Purification from Human Pregnancy Serum of a Low Molecular Weight Mitogen Similar to Placental Lactogen and Growth Hormone

(43280)

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Abstract. **Recently, we isolated from the serum of pregnant women a factor that induced rapid proliferation of a lactogen-dependent rat lymphoma cell line (Nb2). This mitogenic factor is reasonably specific to pregnancy, since it was present in serum samples from second trimester as well as term-pregnant women, but not in those of adult men or cycling females. It is unlikely that this mitogenic activity (referred to as pregnancy mitogen [PM]) is due to contamination by classical lactogens, since acetone fractionation of serum yielded a preparation devoid of placental lactogen and prolactin, as determined by radioimmunoassays. Further purification of acetone precipitates from term-pregnant serum by ion exchange chromatography and gel filtration yielded a mitogenic activity with a relative mol wt of approximately 10,000. PM activity in the NB2 cell bioassay was not affected by the presence of prolactin antiserum. However, its activity was immunoneutralized by coincubation with anti-placental lactogen serum and, to a lesser extent, anti-growth hormone serum. It appears that PM was not generated by our extraction procedure, since gel filtration of whole serum also yielded a bioactive fraction of approximately 10 kDa. PM was further purified to homogeneity by high-performance liquid chromatography. Examination of the preliminary amino acid composition of PM revealed differences from that of a bioactive fragment of growth hormone and a corresponding portion of placental lactogen, suggesting that PM could be either a molecular variant of these hormones or a novel protein.** [P.S.E.B.M. 1991, Vol 197]

A focus of this laboratory has been the characterization of a prolactin-induced lactogen that is released by liver tissue and is present in the serum of nursing rats. This liver lactogenic factor is functionally similar to prolactin (PRL), since it can stimulate casein release from individual mammary epithelial cells in culture (1, 2), but it differs from the hormone in that it lacks mitogenic properties as assessed by Nb2 lymphoma cell proliferation (3). In attempts to identify a similar factor in human serum, a low molecular weight peptide that possesses considerable mitogenic activity was inadvertently discovered. This paper provides a detailed account of the biochemical isolation, purification, and initial characterization of this mitogenic factor. For the sake of simplicity and clarity, this factor will henceforth be referred to as pregnancy mitogen (PM).

Materials and Methods

Reagents. Unless stated otherwise, all chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Tissue culture media and supplements were obtained from Gibco (Grand Island, NY). Solvents used for high-performance liquid chromatography (HPLC) were HPLC grade (Burdick and Jackson, Muskegon, MI). All water was ultrapurified by a Milli-Q Plus Water System (Millipore, Bedford, MA).

Acetone Precipitation of Serum. Blood samples designated as term-pregnant were taken at the time of patient admission (for normal deliveries) to either the

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Received February 14, 1991. [P.S.E.B.M. 1991, Vol 197]
Accepted April 24, 1991.

0037-9727/91/1974-0441$3.00/0

Medical University of South Carolina Hospital or Saint Francis Xavier Hospital, Charleston, SC. Blood samples obtained from women at various stages of pregnancy were taken during routine obstetric examinations performed at the Medical University of South Carolina Hospital. Blood was centrifuged and the serum decanted and stored at −80°C until extracted. Pools of term-pregnant serum (500–1900-ml batches) were mixed with acetone to achieve a final concentration of 60% acetone. This mixture was maintained at 4°C overnight, whereafter the insoluble pellet was removed by slow speed centrifugation (2,500*g*, 15 min). Ice-cold acetone was then added to the supernatant solution to achieve a final concentration of 90% acetone. After two days at 4°C, the bulk of the supernatant was syphoned off and the remaining material was centrifuged (10,500*g*, 15 min), the supernatant was decanted, and the pellet redissolved in water and lyophilized. Recovery ranged from 0.8 to 1.0 mg of 90% acetone precipitate per milliliter of whole serum.

Radioimmunoassays for hPRL and Placental Lactogen. An aliquot of 90% acetone precipitate was assessed for native hPRL and placental lactogen (hPL) content by assay systems performed by Specialty Laboratories, Inc., Santa Monica, CA, using a coated tube assay (Coat-A-Count; Diagnostics Products, Los Angeles, CA).

Ion-Exchange Chromatography. In preliminary ion-exchange runs, acetone precipitate was first dialyzed (3500 mol wt cut-off) against 50 m*M* Tris (pH 7.8) to remove a very low molecular weight material that interfered with optical density readings of column effluents. Diethylaminoethyl cellulose (Whatman DE52; Maidstone, England) was equilibrated with 50 m*M* Tris and placed into a 2.5 × 12-cm column. Approximately 500 mg of the acetone precipitate were dissolved in 50 m*M* Tris (pH 7.8) and loaded onto the column. After elution of unbound material, a linear gradient (consisting of 150 ml of 50 m*M* Tris [pH 7.8], in Vessel I and 150 ml of the same buffer, but containing 0.5 *M* sodium chloride in Vessel II) was started. The flow rate was adjusted to 30 ml/hr and approximately 7-ml fractions were collected. The effluent from this and subsequent columns was monitored by ultraviolet absorption at 280 nm. Fractions were desalted by dialysis (3500 mol wt cut-off) against water, lyophilized, and reconstituted in 3 ml of water prior to bioassay.

