Purification and Characterization of Bovine Placental Lactogen*

(Received for publication, September 16, 1975)

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Bovine placental lactogen (bPL), a polypeptide hormone functionally related to bovine growth hormone (bGH) and bovine prolactin (bPR), has been isolated from placentas by pH and ammonium sulfate precipitation, gel filtration, and ion exchange chromatography on DEAE- and CM-cellulose. The hormone has been purified to approximately 99% homogeneity, as determined by end group analysis. On disc gel electrophoresis at pH 9.0 bPL migrates as a pair of closely spaced bands (RF = 0.517 and 0.541) between the positions of bGH and bPR. Its molecular weight, as estimated by gel filtration on Sephadex G-200 in 6 M guanidine hydrochloride and 6.5 mM dithiothreitol, is 22,150 and its isoelectric point is 5.9. The amino acid composition of bPL closely resembles that of bGH and bPR except for a higher content of serine and glycine and a lower leucine content. Like bPR, it has 2 tryptophans and 6 cysteines, but its COOH-terminal sequence is identical with that of bGH: -Cys.Ala.Phe.OH. By Ouchterlony immunodiffusion, bPL forms lines of partial identity with bGH against bGH antisera and with ovine placental lactogen (oPL) against oPL antisera. In the bPL-antibPL system, oPL forms a line of partial identity while bGH and bPR do not cross-react. However, bPL does not form a precipitin line with bPR antisera. These data would indicate that in terms of structure, and hence molecular evolution, bPL and other subprimate placental lactogens occupy a position more intermediate between growth hormone and prolactin than do the primate placental lactogens.

In 1962, Josimovich and MacLaren (1) reported the existence of a placental protein which cross-reacted with antisera to human growth hormone and which was subsequently identified as human placental lactogen. Although this hormone has been tentatively identified in a number of different mammals (2), only the placental lactogens from the human (3), rhesus monkey (4), and sheep (5, 6) have been purified to homogeneity.

Previous studies in the cow have yielded conflicting evidence concerning the existence of a bovine placental lactogen (bPL). Using immunofluorescent antibodies to human growth hormone, Currie et al. (7) were unable to stain the syncytiotrophoblast of bovine placentas. Furthermore, Tolstoi (8), using Ouchterlony immunodiffusion, could not find a cross-reaction between extracts from bovine placentas and human placental lactogen antisera. However, Fororh and Buttle (9) have reported that bovine cotyledons from term placentas have lactogenic activity when co-cultured with mammary gland explants from midpregnant mice, and they concluded that this activity could not be explained by endogenous prolactin levels. Finally, Gudson et al. (10) fractionated placental extracts from several species and measured the ability of these fractions to inhibit hemagglutination by antibodies to human placental lactogen. Although the bovine extracts did inhibit hemagglutination, they were only 0.88% as potent as human placental lactogen. In view of these conflicting reports, the purpose of this investigation was to determine whether a lactogen was present in bovine placentas and, if its existence were confirmed, to isolate and characterize this hormone.

EXPERIMENTAL PROCEDURE
Materials and methods are described in the adjacent miniprint. Some of the data are presented in a miniprint format immediately following this paper. Details of the Materials and Methods, References, and Tables II and III will be found in the miniprint section. For the convenience of those who prefer to obtain this material in the form of full size photocopies, it is available as JBC Document No. 75M-1270. Orders should specify the title, authors, and reference to this paper, the JBC Document Number, and the number of copies desired. Orders should be addressed to The Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Maryland 20014, and must be accompanied by a remittance to the Journal of $1.00 per set of photocopies.
RESULTS AND DISCUSSION

A summary of the purification protocol is given in Table I. The data are from a representative preparation which began with approximately 1.5 kg of semi-thawed cotyledons from a surgically obtained placenta. Delivered placentas contain only 5 to 10% as much placental lactogen as surgical placentas, and the high hemoglobin content of the former complicates the purification procedure.

bPL eluted from Sephadex G-150 as a single peak ($K_{av} = 0.400$) with an ascending shoulder (Fig. 1), and its position was slightly ahead of that of bGH ($K_{av} = 0.418$). On DEAE-cellulose, two active peaks were consistently seen (Fig. 2): the first, bPL-1, appeared after a gradient from 0.03 M NaCl to 0.05 M NaCl; the second peak, bPL-2, was eluted during a gradient from 0.05 M NaCl to 0.065 M NaCl. The small amount of activity eluted with 1.0 M NaCl has not been further characterized. On CM-cellulose, both bPL-1 and bPL-2 eluted as single, symmetrical peaks during separate gradients (Figs. 3 and 4).

