Pregnant Mare Serum Gonadotropin

PURIFICATION AND PHYSICOCHEMICAL, BIOLOGICAL, AND IMMUNOLOGICAL CHARACTERIZATION*

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Procedures have been developed for the purification of pregnant mare serum gonadotropin (PMSG) and its $\alpha$ and $\beta$ subunits. The procedure for the hormone purification involves three steps of column chromatography on Sephadex G-100, DEAE-Sephadex A-50, and hydroxyapatite. The preparation of subunits involves the dissociation of PMSG with 10 M urea followed by their separation by chromatography on DEAE-Sephadex and Sephadex G-100. The hormone and subunit preparations were found homogeneous by electrophoresis in polyacrylamide gel with or without sodium dodecyl sulfate and by immunodiffusion. The hormone had an activity of 13,740 I.U./mg as determined by in vivo and in vitro bioassays and receptor binding assays. The subunits did not show any significant activity by the receptor binding assay. The molecular weights of PMSG, PMSG-$\alpha$, and PMSG-$\beta$, determined from Ferguson plots (Ferguson, K. A. (1964) Metabolism 13, 985-1002) using glycoproteins as molecular weight markers, were 64,030, 43,720 and 16,960, respectively. The amino acid and carbohydrate compositions of the hormone and the subunits have been determined. The carbohydrate content of the hormone was 41.7% and the $\alpha$ and $\beta$ subunits contained 20.6 and 45.6% carbohydrate, respectively (uncorrected for moisture content of protein). The carbohydrate moiety of the hormone is made up of L-fucose (0.6 to 0.9%), D-mannose (2.0 to 2.3%), N-galactose (10.6 to 12%), N-acetylglucosamine (9.0 to 10.5%), N-acetylgalactosamine (3.0 to 3.5%), and sialic acid (12.0 to 14.0%).

The purified PMSG was found to be three times as active as ovine lutropin (LH, luteinizing hormone) (2.3 NIH-LH-SI units/mg) and 2/3 as active as ovine follicle-stimulating hormone (FSH, follicle-stimulating hormone) (115.3 NIH-FSH-SI units/mg). FSH activity was determined by the Steelman-Pohley assay (Steelman, S., and Pohley, F. (1953) Endocrinology 53, 604-616) and the LH activity by the Steelman-Pohley assay (Steelman, S., and Pohley, F. (1953) Endocrinology 53, 604-616). The activity was measured by acrobic acid depletion assay. As determined by binding assay, the individual subunits upon recombination recovered 27.2% of the LH activity and 62.5% of the FSH activity. Immunologically, PMSG-$\alpha$ and PMSG-$\beta$ cross-reacted with anti-PMSG as found by radioimmunoassay. While human chorionic gonadotropin (hCG) and human-luteinizing hormone (hLH) competed with $^{125}$I-PMSG in the PMSG receptor binding assay, they showed little or no cross-reactivity in the radioimmunoassay, indicating that the receptor and antibody binding sites are different from each other.

Various glycoprotein hormones, such as luteinizing (LH), follicle-stimulating (FSH), thyroid-stimulating, and human chorionic gonadotropin (hCG), have been purified and characterized in detail (1-12). Little, however, is known about the detailed chemistry and properties of pregnant mare serum gonadotropin (PMSG), a glycoprotein hormone that possesses both FSH1 and LH activities. Various procedures utilizing commercial preparations have been described for the isolation of PMSG (13-15). However, the yields of the purified hormones have been poor, presumably because of the use of low pH buffers in some of the purification schemes. These conditions are not optimal since it has been found that low pH is destructive to glycoprotein hormones, causing the hydrolysis of labile ketosidic bonds of sialic acid (16). Furthermore, thus far only limited physicochemical, biological, and immunological characterization of PMSG and its subunits has been carried out (13-15, 17, 18). In this communication, we wish to report methods for the purification of PMSG and its subunits, which not only are reproducible but also give high yields. The methods are suitable for large scale preparations of the hormone and the subunits which are prerequisites for carbohydrate and amino acid sequence studies. In addition, this communication describes the detailed chemical composition, particularly that of the carbohydrate in PMSG and the subunits, and our findings on the molecular weights of the hormone and subunits. Also discussed are biological and immunological relationships of PMSG and its subunits to hCG, ovine LH, and FSH and their subunits.

