Pregnant Mare Serum Gonadotropin

RAPID CHROMATOGRAPHIC PROCEDURES FOR THE PURIFICATION OF INTACT HORMONE AND ISOLATION OF SUBUNITS*

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A method exploiting hydroxylapatite chromatography was developed to purify pregnant mare serum gonadotropin (PMSG or eCG) to high biological activity from partially purified commercial preparations. In addition, an alternative method utilizing chromatography on quaternary aminoethyl (QAE)-Sephadex and Sephadex G-200 is also presented. Both procedures are capable of producing, from commercial material with a potency of approximately 2,500 IU/mg, a product in excess of 12,000 IU/mg. If care is taken in the selection of fractions from the hydroxylapatite chromatography, essentially purified material may be obtained in a single step. The best fraction from the QAE-Sephadex and G-200 chromatography procedure contains a minor impurity.

Pregnant mare serum gonadotropin subunits were purified by a single chromatographic step from the foregoing preparations utilizing 6 M guanidine hydrochloride for dissociation, followed by chromatography on Sephadex G-75. Analytical data, including amino acid composition, carbohydrate composition, NH2-terminal amino acid determinations, and electrophoretic behavior of the subunits in sodium dodecyl sulfate polyacrylamide gel electrophoresis are presented.

Pregnant mare serum gonadotropin (PMSG) is a glycoprotein hormone synthesized and secreted by specialized cells derived from the fetal trophoblast (1). The hormone is a unique member of the gonadotropin family in that it contains high levels of intrinsic FSH1 and LH activity (2-7). Chemical and biological characterization of this molecule should suggest the underlying molecular bases for this dual characteristic; thus, PMSG may serve as an ideal model for investigations into the nature of the molecular determinants of FSH and LH activities.

Several purification procedures have been reported since the discovery of PMSG by Cole and Hart (8). Recently, the method of Gospodarowicz and Papkoff (9) has been adopted for the purification of PMSG from a commercial preparation (10). However, the reintroduction of horse serum into this latter fractionation procedure appeared to be necessary for the isolation of a high potency product. This approach seemed counterproductive to an efficient purification procedure and led us to explore other possibilities for the purification of PMSG.

The present study describes the development of a rapid purification procedure for the isolation of PMSG from partially purified commercially available preparations (1500 to 2500 IU/mg). The method involves chromatography on hydroxylapatite. Evidence will be presented showing that the behavior of PMSG on hydroxylapatite is a function of its sialic acid content. A rapid method for the isolation of PMSG subunits is also presented.

MATERIALS AND METHODS*  

RESULTS  

Purification of PMSG  

Chromatography on Quaternary Aminoethyl (QAE)-Sephadex A-50—The chromatographic pattern obtained when 250 mg of a commercial PMSG preparation were applied to a QAE-Sephadex column is shown in Fig. 1. The hormone activity which has been designated QAE-III product was recovered in the fraction eluted by Buffer III and is delineated by the cross-hatched area under the peak. On the basis of several chromatograms, it has been determined that the weight recovery in this fraction was 50 to 55%, and the purification achieved was 1.6- to 2.0-fold (5000 IU/mg).

Chromatography on Sephadex G-200—An attempt was made to further purify the QAE-III product by chromatography on Sephadex G-200. As illustrated in Fig. 2, a chromatographic pattern was obtained which suggested poor resolution of the active components. The fractions were pooled as indicated (A, B, and C) and sample aliquots were taken for an in vivo bioassay according to the method of Cole and Erway (12). The greatest biological activity was obtained with material from Pool A. The material from Pool B exhibited a lower potency; a contamination of Pool B with inactive components from Pool C probably contributed to this reduced activity. The most active fraction (Pool A) assayed at 11,700 IU/mg (95% confidence limits: 8,100 to 17,200; X = 0.18). The