On disc electrophoresis both bPL-1 and bPL-2 migrate as a pair of closely spaced bands having a mobility ($R_f$) of 0.517 and 0.541 (Fig. 5). The three bands of bGH travel much more slowly than bPL ($R_f = 0.242, 0.316$, and 0.403), while bPR migrates faster ($R_f = 0.661$ and 0.720). The two bands of bPL may represent amide differences, since deamidation is known to generate multiple bands with bGH and bPR (11).

Accurate molecular weight determinations have always been difficult with these hormones because of aggregation problems (12). When bPL-1 and bPL-2 were chromatographed on Sephadex G-200 (superfine) in 6 M guanidine hydrochloride containing 6.5 mM dithiothreitol the $K_{av}$ was identical for each and was consistent with a protein of $M_r = 22,150$ when run with six calibrating proteins, including bovine prolactin, ranging in molecular weight from 70,000 to 14,000. However, in sodium dodecyl sulfate gel electrophoresis, both forms of bPL migrated as if they had a molecular weight of between 59,000 and 62,000.

When bPL was subjected to isoelectric focusing, two bands were seen. The major band (approximately 70% of the sample) had an isoelectric point of 5.86, while that of the minor band was 6.1. On disc electrophoresis with ampholytes, only one band was seen and its isoelectric point was 5.90. Both bPL-1 and bPL-2 migrated identically in this system.

![Fig. 1](http://www.jbc.org/) Chromatogram of 5.27 g of the 45 to 65% ammonium sulfate precipitate on a column (5.0 x 130 cm) of Sephadex G-150 equilibrated and eluted with 0.01 M Tris-HCl, pH 9.0, containing 0.02 M NaCl. The arrows indicate the elution positions of bGH and bPR on a preceding calibration chromatogram. The solid bar indicates the fractions pooled for further purification. The column was eluted at 60 ml/hour and the fraction size was 10 ml.

![Fig. 2](http://www.jbc.org/) Chromatogram of the active Sephadex material on a column (2.5 x 40 cm) of DEAE-cellulose equilibrated with 0.01 M Tris-HCl, pH 9.0, containing 0.02 M NaCl. Immediately after the application of the sample (2.05 g) in the equilibrating buffer, the eluting buffer was stepped to 0.01 M Tris-HCl, pH 9.0, containing 0.03 M NaCl (a), and a 500-ml gradient from 0.03 M NaCl (b) to 0.05 M NaCl (c) was run. This was followed by a 250-ml gradient from 0.05 M NaCl (d) to 0.085 M NaCl (e). Finally, the eluting buffer was stepped to 1.0 M NaCl (f). The solid bars indicate the fractions pooled for study. The column was pumped at 100 ml/hour and the fraction size was 16 ml.

![Fig. 3](http://www.jbc.org/) Chromatogram of 150 mg of bPL-1 on a column (2.5 x 20 cm) of CM-cellulose equilibrated with 0.01 M ammonium acetate, pH 5.5. A 200-ml gradient was run from 0.01 M ammonium acetate without NaCl (a) to 0.01 M ammonium acetate with 0.1 M NaCl (b); a second 200-ml gradient was run from 0.1 M NaCl (c) to 0.2 M NaCl (d) before the eluting buffer was stepped to 1.0 M NaCl (e). The solid bar indicates the fractions pooled for study. The column was pumped at 100 ml/hour and the fraction size was 6.5 ml.

### Table I

Purification of bovine placental lactogen

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Successive yields</th>
<th>Cumulative yields</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. NH₄HCO₃ extr.</td>
<td>mg</td>
<td>µg</td>
<td>ng/µg</td>
<td>%</td>
<td>100</td>
</tr>
<tr>
<td>b. pH ammonium sulfate precipitation</td>
<td>19,072</td>
<td>610</td>
<td>32</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>c. Sephadex G-150</td>
<td>2,046</td>
<td>383</td>
<td>190</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>d. DEAE-cellulose</td>
<td>355.4</td>
<td>172</td>
<td>45</td>
<td>45</td>
<td>41</td>
</tr>
<tr>
<td>bPL-1</td>
<td>149.7</td>
<td>64</td>
<td>430</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bPL-2</td>
<td>205.7</td>
<td>108</td>
<td>530</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. CM-cellulose</td>
<td>46.8</td>
<td>82</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bPL-1</td>
<td>24.0</td>
<td>30</td>
<td>1,310</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>bPL-2</td>
<td>38.3</td>
<td>50</td>
<td>1,310</td>
<td>46</td>
<td></td>
</tr>
</tbody>
</table>

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**Bovine Placental Lactogen**

[Image references and links provided.]
The eluting buffer was stepped to 1.0 M NaCl (e). The solid have almost identical amino acid composition, with the hormone is selectively alkylated the molecule loses all prolactin-like activity in the pigeon crop sac assay, while retaining full growth-promoting activity in the tibia test (21). On the other hand, when the disulfide bond at the COOH terminus is selectively reduced by sulfhydrylysis, prolactin-like activity is preferentially preserved, while growth-promoting activity is lost (22).