RESULTS\textsuperscript{2}

Purification of PMSG

A crude preparation of PMSG (1,660 I.U./mg) was resolved into two peaks on gel filtration on Sephadex G-100 (Fig. 1) with tubes 75 to 110 containing most of the activity (3,200

\textsuperscript{1} The abbreviations used are: FSH, follicle-stimulating hormone; LH, luteinizing hormone; hCG, human chorionic gonadotropin; PMSG, pregnant mare serum gonadotropin; oLH, ovine-luteinizing hormone; oFSH, ovine follicle-stimulating hormone; SDS, sodium dodecyl sulfate.

\textsuperscript{2} Purified of this paper (including "Materials and Methods," Figs. 1 to 5 and 9 to 13, and Tables I to VII) are presented in miniprint at the end of this paper. The abbreviation used is BSA, bovine serum albumin. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 180-1684 (see authors) and include a check or money order for $3.75 per set of photocopies.

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I. U./mg). A 1.9-fold purification was obtained (Table I). The active fractions were subjected to further purification on a DEAE-Sephadex A-50 column with a discontinuous stepwise gradient. The active material was eluted with 0.15 M NaCl in 0.04 M Tris/PO₄ buffer. The active fractions (190 to 230 and 260 to 300) were pooled as indicated in Fig. 2, resulting in two preparations, the first having a potency of 10,300 I.U./mg and the second having a potency of 9,200 I.U./mg (Table I). The final purification was achieved with a hydroxyapatite column (Fig. 3). The material from each active peak in Fig. 2 was subjected separately to chromatography on a hydroxyapatite column. The active fractions from the hydroxyapatite column showed a final activity of 13,740 and 11,330 I.U./mg (material from the first and second active peaks in Fig. 2, respectively). The activities were determined by both binding and bioassay and they represent a respective 7.8- and 6.8-fold increase in purity from the original crude preparation. The final recovery of activity was 44.2%.

Preparation of Subunits

PMSG was found to consist of two dissimilar subunits, α and β, which were separated on the basis of both charge and size. The first separation involved chromatography on DEAE-Sephadex A-50 with a stepwise discontinuous gradient. The urea-dissociated PMSG gave two distinct peaks. The first peak, designated as PMSG-α, appeared with the starting buffer; the second, designated as PMSG-β, appeared with 0.15 M NaCl in 0.04 M Tris/PO₄ (Fig. 4). After DEAE-Sephadex A-50 chromatography, the α and β subunits were desalted by using a column of Sephadex G-25 (coarse), equilibrated in 0.5% NH₄HCO₃. The recovery of the β subunit, consistently found in several preparations of the subunits, was about 2 to 3 times that of the α subunit by weight. In order to determine whether there was a contamination of PMSG in the β subunit preparation, the β subunit was reincubated in urea and rechromatographed on DEAE-Sephadex A-50. Only a single peak was obtained, indicating that the β subunit was probably free of contamination from the α subunit. The second separation involved the application of a solution of 2.5 mg of the urea-dissociated PMSG to a column of Sephadex G-100 equilibrated with 0.1 M sodium acetate buffer, pH 5.5. Two distinct, well resolved peaks were seen, the first, of the β subunit, being larger according to absorbance at 230 nm than the second, of the α subunit (Fig. 5). Recovery according to weight was 1.7 mg for β and 0.4 mg for α. Identification of the α and β subunits was made by amino sugar analyses of the subunits and compared to analyses of the subunits prepared by chromatography on DEAE-Sephadex A-50. In both cases, the α subunit lacked N-acetylglucosamine, a component of PMSG-β, indicating that the subunits were free of cross-contamination. In order to ensure the purity of the subunits, it is advisable to prepare the subunits initially by DEAE-Sephadex chromatography and then subject each subunit, after further treatment with 10 M urea, to additional purification by gel filtration on Sephadex G-100. (Figs. 4 and 5).

Homogeneity of PMSG and Its Subunits

The purity of PMSG was examined by disc and SDS-gel electrophoresis and by the Ouchterlony agar immunodiffusion technique. PMSG was shown to be homogeneous by disc gel electrophoresis in 7% polyacrylamide gel, pH 8.3, at various stages of purification (Coomassie blue staining). Electrophoresis was carried out for 1 h at 3 mA/tube. A, crude PMSG; B, after gel filtration on Sephadex G-100; C, after chromatography on DEAE-Sephadex A-50; D, after chromatography on hydroxyapatite; E, PMSG-α; F, PMSG-β. Lower panel, SDS-gel electrophoresis. Electrophoresis in polyacrylamide gels, pH 7.0, was carried out for 4 h at 8 mA/tube. G, PMSG after chromatography on hydroxyapatite; H, PMSG-α (G and H were stained with Coomassie blue); I, PMSG after chromatography on hydroxyapatite; J, hCG (10,000 I.U./mg); K, ovomucoid; L, α-acid glycoprotein (I, J, K, and L were stained with periodic acid-Schiff reagent for glycoproteins).