Gel Filtration. Bioactive fractions from ion-exchange chromatography were lyophilized, reconstituted in 1.0 *M* acetic acid, and then subjected to gel filtration on a 2.5 × 55-cm column of Sephadex G-75 superfine (Pharmacia, Gaithersburg, MD) in 1.0 *M* acetic acid. The flow rate was 5 ml/hr and the volume collected in each tube was 5 ml. The fractions obtained were lyophilized and dissolved in 3 ml of water prior to bioactivity tests.

High-Performance Liquid Chromatography. Bioactive fractions from gel filtration were pooled, lyophilized, and subjected to analytical high-performance liquid chromatography on an Aquapore RP 300 7-μm C_8 column (2.1 × 30 mm) using an ABI 130-A Separation System (Applied Biosystem, Inc., Foster City, CA). Solvent A consisted of 0.1% trifluoroacetic acid in water and Solvent B of 0.1% trifluoroacetic acid in 80% acetonitrile. Detailed information of the gradient is given in the Legend to Figure 8. All separations were performed with a flow rate of 0.1 ml/min and protein elutions were monitored at 230 nm.

Amino Acid Analysis. PM was hydrolyzed in vapor phase with 6 *M* HCl containing 1.0% phenol at 105°C for 24 hr, followed by derivatization of amino acids with phenylisothiocyanate. Amino acid analysis was performed on a Pico-Tag Amino Acid Analyzer System (Waters, Milford, MA). The results were evaluated by the method of Black and Hogness (4), adapted for an Apple IIe computer.

Bioassay: Nb2 Lymphoma Cell Line. The Nb2 cell bioassay was performed according to the method of Tanaka *et al.* (5) with minor modifications. Nb2 cells were obtained from Dr. P. Gout (University of British Columbia, Vancouver BC, Canada) and maintained as suspension cultures in Fischer's medium supplemented with 10% gelding serum (Crowther Serum Supply, La Para, CO), 10% fetal bovine serum, 100 μ*M* 2-mercaptoethanol (2-ME), 50 units/ml of penicillin, and 50 μg/ml of streptomycin in an atmosphere of 5% CO_2 and 95% air at 37°C. Cell density was maintained between 1×10^4 cells/ml and 8×10^5 cells/ml. Under these conditions, the cell population doubles every 14 hr. Approximately 24 hr prior to use in the bioassay, cells were transferred to medium containing 10% gelding serum, 1% fetal bovine serum, 100 μ*M* 2-ME, and antibiotics to slow their proliferation. After this period, cells were collected and washed three times by centrifugation in assay medium containing 10% gelding serum, 100 μ*M* 2-ME, and antibiotics. Aliquots of the cell suspension (1.8 ml) were placed in 12-well plates (Costar, Cambridge, MA) at a density of 1.85×10^5 cells/ml. Treatments, prepared in 0.2 ml of water (0.2 ml of water served as controls), were added to the cells and the plates were incubated for 40–48 hr. After incubation, the number of viable cells, as assessed by trypan blue dye exclusion, was determined. Values are reported as a percentage of control. Bioactivity of purified fractions from gel filtration was compared to purified preparations of hPRL (NIDDK-hPRL-I-7, AFP-9900), human growth hormone (hGH) (NIDDK-hGH-I-1, AFP-4793B), and hPL (NIADDK-hPL), which were generously provided by the National Institute of Diabetes and Digestive and Kidney Diseases

(NIDDK) and National Hormone and Pituitary Program (NHPP), University of Maryland, School of Medicine (Baltimore, MD).

Immunoneutralization. Assessment of antigenic similarities of PM to hPRL, hGH, and hPL was accomplished by incubating bioactive fractions from gel filtration or the native lactogens with highly specific antisera to these hormones. Antisera were obtained from NIDDK, NHPP, University of Maryland School of Medicine (NIDDK-anti-hPRL-1C-4, AFP-9823102188; NIDDK-anti-hGH-1C-3, AFP-C11981A; NIDDK-anti-hPL-2, AFP-C1801010). In addition, the effectiveness of an anti-hPL obtained from a commercial source (Sigma) was tested. The antisera provided by NIDDK, NHPP were diluted in phosphate-buffered saline containing 0.01% merthiolate, which was cytotoxic. Therefore, prior to use, antisera were dialyzed (3500 mol wt cut-off) against PBS to remove the merthiolate. Anti-hGH and anti-hPL sera from NIDDK, NHPP were used at a working concentration of 1:20,000, while anti-hPRL serum and the anti-hPL serum from Sigma were used at a final concentration of 1:1,000.

Results

Bioactivity of Acetone Precipitates. Preliminary studies were conducted to determine the relative concentration of mitogenic activity in sera obtained from individuals in various reproductive states. Figure 1 demonstrates that acetone precipitates of pooled serum samples obtained between 14 weeks of pregnancy and term stimulated the proliferation of Nb2 lymphoma cells. In contrast, acetone precipitates of sera from men and cycling women were inactive in this bioassay system. The activity demonstrated by acetone precipitates of sera from pregnant women was not attributable to native hPL or hPRL, since both of these lactogens were nondetectable by radioimmunoassay in acetone precipitates diluted to whole serum equivalents (data not shown).