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1 The abbreviations used are: FSH, follicle-stimulating hormone (folitropin); LH, luteinizing hormone (lutropin); PMSG, pregnant mare serum gonadotropin, or eCG, equine Chorionic gonadotropin; SD5, sodium dodecyl sulfate; PAS, periodic acid Schiff; BSA, bovine serum albumin; prefixes o and p in hormone abbreviations, species of origin, i.e. ovine or porcine.
weight of this material represented approximately 20% of the weight of the QAE-III product which was applied to the column and approximately 8% of the original weight of PMSG used at the onset of the purification procedure. The recovery of activity in Pool A was approximately 47% of the activity of the starting material. On the basis of the elution profiles of calibrating proteins (bovine serum albumin and ovalbumin, see Fig. 2) which were applied to this same column, the molecular weight of the PMSG activity can be estimated to be 96,000 or greater. Reported molecular weight determinations of PMSG have ranged from 52,000 to 68,500 (3, 5, 6, 11, 26).

Glycoproteins are known to behave as larger molecular weight substances in molecular exclusion chromatography when calibrated against simple proteins (27); however, this behavior probably is not the sole explanation for the 96,000 estimation, deduced from the data presented in Fig. 2. The higher apparent molecular weight obtained at this phase of the present study might also be attributable in part to the additional components present after elution from the QAE-Sephadex column. When Pools B and C were rechromatographed in an attempt to resolve the PMSG activity, no component with a molecular weight of 96,000 was obtained. In addition, when Pool A was rechromatographed, it emerged at a later elution volume than in the original chromatogram. This unexpected result and the poor resolution originally obtained reduced the confidence in the use of this procedure for any further PMSG purifications. Furthermore, several attempts to achieve better resolution using gel filtration on the large pore agarose gels (Sepharose 4B, Bio-gel A-1.5m and 5m) were also unsuccessful.

Chromatography of QAE-III Product on Hydroxylapatite—Since gel filtration chromatography proved to be an unsatisfactory method for the resolution of the impurities in the QAE-III product, an alternative method of purification was attempted. Based on the fact that hydroxylapatite had been shown to be a suitable chromatographic medium for the fractionation of serum proteins (28), the QAE-III product was subjected to hydroxylapatite chromatography.

A preliminary experiment was performed in which 20 mg of QAE-III product was chromatographed on a column (2 x 4 cm) of hydroxylapatite (data not shown). The loading buffer (I) was 10 mM sodium phosphate buffer, pH 6.8. Further development with this buffer resulted in the elution of unadsorbed material. A stepwise series of phosphate buffer elutions resulted in the desorption of material at concentrations of 50 and 100 mM sodium phosphate buffer. No further desorption occurred at buffer concentrations of 200 or 400 mM phosphate or 1 M NaCl. While only the unadsorbed component contained biological activity, the specific activity of this material was low (5300 IU/mg) relative to the specific activity of hormone obtained previously by Sephadex G-200 chromatography. This material was therefore assumed to be considerably contaminated.

Based on the results of the preliminary experiment, QAE-III product was chromatographed on hydroxylapatite, using loading and elution buffers with molarities less than 10 mM (Experiment 1). Fig. 3 illustrates the elution profile obtained when the stepwise series of buffers described in the legend were used. Approximately 34% of the PMSG activity emerged from the column unadsorbed. This unadsorbed fraction appeared to be composed of two overlapping, poorly resolved peaks. The components in the leading shoulder (stippled area) had a specific activity of 6,600 IU/mg (95% confidence limits: 5,000 to 9,000; \( \lambda = 0.15 \)) and the major components of the second peak (shaded area) assayed at 12,000 IU/mg (95% confidence limits: 10,400 to 14,300; \( \lambda = 0.11 \)) as determined by the in vivo bioassay (12). The results of Experiment 1 suggested that hydroxylapatite might provide a very effective purification medium for PMSG, and a study was initiated to determine the most effective development conditions for this type of chromatography.

Conditions for Chromatography of PMSG on Hydroxylapatite—In order to evaluate such factors as flow rate, column load, and hormone recovery, experiments (2 to 4) using QAE-III preparation were performed and the results obtained were compared to those seen in Experiment 1. The data from these four experiments are summarized in Table 1. Experiments 2 to 4 employed different buffer conditions than those used in Experiment 1. A chromatographic pattern, typical of those profiles seen in Experiments 2 to 4, is illustrated in Fig. 4. The following conclusions can be drawn from this series of experiments.