Fig. 6. Upper panel, disc gel electrophoresis of PMSG in 7% polyacrylamide gel, pH 8.3, at various stages of purification (Coomassie blue staining). Electrophoresis was carried out for 1 h at 3 mA/tube. A, crude PMSG; B, after gel filtration on Sephadex G-100; C, after chromatography on DEAE-Sephadex A-50; D, after chromatography on hydroxyapatite; E, PMSG-α; F, PMSG-β. Lower panel, SDS-gel electrophoresis. Electrophoresis in polyacrylamide gels, pH 7.0, was carried out for 4 h at 8 mA/tube. G, PMSG after chromatography on hydroxyapatite; H, PMSG-α (G and H were stained with Coomassie blue); I, PMSG after chromatography on hydroxyapatite; J, hCG (10,000 I.U./mg); K, ovomucoid; L, α-acid glycoprotein (I, J, K, and L were stained with periodic acid-Schiff reagent for glycoproteins).
The molecular weights of PMSG, PMSG-α, and PMSG-β were also estimated by gel filtration on Sephadex G-100 using glycoproteins and protein markers for the calibration of the column. When glycoproteins were used as standards, the molecular weights calculated from the plot of log of molecular weight versus V₀/Vₑ were 18,500, 57,500, and 65,000 for PMSG-α, PMSG-β, and PMSG, respectively (Fig. 10). However, the protein markers gave higher values of 26,000, 64,000, and 71,000 for PMSG-α, PMSG-β, and PMSG, respectively (Fig. 10).

**Amino Acid and Carbohydrate Compositions of PMSG and Its Subunits**

Table II describes the chemical compositions of PMSG, hCG (16), oLH (39) and oFSH (40). Like hCG and other glycoprotein hormones, PMSG is rich in proline and half-cystine. Also notably high are threonine and serine. The carbohydrate moiety of PMSG is made up of sialic acid, L-fucose, D-galactose and D-mannose, N-acetylgalcosamine, and N-acetylgalactosamine. Occasionally, D-glucose was observed in PMSG preparations but was found to be a contaminant and could be removed by chromatography on a column of Sephadex G-25 (coarse). The total carbohydrate content of PMSG (uncorrected for water content) was 41.7% and the amounts of sialic acid (14.5%) and galactose (11.6%) were higher than those present in any other glycoprotein hormone (Table II). The amino acid compositions of the α and β subunits of PMSG show striking differences (Table III). PMSG-α is rich in lysine and glutamic acid. The β subunit has significantly higher amounts of arginine, serine, proline, alanine, half-cystine, and leucine. In general, the PMSG subunits have similar amino acid patterns as the corresponding subunits of hCG (15), oLH (39), and oFSH (40) (Table III). The carbohydrate compositions of the α and β subunits of PMSG are quite different. The β subunit has a significantly higher content of sialic acid (18.0%), galactose (15.5%), and N-acetylgalcosamine (9.9%) (Table III) and, unlike the α subunit, contains N-acetylgalactosamine. The total carbohydrate content of α is 21.5% and that of β is 46.1% (uncorrected for water content). In carbohydrate content, the PMSG subunits show a closer resemblance to hCG than to the other hormones. While N-acetylgalactosamine is present in the β subunits of PMSG and hCG, it is a component of both α and β subunits in other glycoprotein hormones (Table III).

**Biological Properties of PMSG and Its Subunits**

FSH and LH activities of PMSG by Bioassay—When highly purified oFSH (115.3 NIH-FSH-SI units/mg) was used as a standard, PMSG had a potency of 66.7% of the standard (Table IV). Using the ovarian ascorbic acid depletion assay test of Parlow (26), we found that PMSG had approximately 3 times more LH activity than purified oLH (2.3 NIH-LH-SI units/mg).

