Bovine Placental Lactogen: Molecular Cloning and Protein Structure†,‡

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ABSTRACT: The bovine placenta secretes at least one hormone with prolactin-like and placental growth-hormone-like activity. The cDNA for bovine placental lactogen was isolated from a bovine fetal cDNA library by virtue of its nucleotide sequence homology to bovine prolactin (70%) and identified as such from amino acid sequence obtained from the amino terminus and internal tryptic fragments from the isolated hormone. The cDNA predicts a preprohormone of 236 amino acids, with a signal peptide of 36 amino acids. A single consensus site for N-glycosylation marks a probable site of carbohydrate addition. The encoded hormone is quite distinct from the pituitary hormones, as well as the primate and rodent placental lactogens and other predicted bovine placental hormones. It is 51% similar to bovine prolactin in amino acid sequence, 30% similar to the protein predicted by bovine prolactin-related cDNA I, about 30% similar to the rodent predicted placental hormones, and only about 20% similar to human placental lactogen and bovine growth hormone. Despite its greater similarity to bovine prolactin, sequence homology in the region of 5′ flanking sequences and first exon to bovine prolactin-related cDNA I suggests that bovine placental lactogen may share a common evolutionary origin with this other placentally expressed member of the prolactin gene family.

Prolactin-like activities in the placenta, detected by radioreceptor assays, and stimulation of milk synthesis in cocultures of placental tissue and mammary gland have been described in many species (Talamantes, 1975; Kelly et al., 1976). This lactogenic activity is particularly high during the latter two-thirds of gestation. The hormone thought to be responsible for this activity has been isolated and characterized in the mouse, rat, hamster, sheep, goat, and cow (Arima & Bremel, 1983; Becka et al., 1977; Beckers et al., 1980; Chan et al., 1976; Colosi et al., 1982; Eakle et al., 1982; Murthy et al., 1982; Robertson et al., 1981; Southard et al., 1986). Both the molecular and biological properties of the bovine placental lactogen are different from those of other species. The corresponding hormone in rodents and other ruminants is a protein hormone of 20–22 kDa, similar to the evolutionarily related pituitary hormones, growth hormone and prolactin, whereas that isolated in the cow is 30–32 kDa (Arima & Bremel, 1982; Becker et al., 1980; Eakle et al., 1982; Murthy et al., 1982). In addition, both bioassay and specific radioimmunoassay have shown that this hormone is present at much lower concentrations in the maternal circulation than in other species, although levels in the fetus are similar to others reported (Beckers et al., 1982; Byatt & Bremel, 1987; Schellenberg & Friesen, 1982). Analysis of this unusual placental lactogen has demonstrated considerable microheterogeneity, including two size variants, differing about 2000 in M_r, and about five isoelectric variants (Byatt et al., 1986). Recently, it has been shown that carbohydrates account for about 7000 of the apparent M_r in SDS–PAGE gels; however, the size and p*I* microheterogeneity persist after enzymatic removal of the carbohydrates (Shimomura & Bremel, 1988).

In order to study the molecular basis of the heterogeneity of this bovine placental hormone and provide the basis for structure–activity studies of this gene family, we determined the amino acid sequences of the amino terminus and two tryptic fragments of the isolated hormone and then used the information to identify the bovine placental lactogen (bPL)[1] cDNA reported here. Analysis of the primary structure is consistent with a translational product modified by addition of carbohydrate as well as other as yet undefined posttranslational modifications.

Experimental Procedures

Peptide Sequence Determination. Peptide sequence analyses of HPLC-purified intact bPL, reduced and carboxymethylated bPL (RCM-bPL), and tryptic fragments of RCM-bPL were performed on gas- or liquid-phase protein sequencers (Applied Biosystems Inc.) at the University of Wisconsin Biotechnology Center, Madison, WI. Approximately 0.1–1.2 nmol of peptide was used per analysis with average repetitive yield of 88% and average carryover about 5%. Phenyl isothiocyanate derivatized amino acids were converted to the more stable phenylthiohydantoin forms and identified and quantitated by HPLC with a C_{18} RP column (IBM Instruments).

cDNA Library Construction and Screening. Poly(A)+ RNA was purified as previously described (Kessler & DeLuca, 1985) from frozen bovine cotyledonary tissue from 180-day gestation. Double-stranded cDNA was prepared following priming with oligo(dT)$_{12-18}$ (first library) or both oligo(dT)$_{12-18}$ and random oligomers (second library) by the method of Gubler and Hoffman (1983). Following ligation of *Eco*RI linkers (Huynh et al., 1984), cDNA was inserted into the *Eco*RI site of λgt10 (first library) or λZAP (Stratagene; second library). Both libraries were screened by lifting the plaques to Biodyne A (ICN Radiochemicals) using conditions recommended by the manufacturer modified from Benton and Davis (1977). cDNAs were identified by hybridization to

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‡ The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number J02840.