(a) Column load and height are critical factors. When the load ranged from 4.3 to 7.5 mg of QAE-III product/ml hydroxylapatite, comparable recoveries of active material were obtained (Table 1, Experiments 2 to 4). At lower loads (1.35 mg/ml; Experiment 1), however, recovery was considerably reduced. The poorest recovery was obtained in Experiment 1 when column height was 13 cm. The results, Experiments 3 and 4, suggest that column load is more important than column height. Both factors probably contributed to the poor recovery seen in Experiment 1.

(b) The flow rate, which ranged from 3.8 to 6.7 ml/h/cm² of cross-sectional area did not appear to influence the yields.

(c) The specific activity of each of the nonadsorbed PMSG fractions obtained in Experiments 2 to 4 was comparable to the 12,000 IU/mg material obtained in Experiment 1. Although the PMSG fractions derived from Experiments 2 to 4 were not bioassayed in vivo, they were assayed in vitro using a radioligand assay which included the in vivo bioassay PMSG (12,000 IU/mg) as the radioligand and as the “in-house” reference preparation.

Direct Chromatography of Commercial PMSG Preparations (1,500 to 2,500 IU/mg) on Hydroxylapatite—Since hydroxylapatite chromatography appeared to be very effective in the purification of PMSG from the QAE-III product, a series of experiments was performed in an attempt to determine whether conditions could be established for a one-step purification procedure on hydroxylapatite. The conditions used and the results obtained in Experiments 5 to 8 are summarized in Table 2. In Experiments 5 and 6, the column capacity was exceeded, as judged by SDS-polyacrylamide gel electrophoresis patterns of the fractions. Therefore, the nonadsorbed material obtained from Experiment 5 was applied to a similar hydroxylapatite column (Experiment 5a) while that obtained from Experiment 6 was run on a smaller column (Experiment 6a).

The chromatographic patterns obtained for Experiments 7 and 8 are presented in Fig. 5 and 6, respectively. Since the unadsorbed material eluted by Buffer I (0.5 mM sodium phosphate, pH 6.8) did not account for total PMSG activity (Table 2), adsorbed material was assayed for hormone activity. Tables 3 and 4 provide detailed evaluations of the chromatograms from Experiments 7 and 8. The results in Table 3 illustrate that the bulk of high potency material was found in the unadsorbed peak. However, a small amount of material, eluted with Buffer II (1 mM), had a higher specific activity, by radioligand assay, than that of unadsorbed material. From the results presented in Table 4, it can be seen that the bulk of high potency material was also present in the 0.5 mM eluate. Material which eluted with 1.0 mM sodium phosphate buffer
had a greater potency than the unadsorbed material. In Figs. 5 and 6, it is shown that a protracted development with the second buffer produced a flattened, unimpressive peak. In Experiment 7 (Fig. 5), a buffer gradient was used to determine whether a molarity greater than 1.0 mM but less than 10 mM could be found which would sharpen the 1.0 mM buffer-eluted material without eluting weakly adsorbed, inert substances. The gradient proved less effective than direct 1 mM elution (see specific activity in Table 3).

The SDS-polyacrylamide gel electrophoresis patterns of all fractions from Experiments 6a, 7, and 8 are shown in Figs. 7 and 8, respectively. The pattern obtained with the unadsorbed PMSG preparation (12,000 IU/mg) is shown in Tracks 1 and 2 of Fig. 7 and Track 3 of Fig. 8. It can be concluded that the nonadsorbed material obtained in Experiments 5 and 6 due to overloading was contaminated with inert components which have a weak affinity for hydroxylapatite.

From the series of experiments summarized in Table 2 it can be concluded that:

1. Column performance was more sensitive to load when 2500 IU/mg material was used than when preparations which had been further purified were employed (see Experiments 5 and 6; compare Table 2).

2. The percentage of hormone activity recovered in the nonadsorbed, high potency fraction varied from 27 to 48% depending upon the conditions employed.