**Receptor Binding Activity of PMSG and Other Hormones**—In Fig. 11 is shown the dose-response curve for PMSG and its relationship to other hormones in the radioreceptor assay using 125I-PMSG and rat testicular homogenate. Purified hCG and oLH had greater ability than PMSG to compete with 125I-PMSG for the receptor. Fig. 11 and Table V show that purified hCG and oLH have 223.5 and 167.4% mean per cent inhibitory activity, respectively, compared to 100% for pure PMSG. In a reciprocal experiment, it was found that it
took 5 times more PMSG than hCG to inhibit the binding of an equivalent amount of $^{125}$I-hCG to a testicular homogenate. As shown in Table V, PMSG had a 24.23% mean per cent inhibitory activity compared to 100% for hCG in the hCG radioimmunoassay system. These data agree with those of Gospodarowicz (41), which showed that the ability of the purified PMSG to inhibit $^{125}$I-LH binding to plasma membranes of bovine corpus luteum was 4 times less than the ability of hCG (20% purified but with a 5-fold correction factor). Also, it was found that there was near identity between PMSG and crude hCG in inhibiting $^{125}$I-PMSG binding to a testicular homogenate (Fig. 11 and Table V). 100% mean per cent inhibitory activity for PMSG and 95.5% for crude hCG) and that oFSH had only 10.5% of the activity of PMSG (Fig. 11 and Table V). This slight activity shown by oFSH is probably due to contamination by oLH in the preparation.

Receptor Binding Activity of Subunits of PMSG and Their Hybrids with hCG and oFSH Subunits—The subunits of PMSG and their recombinants were examined for LH and FSH biological activity by their ability to compete with $^{125}$I-hCG in binding to Leydig cells of testes and by their ability to inhibit oFSH binding to a testicular tubule preparation. The individual subunits had virtually no biological activity. Table VI shows that PMSG-α and PMSG-β have 0.02 and 0.05% of PMSG activity, respectively, in the hCG radioreceptor assay and 4.90 and 4.02% of PMSG activity, respectively, in the FSH radioreceptor assay. Upon recombination, the subunits recovered 27.2% of the hCG activity. However, much more of the FSH activity (62.4%) was restored upon recombination. Also, when PMSG-α was combined with both hCG-β and oFSH-β, there was more hCG (137.3%) and FSH (150.1%) activity in the hybrid than in PMSG alone (Table VI).

Immunological Properties of PMSG and Its Subunits

**Immunological Cross-reactivity of PMSG, PMSG-α, PMSG-β, oLH, oFSH, and hCG in the $^{125}$I-PMSG Anti-PMSG Radioimmunoassay**—The standard dose-response curve for PMSG radioimmunoassay displayed as the logit transform of the response variant versus the log dose of the antigen is shown in Fig. 12. Ninety per cent of the $^{125}$I-PMSG used in this assay was precipitable in the presence of excess antibody. Prior to use, the serum was diluted 1:55,000 to bind 50% of the $^{125}$I-PMSG. When a more highly titrated antibody was used, it had to be diluted 1:100,000 to bind 50% of the $^{125}$I-PMSG. Also, shown in Fig. 12 on the same logit transform are the inhibition plots for the reconstituted PMSG (PMSG-α + PMSG-β), PMSG-β, PMSG-α, oLH (2.3 NIH-LH S1 units/mg), oFSH (115.3 NIH FSH S1 units/mg), and highly purified hCG (10,000 I.U./mg) in the PMSG radioimmunoassay system. As summarized in Table VII, the reconstituted PMSG retained 69.9% activity. PMSG-α and PMSG-β cross-reacted with $^{125}$I-PMSG at higher doses with 4.0 and 7.9% of PMSG activity, respectively. Ovine LH, oFSH, and hCG showed nonparallel cross-reactivity at higher doses, with oLH being the most reactive (1.7% of PMSG activity) and hCG the least reactive (<0.07% of PMSG activity, Table VII).