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[1] Abbreviations: bPL, bovine placental lactogen; bPrl, bovine prolactin; bGH, bovine growth hormone; bPRCI, bovine prolactin-related cDNA I.

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nick-translated bovine prolactin cDNA at low stringency (Rigby et al., 1977; Sasavage et al., 1982) or to previously identified placental prolactin-related cDNAs under conditions of high stringency. For low-stringency screening, filters were hybridized in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM sodium phosphate, pH 7.4, and 1 mM EDTA), 5× Denhardt's [1× Denhardt's is 0.02% each bovine serum albumin, Ficoll, and poly(vinylpyrrolidone)], 0.2% sodium dodecyl sulfate (SDS), and 100 μg/mL herring sperm DNA at 62 °C for 14–18 h. Filters were washed in 2× SSC (1× is 150 mM NaCl and 15 mM sodium citrate), in 0.2% SDS at room temperature for 30 min, and twice in 2× SSC and 0.2% SDS at 65 °C for 30 min. For high-stringency conditions to distinguish closely related species, filters were hybridized in 1.25× SSPE, 5× Denhardt's, 0.2% SDS, and 100 μg/mL herring sperm DNA at 68 °C for 12–14 h and washed in 2× SSC and 0.2% SDS at 65 °C for 1 h, followed by 0.1× SSC and 0.1% SDS at 65 °C for 1 h. After screening and plaque purification, inserts of interest from the cDNA in the gt10 library were subcloned into plasmid Bluescript M13 vectors (Stratagene) or recovered in pBluescript SK(−) by the automatic excision process, which is a property of λZAP.

Sequence Analysis. Nucleotide sequences of the entire length of both strands of the cDNAs were determined by the dideoxynucleotide chain-termination method (Sanger et al., 1977), following successive deletions with restriction enzymes, or *Escherichia coli* exonuclease III as modified from Henikoff (1984). Sequences were analyzed by using the University of Wisconsin Genetics Computer Group programs (Devereux et al., 1984).

Hybridization Selection of Poly(A)+ RNA. Linearized, denatured plasmid (containing the cDNA insert) in 2 M NaCl was passed three times through a silanized 15-mL Millipore apparatus containing a nitrocellulose filter (Schleicher & Schuell) that had been soaked in 4× SSC. After washing with 20 mL of 4× SSC, the filter was baked in vacuo at 80 °C for 2 h. The filter was broken into small pieces that were washed by boiling in 1 mL of diethyl pyrocarbonate (DEPC) treated water for several minutes. The filters were lyophilized until dry.

Bovine cotyledonary poly(A)+ RNA (100 μg) from 180-day gestation in 200 μL of hybridization buffer [410 mM NaCl, 20 mM PIPES at pH 6.4, 1 mM EDTA, 0.2% SDS, 125 μg/mL calf liver tRNA (Boeringer Mannheim), and 50% (v/v) deionized formamide] was heated to 70 °C for 10 min and added to the filters. The hybridization was performed at 50 °C for 2 h and followed by 43 °C for another 3 h. The hybridization buffer was removed, and the filters were washed 10 times at 65 °C in 150 mM NaCl, 10mM Tris-HCl (pH 7.5), 1 mM EDTA, and 0.5% SDS and 5 times at 65 °C in the same buffer without SDS.

To elute the RNA, the filters were boiled for 5 min in 300 μL of DEPC-treated water with 10 μg of calf liver tRNA, vortexed, snap-frozen in an ethanol/dry ice bath, and thawed at room temperature. The supernatant was removed and precipitated with 40 μL of 3 M potassium acetate and 1 mL of ethanol at −20 °C overnight. This process was repeated a second time without snap-freezing. The two precipitates were each redissolved in 5 μL of DEPC-treated water and pooled.

Translation and Gel Electrophoresis. The RNA was translated by using a rabbit reticulocyte lysate system (Bethesda Research Laboratories) in the presence of 90 μCi of [^{35}S]methionine (800 Ci/mmol, Amersham) according to the manufacturer's recommendations. The sample was treated with 20 μg/mL bovine pancreatic RNase A (Sigma) at 37 °C for 30 min, and the translation products were separated by one-dimensional discontinuous SDS–polyacrylamide gel electrophoresis (SDS–PAGE; Laemmli, 1970) using a separating gel of 12.5% acrylamide. The following proteins (Bethesda Research Laboratories) were run as molecular weight standards: lysozyme (14 300); β-lactoglobulin (18 400); α-chymotrypsinogen (25 700); and ovalbumin (43 000). Gels were processed for fluorography using EN^{3}HANCE (New England Nuclear) according to the manufacturer's instructions, dried, and exposed to Kodak XAR-5 film for 1–2 days at −80 °C.