Preliminary experiments were conducted (data not shown) to obtain general information about the chemical properties of PM. In short, we found that freezing and lyophilization did not destroy PM activity of acetone-precipitated pregnancy serum. The stability of this substance under these conditions was beneficial because it enabled us to concentrate and store the material frozen for subsequent purification. In contrast, PM activity in acetone precipitate was virtually abolished by treatment with HCl (pH 1.0 for 30 min, followed by neutralization with NaOH and centrifugation to remove the precipitate) and attenuated by heat (95°C for 30 min, followed by centrifugation). Also, it was severely attenuated by a 2-hour incubation with pepsin. The results of these experiments indicated that the lactogen was a protein or peptide.

Further purification of this mitogenic factor was accomplished in three separate batch purifications of serum from term-pregnant women. The first consisted of 2 liters of serum that provided material (through gel filtration) for immunoneutralization experiments and dose-response characterization. The second batch consisted of 5 liters of starting material that was purified through one run of analytical HPLC. This provided information on the appropriate gradient that would ultimately achieve a homogeneous peak. In the third batch, a final 5 liters of serum were purified, which resulted in an homogeneous peak on HPLC and information regarding amino acid composition.

Column Chromatography. Crude extraction of PM by acetone precipitation was followed by anion exchange chromatography (Fig. 2). PM is a negatively charged molecule, in that it eluted from the column at approximately 0.2 *M* NaCl. The bioactive peak was always associated (in 30 runs) with the descending portion of the first major protein peak beyond the breakthrough fractions.

Active fractions from ion exchange were subsequently subjected to gel filtration, with the bioactivity eluting at a relative mol wt of 8,000–12,000 (Fig. 3). No bioactivity was associated with fractions eluting at positions corresponding to mol wt 20,000 or greater, indicating bioactivity was not associated with native hGH, hPL, or hPRL.

Comparison of Dose-Response Characteristics of PM to Known Lactogens. A comparison of dose-response curves for PM (bioactive fractions from gel filtration) and the established lactogens is illustrated in Figure 4. It is clear that the dose-response characteristics of PM are similar to those of hPRL and hGH. Moreover, the maximal efficacy (response) was comparable to those of the pituitary lactogens. This differs from the situation for hPL, which in our hands evoked a maximal response consistently lower than those of the other substances tested.

PM Is Structurally Related to hPL and hGH, but Not hPRL. The effectiveness of PM in the lactogen-dependent Nb2 cell assay suggested that it might possess sequence homology with some of the more established lactogens. In an attempt to explore this possibility, we tested whether antisera to hPL, hGH, or hPRL would affect the activity of purified PM in the Nb2 assay. The effects of hPL and hGH antisera on PM activity are presented in Figure 5. As can be seen, a dilution of anti-hPL serum that was effective in neutralizing the activity of a maximal dose of hPL also abolished the activity of PM. In contrast, the activity of PM was only partially suppressed by the presence of hGH antiserum. Since both hGH- and hPL-antiserum cross-react with the heterologous antigen in radioimmunoassays, we tested their ability to immunoneutralize hGH or hPL (5000 ng) in the Nb2 assay. As would be expected, the activity of the hGH and hPL was attenuated with