The amino acid composition of the members of this hormone family show a remarkable similarity (Table II). The only residues in bPL which differ significantly from either bGH or bPR (serine, glycine, and leucine) would not be expected to alter the more important ionic properties of the molecule, such as the isoelectric point and the net charge at physiological pH. The preservation of net charge is crucial to biological activity, since acetylation of the ε-amino groups of lysine in either bGH or ovine prolactin results in complete inactivation of these hormones (18), while conversion of these side chains to guanido groups does not alter biological activity (19). Although the net charge on bPL is the same as that on ovine prolactin, the markedly reduced leucine content has lowered its average hydrophobicity (20), which may explain its increased solubility as compared to other closely related proteins.

The tryptophan and cysteine residues are of particular interest since they play a key role in the biological activity of these hormones. When the single tryptophan in human growth hormone is selectively alkylated the molecule loses all prolactin-like activity in the pigeon crop sac assay, while retaining full growth-promoting activity in the tibia test (21). On the other hand, when the disulfide bond at the COOH terminus is selectively reduced by sulfhydrylysis, prolactin-like activity is preferentially preserved, while growth-promoting activity is lost (22). With 2 tryptophans and 6 cysteines, bPL more closely resembles pituitary prolactin, and this structural similarity is consistent with its postulated role in mammary development during pregnancy (23).

The presence of two active peaks on DEAE-cellulose chromatography led to speculation that there were two distinct molecular forms of bPL. A precedent for this is seen in monkey placental lactogen, where 2 active molecules differ significantly in amino acid composition (4). By contrast, bPL-1 and bPL-2 have almost identical amino acid composition, with the differences limited to 1 less threonine, proline, and valine in bPL-1. It is possible that bPL-1 represents an enzymatic cleavage product of the native molecule, bPL-2; this phenomenon has been well described for bGH, where a significant number of molecules have up to 4 NH₂-terminal residues removed (24). This hypothesis could not be confirmed by NH₂-terminal analysis, since no 3-phenyl-2-thiohydantoin derivatives were detectable through five cycles of automated Edman degradations on reduced and carboxymethylated bPL. The amino acid composition of bPL resembles that of turtle growth hormone more closely than any other growth hormone of known composition (Table II). With the serine, glycine, and leucine contents remarkably close, the single major difference between the hormones is the higher glutamic acid content of the turtle molecule.

The results of carboxypeptidase digestion are shown in Table III. Although phenylalanine and alanine are rapidly released by carboxypeptidase A, the 15-min aliquot contains 50% more of the former. The homology with bGH also strongly argues for a COOH terminal sequence of -Cys-Ala-Phe-OH. The average yield for phenylalanine (99.7%) provides convincing evidence for the homogeneity of bPL. Except for alanine and aminoethyl-cysteine, no other residues were detected to 5 hours.

bPL also possesses certain immunological characteristics in common with bGH, since it forms a line of partial identity with bGH against bGH antisera (Fig. 6). It also forms a line of partial identity with oPL against oPL antisera, but does not form a precipitin line against bPR antisera. The antisera to bPL produce no precipitin lines with either bGH or bPR; however, oPL does form a line of partial identity with bPL against undiluted bPL antisera (Fig. 7).

The displacement curves for bPL in both the lactogenic and growth hormone radioreceptor assays were parallel to the standards (Figs. 8 and 9), indicating that bPL is binding to the same receptor sites as prolactin and growth hormone, respectively. However, the activity of bPL was low in the lactogenic radioreceptor assay, since 1 mg of pure bPL had the displacing potency of only 1.3 μg of bPR. Inactivation during the purification procedure appears unlikely, since ovine placental lactogen purified in this laboratory by a similar procedure (25) is as potent as human placental lactogen (26) or ovine prolactin in the same assay. Species specificity of the bPL-receptor interaction is not likely since the specific activity remained unaltered in a homologous assay using bovine mammary receptors. Similarly, bPL possessed a low specific activity.

* F. F. Bolander and R. E. Fellows, unpublished observations.

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2706 Fig. 6 (left). Double immunodiffusion of bPL and bGH against undiluted rabbit bGH antiserum (center well). Both bPL-1 (5 mg/ml) and bPL-2 (5 mg/ml) form faint lines of partial identity with bGH (0.2 mg/ml).

Fig. 7 (right). Double immunodiffusion of pituitary and placental hormone against undiluted rabbit bPL antisera (center well). bPL-1 (1 mg/ml) and bPL-2 (1 mg/ml) form lines of identity with each other, oPL (5 mg/ml) forms a faint line of partial identity with bPL, and bGH (7.5 mg/ml) and bPR (7.5 mg/ml) form no lines.