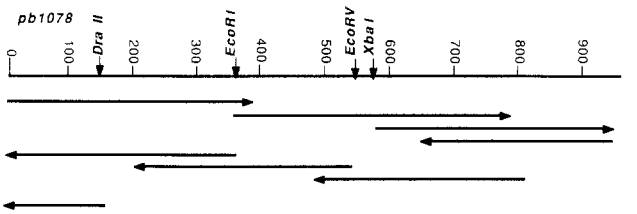

FIGURE 1: Partial restriction endonuclease map and sequencing strategy for pb1078. The sequence obtained from a particular reaction is shown by the length and direction of the arrow.

RESULTS

The bovine placenta transcribes a family of genes related to prolactin (Schuler et al., 1987), as has been reported for the rat and mouse (Duckworth et al., 1986a,b; Jackson et al., 1986; Linzer et al., 1985; Linzer & Nathans, 1985). To determine which of our cDNA clones coded for the described bovine placental lactogen, we obtained amino acid sequence information from the isolated bovine hormone, including both size variants (underlined amino acids shown in Figure 2). Repeated sequence runs were done from the amino terminus, extending 49 residues into the molecule. Sequence information was also obtained from an internal 11 amino acid tryptic fragment containing a tryptophan. An additional short five amino acid fragment was also sequenced. Two residues (alanine and valine) were present in equal molar amounts in the first cycle at the amino terminus. Other residues were unambiguous.

Comparison of the amino acid sequence information from the protein revealed that bovine placental lactogen was more similar to prolactin than the predicted amino acid sequence of those bovine placental prolactin-related cDNAs for which nucleotide sequence information was available (Schuler & Hurley, 1987; Schuler, unpublished observations). The amino-terminal 49 amino acids of bovine placental lactogen were 43% homologous to the pituitary hormone, compared to about 35% with previously characterized cDNAs. The 11 amino acid internal fragment was 64% similar to prolactin, compared to 27–45% for our other placental prolactin-related cDNAs. On the basis of this information, identified placental prolactin-related cDNAs were differentially screened for similarity to characterized cDNAs and prolactin cDNA. This revealed cDNA clones that were more similar to bovine prolactin than others already sequenced.

A partial nucleotide sequence was obtained from the first of these clones isolated (bPL713) after subcloning into Bluescript M13 KS−. This sequence predicted an amino acid sequence identical with that of internal tryptic fragments found above. This cDNA was then used to rescreen the second cDNA library at high stringency to obtain a longer clone. The nucleotide sequence of the resulting clone, bPL1078, is shown in Figure 2. The predicted amino acid sequence matches that of the sequenced protein exactly, with an alanine predicted at the amino terminus of the mature protein.

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GGAATGCCTCCATTCAGTCCTGTTGGGCCATCTCCCCATCAGCAGCAGTCCTCATCCTGGGATTTCTCTCCA   72

ATCCTCATGGCTCCAGCATCTAGCCATCGTGGGCACCAGTGGATTTGTGACCTTGTTCGAGGGTCCTGCCTG  144
      MetAlaProAlaSerSerHisArgGlyHisGlnTrpIleCysAspLeuValArgGlySerCysLeu
      -36             -30                 -20

CTCCTGCTGCTGGTGGTGTCAAATCTACTCTTGTGCCAGGGTGCGGAGGATTATGCACCATACTGTAAAAAC  216
LeuLeuLeuLeuValValSerAsnLeuLeuLeuCysGlnGlyAlaGluAspTyrAlaProTyrCysLysAsn
          -10                  1                  10

CAACCTGGCAACTGCCGGATTCCCCTTCAAAGCCTGTTTGAGAGAGCAACATTGGTGGCTAGCAACAACTAT  288
GlnProGlyAsnCysArgIleProLeuGlnSerLeuPheGluArgAlaThrLeuValAlaSerAsnAsnTyr
                20                  30

AGGCTCGCCAGGGAAATGTTCAATGAATTTAATAAACAGTTTGGCGAGGGCAAAAACTTCACTTCCAAGTTC  360
ArgLeuAlaArgGluMetPheAsnGluPheAsnLysGlnPheGlyGluGlyLysAsnPheThrSerLysPhe
          40                  50