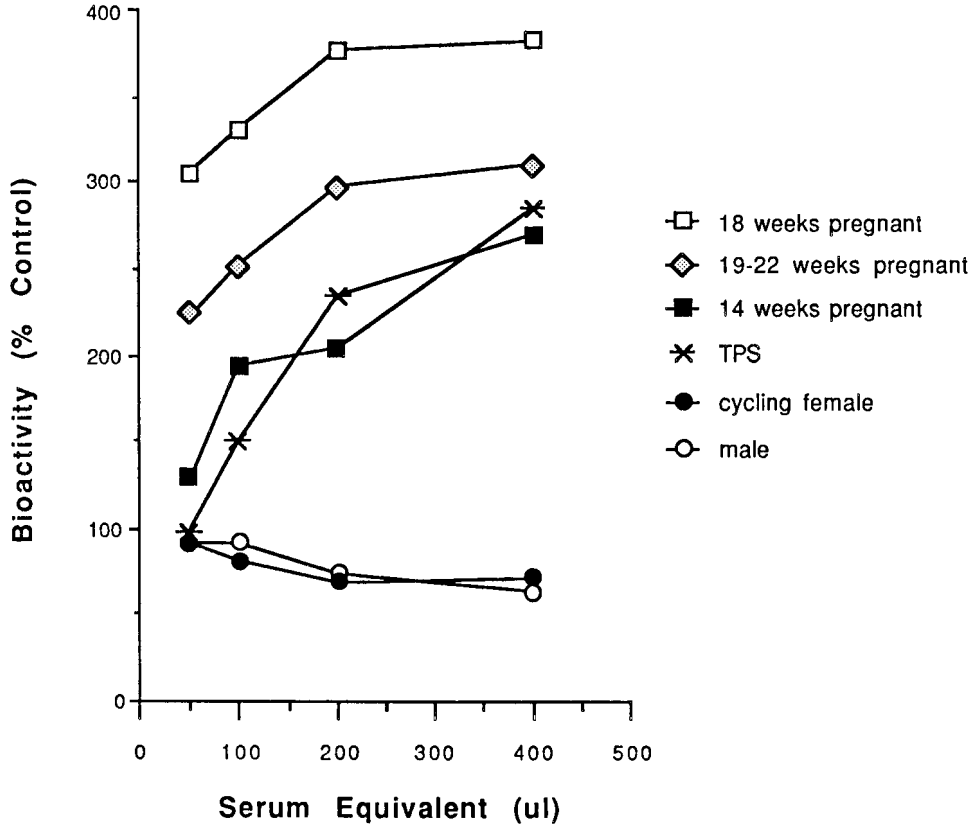

Figure 1. Comparison of mitogenic activity of 90% acetone precipitates of pooled serum samples from women at 14 weeks of pregnancy, 19–22 weeks of pregnancy, and term (TPS) with that of serum acetone precipitates from randomly cycling women and men. In this and subsequent figures, values are expressed as percentage of control and each point represents the mean of triplicate determinations. Data presented are representative of those obtained in two additional experiments.

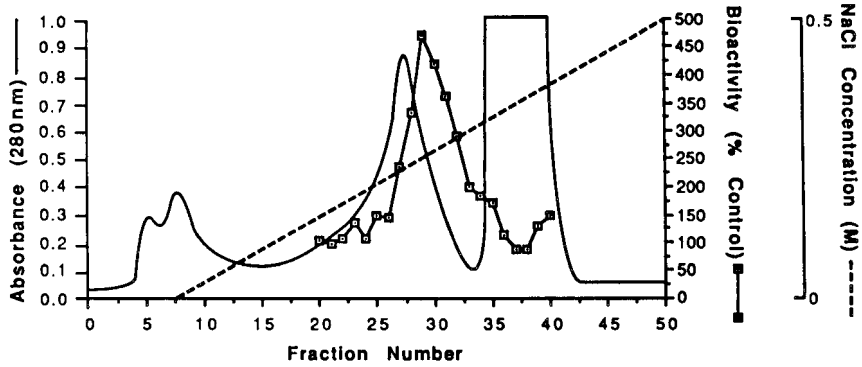

Figure 2. DE52 cellulose chromatography of 90% acetone precipitates of serum from term-pregnant women. The column (2.5 × 12 cm) was equilibrated with 50 m*M* Tris (pH 7.8) and eluted using a linear gradient (300 ml) at 0–0.5 *M* NaCl (shown as a dashed line). Solid lines represent the absorbance at 280 nm of the protein eluted. Bioactivity of each fraction is denoted by open squares.

heterologous antiserum, but not completely abolished (data not shown). The effectiveness of hPRL (which does not cross-react with either antisera) indicates that the actions observed were specific and the effect was not due to a toxic effect of the antisera on Nb2 cells. To control for the possibility that these effects were peculiar to the antibody preparations tested, we repeated these experiments with a different antiserum to hPL, and virtually identical results were obtained (data not shown). On the other hand, an antiserum capable of abolishing hPRL activity had no effect on the actions of purified PM (Fig. 6). Thus, it appears that PM shares epitopes common with hPL and, to a lesser extent, with hGH. However, this immunological similarity does not extend to hPRL.

PM Is Not an Extraction Artifact. The immunological similarities between PM and the lactogens hPL and hGH raised the possibility that the 10-kDa mole-

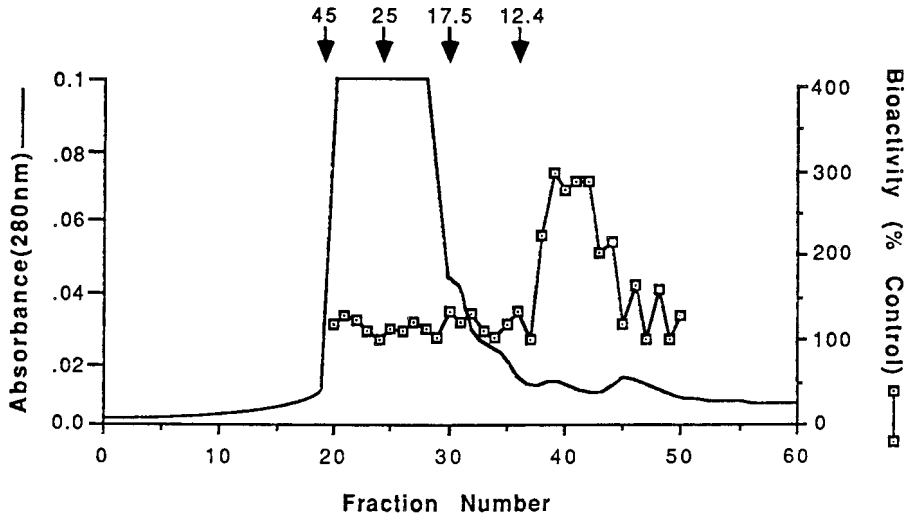

Figure 3. Gel filtration of bioactive fractions from ion exchange chromatography. Lyophilized fractions were acidified and loaded on a Sephadex G-75 superfine column (2.5 × 55 cm) in 1.0 *M* acetic acid. Protein eluted from the column is shown as a solid line, whereas bioactivity of each fraction is represented by open squares. Molecular weight markers were loaded following the sample run and their relative elution position is represented by the arrows (ovalbumin, mol wt 45,000; chymotrypsinogen, mol wt 25,000; myoglobin, mol wt 17,500; cytochrome C, mol wt 12,400).