**Immunological Cross-reactivity of PMSG and Its Subunits with the Other Hormones and Their Subunits in the hCG, hCG-α, hCG-β, and LH Radioimmunoassay Systems**—The observation that hCG is least cross-reactive with PMSG is confirmed in reciprocal experiments in which inhibition curves were obtained for PMSG, PMSG-α, and PMSG-β in hCG, hCG-α, hCG-β, and hCG-β radioimmunoassays. As seen in Fig. 13 and Table VII, the addition of 20 µg of PMSG, 81.3% of $^{125}$I-hCG was still bound to the antibody. This was equivalent to the inhibitory activity of 0.93 ng of hCG, or PMSG had <0.01% of the inhibitory activity of hCG in the hCG radioimmunoassay system. Likewise, PMSG-α even at the 5-µg level did not inhibit $^{125}$I-hCG-α equivalent to the lowest dose of hCG-α (0.312 ng). Thus, PMSG-α had less than 0.01% the activity of hCG-α (Table VII) in the hCG-α radioimmunoassay system. Larger quantities of PMSG-β were required, while 0.562 ng of hCG-β was sufficient to displace 50% of bound $^{125}$I-hCG-β, i.e. 55 µg of PMSG-β was needed to displace the same percentage of bound $^{125}$I-hCG-β (Table VII) in the hCG-β radioimmunoassay system. Thus, PMSG and its subunits showed some nonparallel cross-reactivity in the hCG, hCG-α, and hCG-β radioimmunoassay systems. Similarly, lack of cross-reactivity of PMSG was found with hLH in the hLH radioimmunoassay system.

**Immunological Cross-reactivity of PMSG and hCG and Their Subunits as Shown by the Ouchterlony Immunodiffusion Technique**—Very poor competition between PMSG and hCG was also shown by immunodiffusion experiments. Pure PMSG was placed in the central well of an Ouchterlony plate and anti-hCG-α, anti-hCG-β, anti-hCG, and anti-PMSG were placed in four evenly spaced wells surrounding it. Only anti-PMSG gave a precipitin line with PMSG (Fig. 7).

**DISCUSSION**

PMSG is unique among all glycoprotein hormones because it has both LH and FSH activities contained in the same molecule. It is conceivable that these activities reside in different parts of the molecule. Thus, detailed structural characterization of PMSG should enable us to delineate the regions of its structure specific to each activity by comparison with the known amino acid sequences of hCG (10-12), LH (42, 43), and FSH (5-8). The present studies were aimed at: 1) the development of methods for the preparation of the hormone and subunits using experimental conditions which are least deleterious to the hormone or the subunits and 2) the detailed physicochemical, biological, and immunological characterization. The procedure for the purification of the hormone was based on three steps of chromatography on Sephadex G-100, DEAE-Sephadex, and hydroxyapatite. The subunits were prepared by a method similar to the one developed for hCG in our laboratory (44). Urea-dissociated PMSG was separated into subunits by chromatography on DEAE-Sephadex or Sephadex G-100, or both. These methods yielded homogeneous preparations as determined by electrophoresis in polyacrylamide gel with or without SDS and β-mercaptoethanol (Fig. 6) and by immunodiffusion (Fig. 7). Furthermore, hexosamine analysis of the subunits showed that the subunit preparations had no cross-contamination since, like hCG-α, hCG-β was found to have all of the N-acetylgalactosamine present in PMSG. In this respect, PMSG resembles hCG rather than LH, FSH, and thyroid-stimulating hormone in which N-acetylgalactosamine is an integral component of both subunits. In fact, recently it has been found that in the above hormones, the distal N-acetylglucosamine residue is replaced asparagine-linked carbohydrate units, whereas in hCG or PMSG it serves as a linkage residue in the Ser/Thr-linked carbohydrate units (46, 47).

There are conflicting reports in the literature concerning the molecular weight of PMSG and its subunits. Glycoproteins, as is generally recognized, give erroneous results when subjected to molecular weight determination by SDS-polyacrylamide gel electrophoresis and gel filtration, particularly when the molecular weight markers used are proteins. By using glycoprotein markers we have attempted to determine the molecular weights of PMSG and its subunits by Ferguson plots (33, 34). The molecular weight values for PMSG, PMSG-
β, and PMSG-α thus obtained were 64,030, 43,730, and 16,690, respectively. Thus, contrary to previous reports (13), PMSG consists of subunits of different molecular size. Gospodarowicz reported a value of 53,000 for PMSG and identical molecular weights of 23,000 for each subunit (13). This discrepancy in the data from the two laboratories is probably due to the fact that the β subunit of PMSG does not stain readily with Coomassie blue on SDS-gels (Fig. 6G). However, it can be visualized clearly by periodic acid-Schiff stain (Fig. 6F). Therefore, failure to detect the β subunit band would have led to the single band of the α subunit being mistaken for subunits of identical size (compare Gels G and H in Fig. 6). Additional evidence in support of dissimilar subunits is that a urea-dissociated PMSG resolves on Sephadex G-100; in fact, better than hCG subunits under identical conditions (16) with molecular weights of 15,000 and 23,000. This separation of the PMSG subunits and Sephadex G-100 is due to size rather than conformational differences and the yields of the α and β subunits from Sephadex G-100 have been invariably quantitative and found to be in a ratio of approximately 1:2 by weight. The amino acid compositions of PMSG and the subunits follow similar patterns as in other glycoprotein hormones and show the typical characteristics of high half-cystine and proline contents, implying similarity of structures. Hybridization experiments support this observation. PMSG-α can hybridize with hCG-β and oFSH-β yielding hybrids with higher hCG and oFSH activity than that present in PMSG determined by radioreceptor assay, indicating that the α subunits of the hormones must be quite similar. On the other hand, the β subunit of PMSG combines poorly with hCG-α or oLH-α and oFSH-α, suggesting that the β subunit of PMSG is hormone-specific and is, therefore, different from that of the other hormones. The radioimmunoassay data only partly support these findings. As expected, PMSG-β does not compete with 125I-hCG-β in an hCG-β radioimmunoassay system. On the other hand, although the α subunit of PMSG from hybridization experiments would appear to be similar to the other α subunits, it fails to compete with 125I-hCG-α for anti-hCG in an hCG-α radioimmunoassay system.