ATCAACAGCTGCCACACCGAATTCATGACTACCCCTAATAACAAAGAAGCAGCTGCAAATACAGAGGACGAA  432
IleAsnSerCysHisThrGluPheMetThrThrProAsnAsnLysGluAlaAlaAlaAsnThrGluAspGlu
  60                  70                  80

GCCCTGTTGAGGTTGGTTATCAGTTTGCTCCACTCGTGGGATGAACCTCTGCATCAGGCAGTCACAGAGTTG  504
AlaLeuLeuArgLeuValIleSerLeuLeuHisSerTrpAspGluProLeuHisGlnAlaValThrGluLeu
            90                  100

TTGCACAGGAATGGAGCCTCACCTGATATCTTGGCAAGGGCTAAAGAGATTGAGGACAAGACCAAAGTACTT  576
LeuHisArgAsnGlyAlaSerProAspIleLeuAlaArgAlaLysGluIleGluAspLysThrLysValLeu
      110                 120                 130

CTAGAAGGTGTGGAAATGATACAAAAAAGGGTTCATCCTGGAGAGAAGAAGAACGAGCCCTATCCAGTGTGG  648
LeuGluGlyValGluMetIleGlnLysArgValHisProGlyGluLysLysAsnGluProTyrProValTrp
            140                 150

TCAGAAAAGTCCTCCCTGACAGCAGACGATGAGGATGTGCGCCAAACTGCCTTTTATAGAATGTTCCACTGC  720
SerGluLysSerSerLeuThrAlaAspAspGluAspValArgGlnThrAlaPheTyrArgMetPheHisCys
      160                 170

CTACACAGGGATTCGAGTAAAATTAGCACCTACATCAATTTGCTTAAGTGCCGATTCACCCCATGCTAAGCC  792
LeuHisArgAspSerSerLysIleSerThrTyrIleAsnLeuLeuLysCysArgPheThrProCys
  180                 190                 200

CACAATTAACCCAACCAGTCCTGAGATGGTTAGTGATGATCCATCCCGTCAAAAGCTTCTTTGAGTTTTATA  864

GCTCTTTAATGCATGTTTGGGTGTAATGGGTCTCATCTGAAACAAAATAAACACAGATTCTGTAGAGATGTC  936

AAAATCTAAAAA 948
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FIGURE 2: Nucleotide sequence pb1078. The predicted amino acid sequence for bPL is shown below the nucleotide sequence. Amino acid sequence obtained from the isolated protein is underlined. The consensus sequence for N-glycosylation is underscored twice. The polyadenylation signal AATAAA is underlined with a dashed line. An arrowhead marks the site of signal peptide cleavage.

bPL cDNA described here is somewhat more closely related to bovine prolactin cDNA (70% nucleotide homology; Sasavage et al., 1982) than to the previously described bovine prolactin-related cDNA I (bPRCI, 62% nucleotide homology; Schuler & Hurley, 1987). Although the bPL cDNA is more similar overall to prolactin, the 5′ untranslated region and first exon are much less similar to the pituitary hormone even after allowing for a difference in the splicing site between the first and second exon (Figure 3). In contrast, the homology to bPRCI persists into this region (68%). Similarity to both prolactin and bPRCI is maintained through the 3′ untranslated region, and the site of the polyadenylation signal (Proudfoot & Brownlee, 1976) is conserved among these related genes.

This cDNA encodes an open reading frame from the beginning of the clone to the termination codon at nucleotide positions 787–789. A single AUG is present in the 5′ region of this clone at nucleotide positions 79–81, which presumably is the initiating methionine. This predicts a preprohormone of 236 amino acids. The amino terminus of the predicted protein is strongly hydrophobic, consistent with an apparent function as a signal peptide. Our amino acid sequence of the isolated hormone pinpoints the site of cleavage of the signal peptide following the glycine at amino acid 36. The predicted mature hormone is 200 amino acids, with a molecular weight of 23 500. It has six cysteine residues in positions similar to those in bovine prolactin, which presumably allow formation