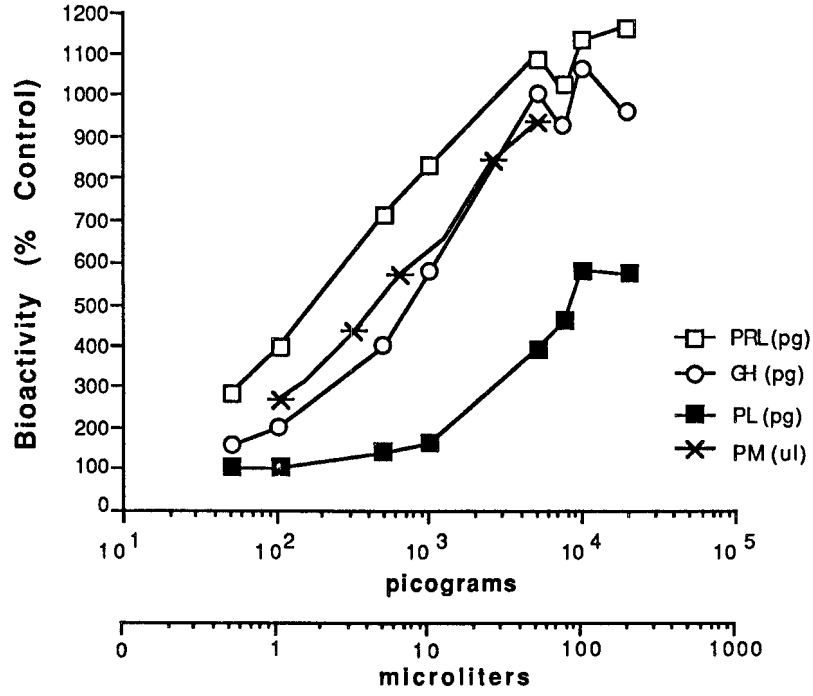

Figure 4. A comparison of dose-response curves for pregnancy mitogen (μl of material purified by gel filtration) and established human lactogens. Each point represents the mean of triplicate wells within a single representative experiment.

cule may have arisen as an artifact of extraction (i.e., a cleavage of the hPL or hGH molecule). Therefore, we subjected unextracted serum to gel filtration in an attempt to determine whether the 10-kDa peptide was present. This was accomplished by acidifying 4 ml of term-pregnancy serum (a pool from eight different individuals) to the pH of the column effluent, and subjecting this preparation directly to gel filtration. The biological activity recovered in the various fractions is plotted in Figure 7. Two distinct regions of biological activity were obvious. The first of these eluted at a point consistent with the molecular weights of hPL, hGH, and hPRL. The second peak of activity had an apparent molecular weight identical to that obtained when serum was subjected to acetone fractionation and ion exchange chromatography prior to gel filtration.

Purification of PM to Apparent Homogeneity. Bioactive fractions from gel filtration were further purified by HPLC. Isolation of bioactivity associated with a symmetrical and homogeneous peak was achieved by

