table 1

Amino acid composition of oPL, oGH, oPRL and hPL[a]

Amino acid	oPL-I	oPL-II	oPL[b]	oPRL[c]	oGH[d]	hPL[e]
Lys	14.6 (15)	16.3 (16)	14	9	13	9
His	5.9 (6)	6.4 (6)	4	8	3	7
Arg	9.7 (10)	9.7 (10)	10	11	13	11
Asp	18.3 (18)	18.5 (19)	19	22	16	22
Thr	11.6 (12)[f]	12.2 (12)[f]	10	9	12	12
Ser	16.9 (17)[f]	16.5 (17)[f]	15	15	12	18
Glu	24 (24)	24 (24)	24	23	25	25
Pro	9.9 (10)	9.6 (10)	10	11	8	5
Gly	14.7 (15)	10.2 (10)	15	11	10	7
Ala	12.2 (12)	11.7 (12)	13	9	14	6
½-cystine	6.4 (6)[g]	N.D.	6	6	4	4
Val	10.7 (11)	12.3 (12)	12	10	7	7
Met	1.5 (2)	1.7 (2)	5	7	4	6
Ile	10.0 (10)	11.3 (11)	10	11	7	7
Leu	17.1 (17)	15.8 (16)	13	22	22	25
Tyr	5.5 (6)	6.2 (6)	4	7	6	8
Phe	5.9 (6)	6.4 (6)	7	6	13	11
Trp	2.4 (2)[h]	2.3 (2)[h]	2	2	1	1
Total	199	196	193	199	191	191
Calculated M_r	22103	21927	21418	22720	21806	21600

[a] Values for amino acids are expressed as residues per molecule with nearest integral numbers in parentheses
[b] From [13]
[c] From [22]
[d] From [23]
[e] From [24]
[f] Extrapolated to zero time of hydrolysis
[g] Determined as cysteic acid after oxidation with performic acid [16]
[h] Determined by the spectrophotometric method in [17]

have almost identical residues except that oPL-I has a slightly higher content of glycine. Our oPL preparations are similar to that of Hurley et al. [13] except that our oPLs have a slightly higher content of leucine and tyrosine and a lower amount of methionine residue. Although our oPLs are not identical with oPRL in amino acid composition, they are closer to oPRL than to oGH in content of histidine, arginine, proline, valine, isoleucine, phenylalanine and tryptophan. oPLs are closer to oGH in content of lysine, aspartic acid, threonine, methionine and tyrosine. In addi-

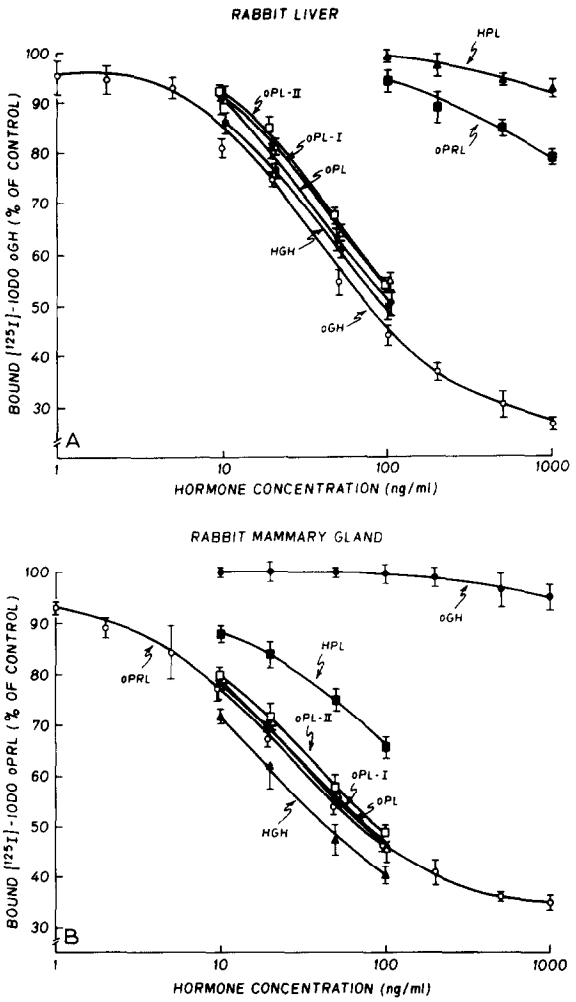

Fig.3. Radioreceptor assay for growth hormone-like activity (RRA-GH) using iodinated ovine growth hormone, oGH (NIADDK-oGH-I-3) and rabbit liver particulate fractions as tracer, standard and receptors, respectively (A). Radioreceptor assay for prolactin-like activity (RRA-PRL) using iodinated ovine prolactin, oPRL (NIAMDD-oPRL-I-1) and rabbit mammary gland particulate fractions as tracer, standard and receptors, respectively (B).