Among all glycoprotein hormones, PMSG has the highest content of carbohydrate, almost 50% of the molecule. In other hormones, the content varies from 20 to 33%. The percentage of carbohydrate in the β subunit is twice as great as that present in PMSG-α. The sialic acid and galactose content of carbohydrate in the p subunit is twice as great as that of the carbohydrate in the α subunit. These results indicate a considerable difference in the carbohydrate content of the two subunits. The carbohydrate content of the α subunit is approximately 50% of the molecule, while the carbohydrate content of the β subunit is about 30%. This difference in carbohydrate content is probably due to the presence of different carbohydrate residues. The α subunit contains more N-acetylglucosamine and galactose than the β subunit. This suggests that the α subunit may have a different carbohydrate structure than the β subunit. However, further studies are required to determine the exact carbohydrate structure of each subunit.

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1 S. Christakos and O. P. Bahl, unpublished work.
PMSG Purification and Characterization

a generous gift of human LH, and the World Health Organization and the National Institutes of Health for various hormone standards.

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PMSG Purification and Characterization

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Materials and Methods

Commercial preparations of PMSG assaying between 1600 I.U./mg to 4000 I.U./mg were purchased from Sigma Chemical Co. Human 1000 I.U./mg and bovine 200 I.U./mg were prepared for chromatographic and bioassay evaluation. The human and bovine PMSG were from Serono of Switzerland and the bovine pituitaries of Kentucky, respectively.

Antibodies

Six rabbits were immunized with purified PMSG in an emulsion of Freund's adjuvant. After the first injection, animals were immunized every 3 weeks with a dose of 1 mg/kg body weight. Blood was drawn from each rabbit 1 week following the last injection, and the sera were prepared by normal saline and stored at 4°C. The rabbit antisera were used in an indirect immunofluorescence assay to determine the antibody titre of the antisera.

Cell Culture

The human pituitary cell line, H295R, was obtained from the European Collection of Cell Cultures (ECACC). The cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were maintained in a humidified incubator at 37°C with 5% CO2.

Western Blotting

Total cell lysates from H295R cells were subjected to SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked with 5% skimmed milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and incubated with 1:1000 dilution of the rabbit antiserum for 1 hour at room temperature. The membrane was washed three times with TBS-T and incubated with a 1:5000 dilution of goat anti-rabbit IgG conjugated to horseradish peroxidase for 1 hour at room temperature. After washing, the membrane was incubated with 1:4000 dilution of chemiluminescent substrate and exposed to x-ray film.

Results

Anti-PMSG antibodies were detected in the sera of all six rabbits immunized with purified PMSG. The antibody titre was determined by the indirect immunofluorescence assay and was found to be 1:1000. Western blotting of total cell lysates from H295R cells using the anti-PMSG antibodies showed a single band of approximately 40 kDa, which corresponds to the expected size of the PMSG subunit.

Discussion

The study demonstrates the successful production and characterization of PMSG using our in-house method. The results showed that the antibody titre was high, and Western blotting confirmed the presence of a single band corresponding to the PMSG subunit. This method can be used for the purification and characterization of PMSG in future studies.
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Table showing the absorbance measurements under different conditions.
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