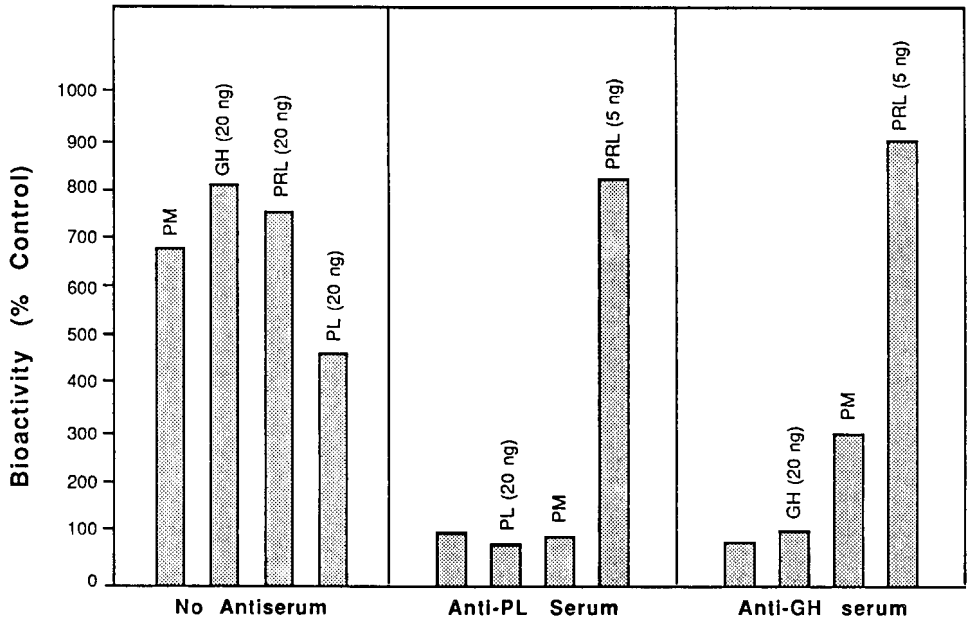

Figure 5. Bioactivity following coincubation of a maximal dose of purified pregnancy mitogen (PM after gel filtration), with anti-hPL serum and anti-hGH serum (NIDDK, NHPP; 1:20,000). Coincubation of antiserum with maximal doses of homologous antigen (hPL or hGH) or heterologous antigen (hPRL) were used as internal controls for potency and specificity.

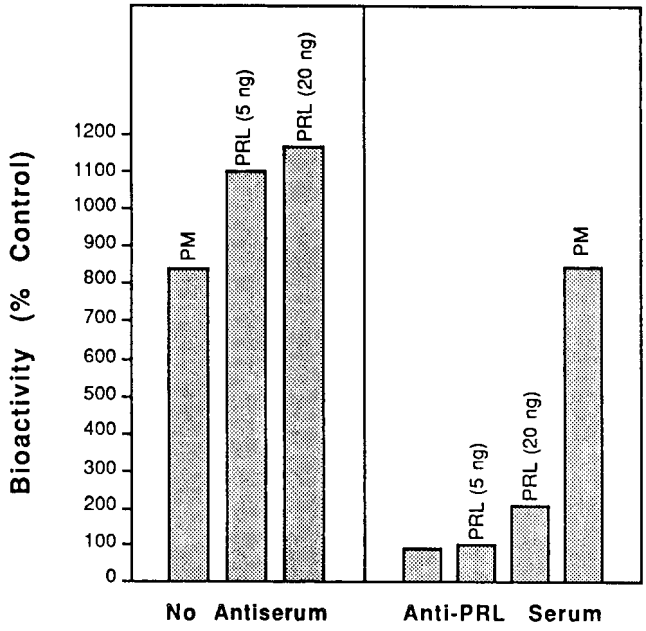

Figure 6. Bioactivity of a maximal dose of purified pregnancy mitogen or hPRL following coincubation with anti-hPRL serum (NIDDK, NHPP; 1:1000).

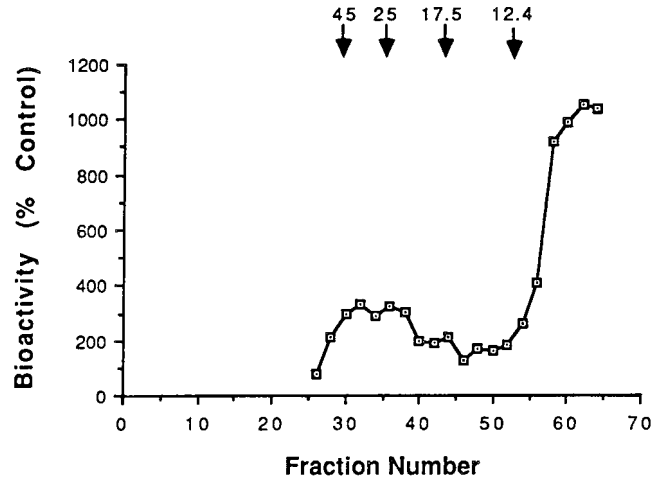

Figure 7. Bioactivity of acidified whole serum (4 ml) from term-pregnant women after gel filtration on Sephadex G-75 superfine column in 1.0 *M* acetic acid. Arrows denote the elution of molecular weight markers (same as in Fig. 3).

three sequential HPLC runs as depicted in Figure 8. In the initial run (Fig. 8A), bioactivity was not associated with a distinct protein peak, but eluted at the descending portion of the last peak. The late elution of PM suggests that it is a highly hydrophobic molecule. Rechromatography (Fig. 8B and C, respectively) of the bioactive fraction resulted in a single symmetrical peak possessing bioactivity, indicating PM was purified to apparent homogeneity.

Amino Acid Analysis of PM. All of the purified fraction (20 pmol) was hydrolyzed and subjected to amino acid analysis in a single test (Table I). This material represented 5 liters of serum. Given that antigenic similarities exist among PM, hPL, and hGH, an amino acid composition of a previously reported fragment of hPL (residues 1–99) and a comparable fragment of hGH (residues 1–99) are also included in Table I. The amino acid composition of PM differs from both hPL (residues 1–99) and hGH (residues 1–99).