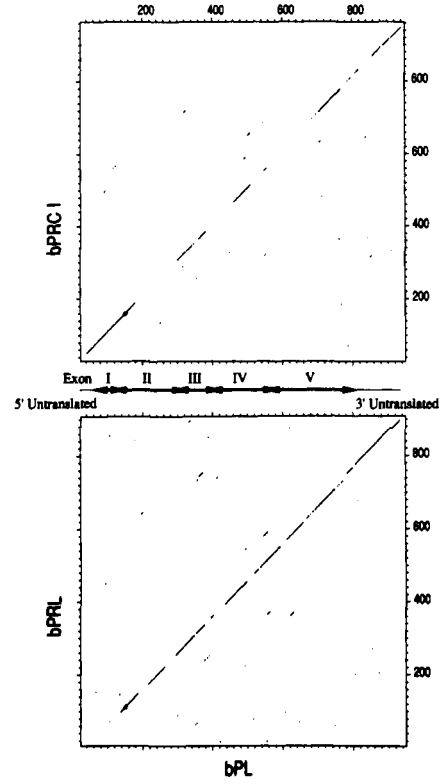

FIGURE 3: Comparison of the nucleotide sequences of bovine prolactin-related cDNA I (bPRCI; Schuler & Hurley, 1987) and bovine prolactin (bPrl; Sasavage et al., 1982) to the bPL cDNA using the program Dotplot, which identifies regions of homologies (Devereux et al., 1984). (A) Comparison of bPL cDNA, horizontal axis, to bPRCI, vertical axis. (B) Comparison of bPL, horizontal axis, to bPrl, vertical axis. An approximation of structure of bPL mRNA is diagrammed on the basis of conserved gene structure for this family (Miller & Eberhardt, 1983).

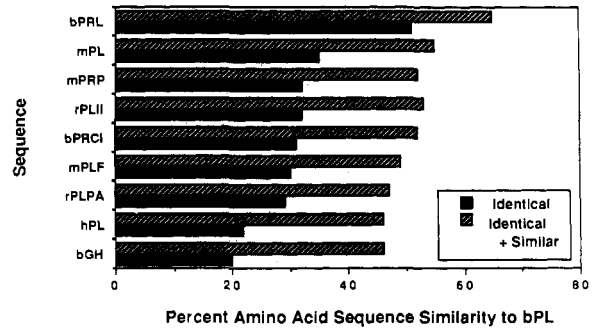

FIGURE 4: Amino acid sequence similarity to the secreted bPL. Similar amino acids are identified by the point acceptance mutation matrix of Dayhoff (1972), as modified by Gribskov and Burgess (1986).

of three disulfide bonds analogous to the pituitary hormone. Similarly, two tryptophans are present in the predicted protein, in positions similar to those in bovine prolactin. The predicted amino acid sequence also reveals a site of potential N-glycosylation (Asn-Phe-Thr) at residues 53–55 of the mature protein (Bahl & Shah, 1977). This site is apparently a location of some of the carbohydrate described for this protein (Shimomura & Bremel, 1988).

Many of the features of the bPL protein as predicted from the cDNA sequence are similar to the other described members of this gene family, resembling bovine prolactin most closely. As suggested by the partial amino acid sequence derived from the protein, the total amino acid sequence predicted from the cDNA shows a higher level of homology to prolactin than do the other related bovine placental cDNAs (Figures 4 and 5; unpublished results). It is not more closely related to the

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                                                                      60
  bPL  MAPASSHRGH QWICDLVR.. GSCLLLLLVV SNVLLCQGAE DYAPYCKNQP GNCRIPQSL
  bPRL ..........  .mdskgssqk   r         l    vv s.t v p g     qvs rd
  mPL  ..........  mklslsqpcs f ga    ●    l vwekvt slp..... . y l te
  rPLII ..........  .vqls tqpc f gt   m a   tl  weqvt s........a p y mstg
  mPRP ..........  .mlps iqpc s gt  ● lm   lf ...w  kvssapi as eavlsd kd
  bPRCI     p f      tynp  ..        lm   l     .k scpscgpdvf vsl ksftdr
  mPLF ..........  .mlpssiqpc swi      n  sl wknv s ..f m amrn  r fmsfedt
  rPLPA ..........  .mhls shqw s wtv   l    l  ..... .......... ...wenta a
  bGH  ..........  .......... ....mmaagp rts  laf l lcl wtqvvg afpams sg

                                                                     120
  bPL  FERATLVASN NYRLA..... ........RE MFNEFNKQF. ..GEGKNFTS KVINSCHTEF
  bPRL  d  vm shy ihd s..... ........s        d ry. ..aq  g it mal     ss
  mPL  yq viv sh  ahd  ..... ........sk a m  emk . .. rtawtyg lmlsp   aa
  rPLII yq vvelshy thd  ..... ........sk v i  dmk g rtvwth l.. .mlsp   aa
  mPRP   n  vlsge msk gvimrk effmnsfss  t  kiildlh kst n...it  af     vp
  bPRCI  mn aslshd f n sti... ..........       deky. ..aq  lyyi n tk    ns
  mPLF   l gslsh  .......... ...isievs  l    e hy. ..snvsglrd  spmr n s
  rPLPA mrakl nvh  ytsygdtwnq aikisqdmnq yisdlsthvk ifyaqgrgfe rrttr   ss
  bGH   an v r qh lhq  a.... .........d t k  ertyi pe qrysiqn tqvaf fs t
         *    *  *                                                   *