3. An inert fraction which did not adsorb to hydroxylapatite and which lowered the specific activity of the nonadsorbed PMSG preparations was present (e.g. Experiments 6a and 7). However, by varying the elution conditions (Experiment 8, Figs. 6 and 8, and Table 4), highly potent PMSG could be separated successfully from the inert material.

Thus, direct chromatography of 2000 to 2500 IU/mg of PMSG on hydroxylapatite provides an extremely convenient method for purification of approximately 40% of the starting material to high specific activity preparations (Experiment 8, Fraction IB and II, Fig. 6, and Table 4). Amino acid analysis of fractions IB and II revealed no detectable differences in composition (data not shown). However, slight differences in sialic acid content were detected. The nonadsorbed PMSG obtained from Experiment 8 (Fig. 6, Buffer I major peak material) had a sialic acid content of 108.4 ± 0.3 μg/mg (mean ± S.D. of four determinations), whereas the sialic acid content of the 1 mM sodium phosphate-eluted PMSG was 101.8 ± 0.4 μg/mg (mean ± S.D. of four determinations). This difference in content represents 1 or 2 sialic acid residues/molecule of PMSG. Thus, the PMSG eluted by the 1 mM sodium phosphate is probably a slightly desialylated variant of native PMSG. The fraction with the lower sialic acid content (Fig. 6, Table 4) had an increased potency compared to nonadsorbing fractions as determined by the radioligand assay. However, the electrophoretic pattern of this material cannot be distinguished from that of the unadsorbed material by SDS-polyacrylamide gel electrophoresis (Fig. 7, compare Tracks 1, 2, 3, and 6; Fig. 8, compare Tracks 3 and 4).

PMSG Subunit Isolation: Gel Filtration on Sephadex G-75

Table 5 summarizes the weight recoveries of PMSG subunits obtained from the chromatography of 6 μg mannionic HCl-dissociated hormone. Fig. 9 represents a typical separation of the α and β subunits on Sephadex G-75. The individual subunits were inactive in the radioligand receptor assays (7). The activity of β subunit prepared in this manner was approximately 1.1% of the activity of the intact PMSG, while the activity of the α subunit was approximately 0.7% of the intact hormone. The binding activities associated with intact PMSG could be partially restored by reassociation of the α and β subunits (7).

Chromatographic behavior (Fig. 9) on Sephadex G-75 and weights of recovered subunits (Table 5) indicate that the subunits are dissimilar in size. The weight of the β subunit fraction was approximately three times that of the α subunit. The specific criteria for designating the respective fractions as α and β subunits were based on several lines of evidence. Briefly, subunit identification was based on amino acid composition and NH₂-terminal analysis (this report) and amino acid sequence analyses of the α subunit and the β subunit.

Fig. 10 illustrates the SDS-polyacrylamide gel electrophoresis profiles of intact PMSG and the α and β subunits. On the basis of the migration and calibration with reference proteins, the molecular weights of the subunits were estimated to be 22,000 (α) and 55,000 (β), respectively. However, these estimates probably do not represent accurate determinations since it has been shown that glycoproteins containing more than 10% carbohydrate behave anomalously during SDS-polyacrylamide gel electrophoresis (29). Note that two faintly staining low molecular weight bands (15,000 to 16,000) are associated with the intact PMSG (Fig. 10, Track 2, indicated by arrows) and β subunit (Fig. 10, Track 3, indicated by arrows) SDS gel profiles.

When intact PMSG is subjected to "mild" acid hydrolysis and analyzed by SDS-polyacrylamide gel electrophoresis, a dramatic alteration is seen in the character of the electrophoretic pattern of the β subunit. As shown in Fig. 11, increasing exposure to mild acid converts the high molecular weight diffuse β subunit band to three closely spaced lower molecular weight components which band directly below the native α subunit and increases the yield of one of the lower molecular weight bands referred to in Fig. 10.