Discussion

The data presented here describe a 10-kDa peptide which we designate PM. This peptide possesses mito-

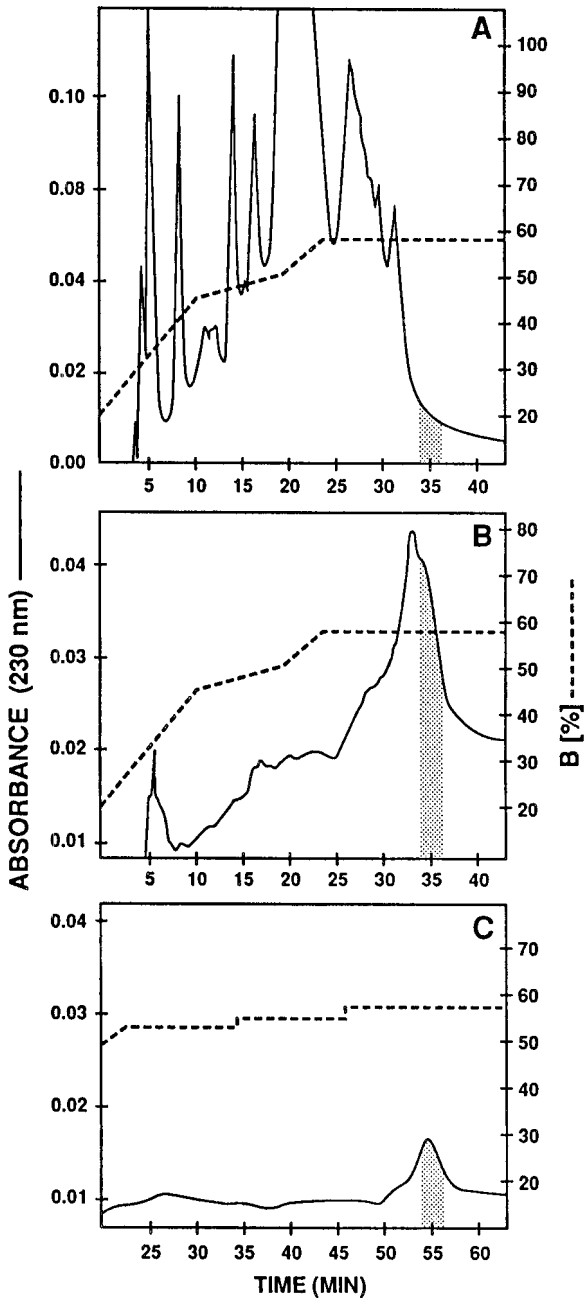

Figure 8. Analytical high-performance liquid chromatography of purified pregnancy mitogen on an Aquapore RP 300 7-μm column (2.1 × 30 mm) using the solvent systems A, 0.1% trifluoroacetic acid in water, and B, 0.1% trifluoroacetic acid in 80% acetonitrile. The proteins (measured at 230 nm, represented by solid lines) were separated using the gradient denoted by the dashed line. For HPLC runs represented in Panels A and B, linear gradients were used consisting of 20–45% B for 10 min, followed by 45–50% B for 10 min and 50–57.8% B for 5 min, then maintained at 57.8% B for 30 min. The gradient in Panel C was identical to that of Panels A and B during the first 22 min (up to 53.3% B), then a step gradient was used; 53.3% B for 10 min, 55.0% B for 10 min, and 57.8% B for 30 min. Panel A is a representative chromatogram of an initial HPLC run, comprising one eighth of the purified material obtained from 5 liters of pooled serum from term-pregnant women. The shaded area represents bioactive fractions which were pooled from seven individual HPLC runs and rechromatographed (Panel B). Bioactive fractions from rechromatography (shaded area) were rerun (Panel C) resulting in a bioactive peak that appeared to be symmetrical.

Table I. Amino Acid Analysis of Pregnancy Mitogen and Theoretical Fragments of PL and GH

	Pregnancy mitogen	Theoretical fragments	
		PL (1-99)[a]	GH (1-99)
Asp/Asn	5.17 (5)[b]	8	7
Thr	6.01 (6)	6	5
Ser	7.19 (7)	11	11
Gln/Glu	16.00 (16)	16	17
Pro	4.37 (4)	5	7
Gly	5.37 (5)	0	0
Ala	6.81 (7)	5	5
Cys	ND[c]	1	1
Val	5.74 (6)	3	2
Ile	1.77 (2)	5	5
Leu	9.60 (10)	13	15
Tyr	1.99 (2)	3	3
Phe	4.85 (5)	7	8
His	2.11 (2)	4	2
Lys	5.68 (6)	3	3
Arg	3.16 (3)	5	6
Met	ND	3	1
Trp	ND	1	1
Fit factor	0.359 (at mol wt 9560)		

[a] See ref. 10.
[b] Values in parenthesis represent the closest integers.
[c] ND, not determined.