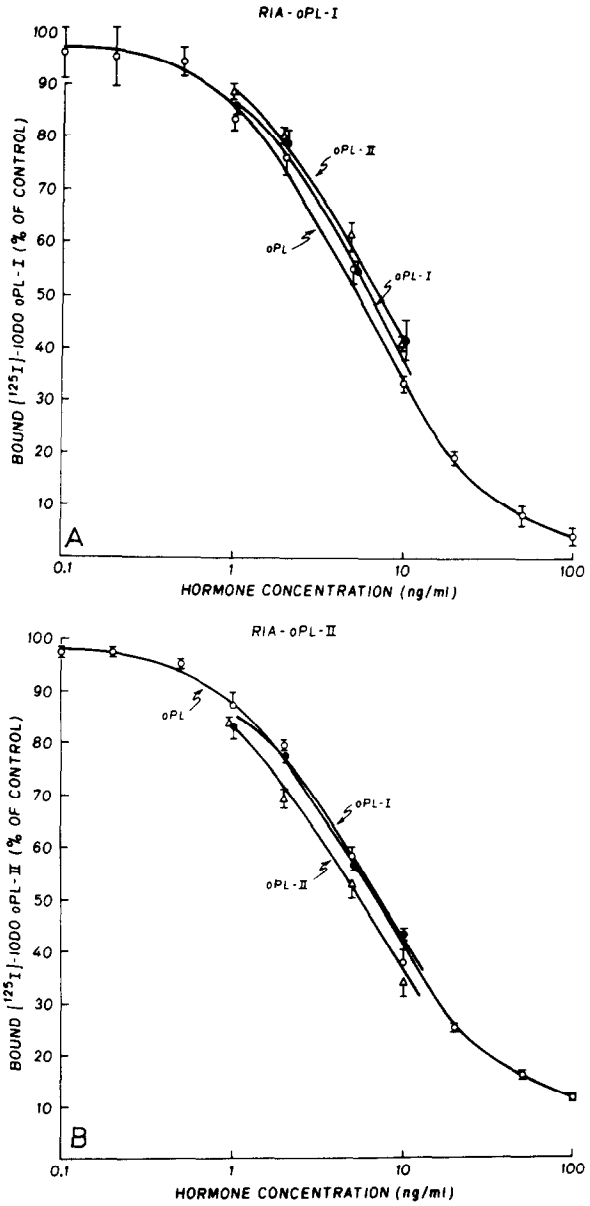

Fig.4. A double-antibody radioimmunoassay for oPL using iodinated oPL-I (A) and oPL-II (B) as tracer.

tion, our oPL preparations have almost no similarity to hPL except in content of histidine, arginine, threonine, serine and glutamic acid which are nearly the same. The estimated total number of residues in oPL-I and oPL-II are 199 and 196, respectively. The minimum M_r values of oPL-I and oPL-II, calculated from the amino acid composition, are 22103 and 21927, respectively.

When the binding activities of oPL-I and oPL-II are analyzed in RRA-GH and RRA-PRL as shown in fig.3A and B, respectively, it is evident that oPL-I and oPL-II, like hGH, are both active in RRA-GH and in RRA-PRL. Furthermore, both oPL-I and oPL-II were equally active in both assays.

Finally, oPL-I and oPL-II were tested in the RIA for oPL (antibodies raised in rabbits using oPL preparation without final purification by HPLC) using iodinated oPL-I and iodinated oPL-II as tracers as shown in fig.4A and B, respectively. These results showed that oPL-I and oPL-II were immunologically similar and equally potent in displacing the tracer.

4. DISCUSSION

By employing RRA-GH and/or RRA-PRL, various investigators have successfully isolated ovine placental lactogen from ovine placenta within the last 10 years [1–4]. To our knowledge, however, no investigator has reported the use of HPLC to purify oPL. Thus, this paper is the first report of the use of HPLC for purification of oPL. It is apparent that HPLC gives superior separation to conventional ion-exchange chromatography (i.e. using ion-exchange cellulose). One of the disadvantages of using HPLC, however, is the relative low yield of the purified oPL. In our hands, we consistently had no more than 10–20% recovery in dry weight and in GH-like activity by using HPLC. Thus, we could only obtain approx. 200–300 μg pure oPL from 2–3 kg ovine placenta (90–120 days gestation). On the other hand, we were very surprised that oPL was resolved into two forms, designated oPL-I and oPL-II. These two forms of oPL differ only in minor amino acid residues (table 1). These studies suggest that oPL-I and oPL-II are probably also identical in primary amino acid sequence with only a minor amino acid substitution. Nevertheless, more studies on the chemical structure of oPL-I and oPL-II are definitely required to answer this question.

It is interesting that both oPL-I and oPL-II were equally active in both RRA-GH and RRA-PRL (fig.3A,B). Furthermore, like hGH, both oPL-I and oPL-II were active in both RRAs, whereas oGH and oPRL were active in only one RRA. These studies suggest that oPL is more similar to hGH structurally than to oGH and/or oPRL. At present, we do not know why the oPL-I and oPL-II preparations were only as active as the oPL preparation without purification by HPLC. One possible explanation might be that, during the final purification procedure using HPLC, some of the oPL might have been denatured.

When tested in the RIA using iodinated oPL-I and oPL-II as tracers as shown in fig.4A and B, respectively, oPL-I and oPL-II were immunologically the same. Thus, these studies further support the hypothesis that, like hPLs which are derived from two identical mRNAs [25,26], oPL-I and oPL-II might also be derived from two similar (if not identical) mRNAs.

In conclusion, our studies demonstrate that oPL is purified to homogeneity and resolved into two forms by HPLC using an anionic exchange column. These two forms of oPL are similar in apparent M_r, amino acid composition and immunological properties. Finally, our receptor-binding studies demonstrate that oPL-I and oPL-II are equally active in both RRA-GH and RRA-PRL, suggesting that these molecules could be derived from similar (if not identical) mRNAs. Indeed, further studies along these lines are definitely required.

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