                                                                     180
  bPL  MTTPNNKEAA ANTEDEALLR LVISLLHSWD EPLHQAVTEL LHRNGASPDI LARAKEIEDK
  bPRL  lp  ed  q  qq hh v ms  ilg  r  n d  yhl   v rgmk  pda    s  i   ee
  mPL   il  e s qv hq ts d  k vs ti qa e    khm aav aalphvpdtl  s t  l er
  rPLII  ip  e s qv hqaks d  k vs ti qa q    khi aav atlpdg dtl  s t  l er
  mPRP  inv etv dv rk sf ef k m lhm la k    khl     salpecpyrl  sk ea  a
  bPRCI fha eerdiv qq ni d sk wtlv  y  n n   hl     q mkel naf  ss trf nm
  mPLF  lp  e   q  rl hya   k sgamisda e s  ddl s   stiknvpdi  isk td kk
  rPLPA lss e   q  qqfql v  g  sh   qa l n   hlwa m cd l st pt  yk lmlkes
  bGH   ipa tg ne  qqks le    isll iq  l g  .....qf  s vftnslv fgtsdrvye
          *

                                                                     240
  bPL  TKVLLEGVE. .MIQKRVHPG EKKNEPYPVW SEKSSLTADD EDVRQTAFYR MFHCLHRDSS
  bPRL n r    m . .  fgq i   a et       glp  qtk    a ys   n ll   r
  mPL   iqg    lk. .i fn  y   avasd. tf   aw d qss   stkns lrt lwr vr  th
  rPLII iqg    l . .t ls  q   avgsd. tf    w d qss  kstkngvlsv lyr mr  th
  mPRP  n d   yi.. ir isk n a i e  d  t   dld  ksa  ketqff l m fsf  ri le
  bPRCI sek qafi r qfskii pvl ntmiqarss  tglp  mssa   r hse  n l y  r   r
  mPLF  inavrn  na l stmlqngd  e kn .... ..awf qs n   a ihsl g  is  dn fk
  rPLPA nik  daikn .. a kgnfe inqkan ta    lgf qspn r t yf   n l   kk  n
  bGH   l d e  ila l ..reled  tpragqilkq tydkfd nmr s dallkn g lls frk ih

                                263
  bPL  KISTYINLLK CRFTPC.... ...
  bPRL   d  lk  n   iiynnnc. ...
  mPL   vdn lkv     dvhnnnc. ...
  rPLII  vdnflkv    diynnnc. ...
  mPRP  tvdflv  f   lllyddvcy sgf
  bPRCI  vdm  kiit  thktc... ...
  mPLF   vdi l v    ymlkidnc. ...
  rPLPA  nvem lk     lirskc.. ...
  bGH    te  lrvm    rfgeasca f..
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FIGURE 5: Comparison of the amino acid sequence predicted by bPL to that of bovine prolactin (bPRL; Sasavage et al., 1982), mouse placental lactogen (mPL; Jackson et al., 1986), rat placental lactogen II (rPLII; Duckworth et al., 1986a), mouse proliferin-related protein (mPRP; Linzer & Nathans, 1985), bovine prolactin-related cDNA I (bPRCI; Schuler & Hurley, 1987), mouse proliferin (mPLF; Linzer et al., 1985), rat prolactin-like protein A (rPLPA; Duckworth et al., 1986b), and bovine growth hormone (bGH; Woychik et al., 1982). Gaps, marked by dots, have been introduced to maximize homologies. Numbers correspond to amino acid position of the aligned sequences. Residues of the related proteins are shown only where they differ from bPL. Asterisks mark those residues proposed to be important for lactogenic function (see Discussion). Underlined regions correspond to clusters of residues conserved among lactogenic hormones (P1, P3, P5, P6, P9, respectively; Nicoll et al., 1986).

prolactin-like placental hormones in other species, although it is more similar to the rodent placental lactogens (rPLII and mPL) than the other predicted bovine placental hormones like bPRCI. Like the rodent placental lactogens, and unlike the human placental lactogen, it has low homology to bovine growth hormone.

The size of the hormone encoded by this cDNA is similar to characterized members of the prolactin–growth hormone gene family, including bovine prolactin. The isolated glycoprotein hormone consists of two size forms differing by about 2000 in M_r. This size difference persists following enzymatic removal of sugar residues. The smaller is approximately the same size as that predicted by the cDNA clone described here. Several possibilities may account for the two sizes of the deglycosylated hormone. They may be products of closely related genes or alternatively spliced RNAs from a single gene. Alternatively, additional posttranslational modifications of the protein may have contributed to the mobility differences observed by SDS–PAGE gels in these studies.