genic activity in a lactogen-dependent assay system and shares epitopes common to both hPL and hGH. These biological and antigenic similarities suggest that this low-molecular weight factor could be a fragment of a native lactogen. Indeed, a precedent for proteolytic degradation of hGH to low-molecular weight bioactive fragments has been documented (6–8). But when one considers that PM is found only in serum samples of pregnant women and not those of men or reproductively cycling women, and that hPL concentrations markedly increase during pregnancy whereas those of circulating hGH do not change appreciably, the most likely parent protein for a putative fragment is hPL (mol wt 21,600), which circulates at extremely high concentrations from the first trimester to term (0.3–6.8 μg/ml; for review, see Ref. 9). Consistent with this view is the observation of Neri and co-workers (10), who reported the presence of nicked components of purified hPL. Four fragments were detected when hPL was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the presence of 2-ME. The four fragments were designated as α, β, γ, and δ and had apparent molecular weights of 16,000, 12,200, 8,800, and 5,900, respectively. The presence of these fragments suggested two cleavage sites within the large loop of hPL, one creating fragments α and δ (combined mol wt 22,000) and another creating the β- and γ-components (combined mol wt 21,000). Unfortunately, information regarding the bioactivity of these fragments was not provided. Therefore, in the present study, extrapolation

of functional data about the bioactivity of hGH fragments was used to predict possible bioactive fragments of hPL, because hPL is both structurally and functionally related to hGH (for review, see Ref. 11). In this regard, a 15-kDa N-terminal fragment of hGH generated by protease treatment and reduction was found to retain activity in both somatotropic and lactogenic assay systems. In contrast, a 7-kDa C-terminal fragment that was generated simultaneously was devoid of activity. These observations, coupled with amino acid-substitution experiments performed on both hGH and hPL, confirm that the N-terminal residues are essential for bioactivity.

Inasmuch as the N-terminal portion of both hGH and hPL govern bioactivity and the apparent molecular weight of PM is approximately 10,000, comparisons of amino acid compositions were made with that of the β-fragment of hPL (N-terminal residues 1–99) reported by Neri *et al.* (10) and a comparable fragment of hGH (residues 1–99), both of which have approximate molecular weights of 12,000. Although our data regarding the composition of PM is preliminary, initial comparisons revealed differences among PM, hPL (residues 1–99), and hGH (residues 1–99). Both hPL (residues 1–99) and hGH (residues 1–99) are devoid of Gly, whereas PM contains five Gly residues. In addition, the ratio of Lys to Arg (6:3) in PM is the reverse of that found in hPL (residues 1–99) or hGH (residues 1–99). Although PM possesses both antigenic and biological characteristics similar to hPL and hGH, the preliminary data presented herein suggest that PM might be a protein separate and distinct from these known lactogens.

Additional evidence which would suggest the uniqueness of PM is the fact that purification procedures were conducted under nonreducing conditions. In all previous reports, fragments could be identified only when disulfide linkages (found between Cys residues 79–181 and 208–215 for both hPL and hGH) were reduced by 2-ME. If indeed PM is an N-terminal fragment of hPL or hGH (the bioactivity of which is dependent on residues 1–134) (11), then one would expect, under nonreducing conditions, the formation of homodimers (by Cys 79) and an apparent molecular weight far greater than 10,000. Taking this into account, along with the preliminary data regarding amino acid composition, it is tempting to speculate that PM may be a novel protein.

The possibility that PM is a fragment of a native lactogen cannot be totally discounted until PM is sequenced. Therefore, the question arises: Is PM an extraction artifact? This concern is a legitimate one since it has been shown recently that a biologically active 16-kDa fragment of PRL could be generated by acid proteases during a preparative dialysis step (12). To determine whether PM was a result of our extraction procedure, whole serum was acidified and subjected directly to gel filtration, thus circumventing both acetone fractionation and ion exchange chromatography. As anticipated, fractions from gel filtration of unextracted serum that eluted in the range of 20,000–22,000 (mol wt of native lactogens) were active in stimulating Nb2 cell proliferation. It was surprising, however, that the relative bioactivity was greatest in fractions corresponding to molecular weights of less than 12,400. Therefore, activity similar (if not identical) to PM is present in whole serum.

The existence of lactogen-like molecules distinct from hPL and hGH is not without precedent in humans. Hennen and colleagues (13, 14) reported the presence in normal placenta of a GH variant (hGH-V) which shares the N-terminal epitope but lacks an internal epitope of pituitary GH. While this molecule binds to GH receptors in pregnant rabbit liver, its biological activity remains to be established. In a related report, screening of a placental cDNA library with a hGH-V-specific oligonucleotide revealed the existence of a unique mRNA that predicts the expression of yet another GH variant that differs considerably from hGH-V and native pituitary GH (15). The same group that identified the initial hGH-V also used two well-characterized polyclonal antisera to measure immunoreactive hPL in extracts of a placenta obtained from a donor lacking the genes for hPL and the human placental GH variant. Surprisingly, one of their antisera detected considerable amounts of a placental lactogen-like material, whereas the other did not (16). It seems, then, that the presence of a new mitogen in placenta may have been "unmasked" by the absence of native hPL. In light of the recent characterization of lactogen-like molecules that are distinct from the traditional lactogens—hPL, hGH, and hPRL—the existence of yet another such compound, PM, does not seem unreasonable.

This work was supported by National Institutes of Health Grant DK 38215 (L. S. F.) and National Research Service Award DK 08420 (R. D. K.). The authors wish to thank William J. Faught for his superior technical assistance, Thomas F. Figliorelli for diligent sample collection, and Mary Ackerman for her patience in preparing the manuscript.

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