Table IV

Comparison of physical and immunological properties of bovine growth hormone, placental lactogen, and prolactin

<table>
<thead>
<tr>
<th>Property</th>
<th>bGH</th>
<th>bPL</th>
<th>bPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>21,500</td>
<td>22,150</td>
<td>22,800</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>6.85</td>
<td>5.9</td>
<td>5.73</td>
</tr>
<tr>
<td>Tryptophan content</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cysteine content</td>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>COOH terminus</td>
<td>Cys-Ala</td>
<td>Cys-Ala</td>
<td>Asn-Asn</td>
</tr>
<tr>
<td>Net charge (pH 7.4)</td>
<td>+0.5</td>
<td>-6</td>
<td>-6</td>
</tr>
<tr>
<td>Pharmacophores (cal/residue)</td>
<td>1,139</td>
<td>935</td>
<td>1,113</td>
</tr>
<tr>
<td>Cross-reaction to:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-bGH antiserum</td>
<td>Complete</td>
<td>Partial</td>
<td>None</td>
</tr>
<tr>
<td>Anti-bPR antiserum</td>
<td>Complete</td>
<td>None</td>
<td>Complete</td>
</tr>
<tr>
<td>Anti-bPL antiserum</td>
<td>None</td>
<td>Complete</td>
<td>None</td>
</tr>
<tr>
<td>Anti-oPL antiserum</td>
<td>Partial</td>
<td>Partial</td>
<td>None</td>
</tr>
</tbody>
</table>

*Calculated from the amino acid composition and the data given in Edsall (29).
*Calculated for ovine prolactin (30), since all of the amides have not been placed for bPR.
Average hydrophobicity (H_Phi) was calculated by the method of Bigelow (30).

Our conclusion is that bPL is structurally intermediate between bGH and bPR (Table IV) and may represent a direct evolutionary line from the primitive precursor which gave rise to both bGH and bPR, rather than recently diverging from growth hormone (28). The very close similarity between the amino acid compositions of bPL and turtle growth hormone further suggests that the structure of bPL has been evolutionarily conserved.

Acknowledgments—The authors are grateful to the Hormone Distribution Program, National Institute of Arthritis, Metabolism and Digestive Diseases, for the bovine growth hormone and prolactin, to Dr. L. C. Ulberg, Reproductive Physiology Research Laboratory, North Carolina State University, for the delivered bovine placentas, and to Dr. Stuart Handwerger for the oPL antisera.

activity in the growth hormone radioreceptor assay, where 1 mg of bPL was equivalent to only 630 ng of bGH. However, biological studies are in progress to investigate its potency, since discrepancies between biological and radioreceptor assay activity have been reported (27).
REFERENCES

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SOMALATIONAL SUPPLEMENTARY

MATERIALS AND METHODS

Materials - Bovine growth hormone (MHH-NH-315) and bovine prolactin (MHH-NH-348) were further purified on a column of Sephadex G-50 as previously described (11). Prolactin hydrochloride (U.S. from J. Baker Chemical Co.) and ammonium sulfate ( Technical grade) were obtained from Eastern Kodak and were crystallized from hot chloroform/ethanol (70:30) and hot acetone. Glyceryl trimethylammonium chloride and N-lauroylsarcosine (3M solution) were obtained from Sigma Chemical Co.; sodium citrate buffer was prepared from the same stock. Other materials were purchased locally.

Procedures - Protein concentrations were determined by the method of Lowry et al. (13). Prolactin was assayed by the method of Mollon and Gaboriau (5). The activity of purified PRL was also measured in a mammalian growth hormone assay (15) using bovine growth hormone as both the tracer and the standard.

Purification - All steps of the purification procedure were done at 4°C in the presence of sodium azide. Protein concentrations were determined by the method of Lowry et al. (4) and were calculated from the protein content of each incubation. The bovine placentas were removed from the uteri of 3-4 mo sows and were cut into small pieces. A 1:10 (w/v) homogenate of homogenate was prepared and was centrifuged at 10,000 g for 30 min. The supernatant fluid was dialyzed against 0.1 M Tris-HCl, pH 7.4, containing 0.05 M sodium chloride and was then dialyzed against distilled water. The lyophilized material was stored at -20°C.

Radioimmunoassay - The purification of bovine placentals lactogen was monitored by the radioimmunoassay method of Mollon and Gaboriau (5). The activity of purified PRL was also measured in a mammalian growth hormone assay (15) using bovine growth hormone as both the tracer and the standard.

REFERENCES

Purification and characterization of bovine placental lactogen.
F F Bolander, Jr and R E Fellows


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