To examine the first possibility, mRNA corresponding to bPL713 was hybrid-selected and translated in vitro. The product is shown in Figure 6 as a single band about 27 kDa,

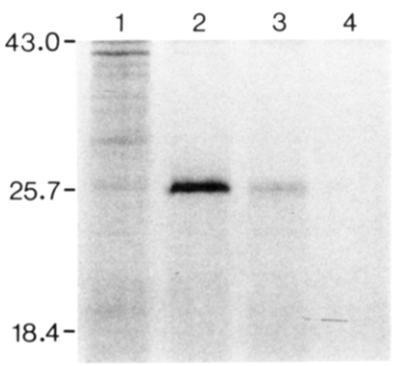

FIGURE 6: In vitro translation of hybrid-selected mRNA. Total and poly(A)+ RNA hybrid-selected as described under Experimental Procedures were translated in vitro by using rabbit reticulocyte lysate in the presence of [^{35}S]methionine, and the products were resolved by SDS–PAGE. (Lane 1) Translation of total cotyledonary poly(A)+ RNA from 180-day gestation. (Lane 2) Translation of poly(A)+ RNA hybrid-selected with bPL cDNA. (Lane 3) Translation of poly(A)+ RNA hybrid-selected with bPRCI. (Lane 4) Translation with no RNA added.

similar in mobility to bPRCI and consistent with the size of the predicted preprohormone (26 894 Da). Additional exposures and two-dimensional gel analysis gave no indication of two-sized products corresponding to the two reported hormone sizes (unpublished data).

Discussion

We have described a cDNA for bovine placental lactogen, which describes a protein more closely related to prolactin than either bovine placental prolactin-related cDNA I or growth hormone. The similar size and predicted secondary structure of bPL compared to prolactin is consistent with the chromatographic and immunological behavior of this protein (Arima & Bremel, 1983; Murthy et al., 1982). These findings are consistent with evolution of prolactin-related genes expressed in the placenta of all nonprimate species, in addition to rodents. In contrast, human placental lactogen is closely linked and very similar in sequence to human growth hormone (Miller & Eberhardt, 1983; Shine et al., 1977).

Although this cDNA is homologous to bovine prolactin throughout the region coding for the mature hormone, the 5′ untranslated region and sequence corresponding to the first exon of bPL are different from that of prolactin (Sasavage et al., 1982) and much more homologous to that of bPRCI (Schuler & Hurley, 1987) as shown in Figure 3. This suggests that the two placentally expressed genes may have arisen from a common gene duplicated from prolactin, which acquired in this process a first exon and 5′ flanking region distinct from the parent gene. Subsequent divergence of the two placentally expressed genes, and perhaps evolutionary pressure on bPL to remain similar to prolactin, may have resulted in the modern bovine genes. Study of the chromosomal sequences corresponding to these prolactin-related cDNAs will provide insights into their molecular evolution.

Bovine placental lactogen is a glycoprotein, unlike the pituitary members of this gene family and the placental lactogens of rodent species but similar to the other predicted bovine and rodent placental hormones (Duckworth et al., 1986b; Linzer et al., 1985; Linzer & Nathans, 1985; Schuler & Hurley, 1987). The presence of a consensus site for N-glycosylation suggests a likely site for addition of carbohydrates to the protein. In vitro translation of mRNA hybrid-selected by using our bPL cDNA reveals a single band at 27 kDa, in contrast to the two size forms of the protein isolated from placental secretory granules. This suggests that the variations in the

isolated protein are not due to different genes or differential splicing but rather to posttranslational modifications. We cannot exclude the possibility that the original protein isolate contained two discrete proteins, only one of which was amenable to sequencing. However, the complete match of both the amino-terminal and internal peptide amino acid sequence with the protein predicted by the cDNA indicates that this is not the case. The nature of the modifications that might cause different mobilities in SDS–PAGE is currently under investigation. The basis for the charge heterogeneity described for the protein (Byatt et al., 1986) is not apparent from these studies, although O-glycosylation, deamidation, and/or phosphorylation could account for the observations. Deamidation has been described for prolactin in several species (Haro & Talamantes, 1985; Lewis et al., 1985). However, all of the glutamine and asparagine residues predicted by the sequence of the cDNA were intact in the sequenced protein.

In the growth hormone–prolactin family of hormones, the protein domains responsible for interaction with their receptors are not yet understood despite extensive study. Many investigators have tried to pinpoint specific residues that may be important in the binding to lactogenic receptors. Despite its ability to bind to rabbit mammary gland prolactin receptors, bPL does not exhibit many of the amino acids that have been postulated as necessary for receptor binding. For example, bPL has no histidines at either equivalent of positions 27 or 30 (Andersen & Ebner, 1979), nor does it have a serine at position 62 (Kohmoto et al., 1984). It does, however, have a threonine at position 65 and a glutamic acid at position 20, a conservative substitution for aspartic acid (Dayhoff, 1972; Kohmoto et al., 1984). Nonetheless, the absence of many of these residues in bPL and the apparent low amino acid homology between bPL and the placental "lactogenic" hormones of other species are consistent with a more complex interaction with the receptor. Nicoll and co-workers (1986) have defined five domains that are conserved in lactogenic hormones (underlined in Figure 5). The deduced bPL amino acid residues are identical with or closely related to the consensus lactogenic residues, 66%, 66%, 57%, 80%, and 66%, respectively, in these clusters compared to 100%, 88%, 86%, 100%, and 78% for bPrl. However, the residues most highly conserved in their study are identical or acceptable replacements in bPL. The level of similarity within these regions is similar to the overall similarity to bPrl including acceptable substitutes (65%). Much work will have to be done before the nature of the interaction of these hormones with their respective receptors is understood. Availability of primary amino acid and nucleotide sequences for these placental lactogens and the other sequenced prolactin-related placental hormones in different species will provide the tools to resolve this question. It is noteworthy that bPL will also recognize growth hormone receptors (Arima & Bremel, 1982; Beckers et al., 1980; Murthy et al., 1982), as does ovine PL (Chan et al., 1976). However, the description of a distinct receptor for the ovine placental lactogen (Emane et al., 1986; Freemark et al., 1987) suggests that a receptor other than the classical lactogenic and somatotropic receptors may mediate the actions of these hormones.

Most of the studies of this hormone have been done in heterologous species. Little is known of the biological activities of bPL in the bovine species (Bremel & Schuler, 1987). Studies in homologous systems are needed to define the target tissues and receptors mediating the actions of these placental hormones with respect to fetal development and maternal adaptations to pregnancy. Availability of a large supply of pure recombinant protein will greatly aid these studies.

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Inactivation of Monoamine Oxidase A by the Monoamine Oxidase B Inactivators 1-Phenylcyclopropylamine, 1-Benzylcyclopropylamine, and *N*-Cyclopropyl-α-methylbenzylamine†

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ABSTRACT: Three known mechanism-based inactivators of beef liver mitochondrial monoamine oxidase (MAO) B are tested as inactivators of human placental mitochondrial MAO A. 1-Phenylcyclopropylamine (1-PCPA), 1-benzylcyclopropylamine (1-BCPA), and *N*-cyclopropyl-α-methylbenzylamine (*N*-CαMBA) are time-dependent irreversible inactivators of MAO A. The K_I values for 1-PCPA and *N*-CαMBA, analogues of the MAO B substrate benzylamine, are much higher with MAO A than with MAO B. Evidence is presented to show that 1-PCPA inactivates MAO A by attachment to the flavin cofactor, unlike the reaction with MAO B in which 1-PCPA can attach to both a cysteine residue and the flavin [Silverman, R. B., & Zieske, P. A. (1985) *Biochemistry 24*, 2128-2138]. The reaction of 1-BCPA with MAO A was too slow to study in detail. *N*-CαMBA exhibits the same properties toward inactivation of MAO A that it does for inactivation of MAO B. Attachment in both cases is shown to be to one cysteine residue per enzyme molecule. The results with 1-PCPA indicate that the active site topographies of MAO A and MAO B are different. The ability of *N*-CαMBA to undergo attachment to a cysteine residue in both MAO A and MAO B may lead the way toward peptide mapping of the two isozymes in order to determine differences in their primary structures.

Monoamine oxidase (MAO)[1] is one of the enzymes responsible for the catabolism of biogenic amines. Inhibitors of MAO have been used clinically for the treatment of depression for almost 30 years (Zeller, 1959). Tranylcypromine (*trans*-2-phenylcyclopropylamine), a member of the cyclopropylamine class of MAO inactivators that is currently in use as an antidepressant drug (Baldessarini, 1985), was shown to be a mechanism-based inactivator (Silverman, 1988) of MAO (Paech et al., 1980; Silverman, 1983). Other cyclopropylamine analogues, e.g., *N*-cyclopropylbenzylamine

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[1] Abbreviations: MAO, monoamine oxidase (EC 1.4.3.4); 1-PCPA, 1-phenylcyclopropylamine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); 1-BCPA, 1-benzylcyclopropylamine; *N*-CαMBA, *N*-cyclopropyl-α-methylbenzylamine.

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