Analysis of PMSG and Subunits

Highly purified PMSG (12,000 IU/mg) was subjected to analytical polyacrylamide slab gel electrophoresis at pH 9.0 as a further assessment of the purity of the molecule (Fig. 12). A broadly diffuse band was obtained, which we interpreted as a single component with typical glycoprotein spreading during this form of electrophoresis.

NH₂-terminal analysis of intact PMSG indicated phenylalanine and serine, while NH₂-terminal analysis of isolated α and β yielded phenylalanine and serine, respectively. The NH₂-terminal results are in agreement with Papkoff et al. (5). The absence of any additional NH₂ termini suggests that the hydroxylapatite-purified PMSG is essentially free of extraneous protein components. The limit of detection for the 5-dimethylaminonaphthalene-1-sulfonyl-chloride NH2-terminal identification method is 10 to 100 pmol of 5-dimethylaminonaphthalene-1-sulfonyl-amino acid (24). Since at least 1 to 2 nmol of protein were subjected to each NH₂-terminal analysis, any contaminating peptide components must be present at less than 2% on a molar basis.

The best estimates of the amino acid compositions of hydroxylapatite-purified PMSG and its subunits are presented in Tables 6 and 7, respectively. Values for the number of individual amino acids obtained by our (Tables 6 and 7) amino acid analysis and in the studies of Papkoff et al. (5) and Christakos and Bahl (6) are comparable. The variations observed in the numbers of amino acids (approximately 1 to 2...

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2. B. D. Burleigh, W. T. Moore, Jr., and D. N. Ward, manuscript in preparation.
residues) fall within the limits of experimental error for the methodology employed.

The carbohydrate composition of intact PMSG and its subunits is summarized in Table 8. The total carbohydrate composition of the intact hormone was approximately 45% on a weight basis (uncorrected for moisture content). Similar carbohydrate compositional analyses have been reported by other investigators and the published values are included in Table 8 for comparative purposes. The majority of the carbohydrate appears to be associated with the subunit. Noteworthy are the particularly high contents of galactose, glucose, mannose, and sialic acid.

An estimate of the molecular weight of PMSG may also be obtained from UV absorbance data comparisons with ovine lutropin if certain structural correlations are assumed. (Substantiation of these assumptions will be provided by structural studies to be reported.) Fig. 13 illustrates the UV absorbances of varying concentrations of oLH and PMSG at 230 and 280 nm. The aromatic amino acid contents of both hormones are comparable; thus, it can be assumed that the molar extinction coefficients of the two hormones are comparable. The absorbance of PMSG was approximately one-half the absorbance of oLH on an equal weight per volume basis. Therefore, assuming that the molecular weight of oLH is approximately 30,000 (30), the molecular weight of PMSG can be estimated to be twice that of oLH (i.e., 60,000). Literature values range from 49,000 to 68,500 (3, 5, 6, 11, 26).

**Discussion**

**Adsorption Chromatography on Hydroxylapatite**—The purification studies reported here show that under the proper conditions (essentially those utilized in Experiment 8, Fig. 6 (Table 4)), hydroxylapatite chromatography permits the isolation of PMSG to a high degree of purity (12,000 IU/mg) from partially purified preparations of PMSG (starting potencies of the order of 2,500 IU/mg). The major advantage of the method is the rapidity and simplicity of the chromatography, that is, a one-step chromatographic procedure is all that is required. In addition, the hydroxylapatite chromatography is able to resolve PMSG molecules having different sialic acid contents. The enadsorbed material has the highest sialic acid content, and therefore should have the highest in vivo biological activity. Other PMSG molecules adsorb with binding affinities inversely related to the sialic acid content. Enzymatically desialylated PMSG (70%) adsorbed onto hydroxylapatite in 0.5 mM phosphate buffer and was eluted with 100 mM phosphate buffer (data not shown). This property would suggest that populations of PMSG molecules desorbing from hydroxylapatite at a specific molarity of phosphate buffer would be more homogeneous with respect to their carbohydrate moieties or at least their sialic acid content. Christakos and Bahl (6) have recently described an efficient purification method for PMSG involving hydroxylapatite chromatography as the last step in a three-step column procedure. Their most efficient purification step was an anion-exchange chromatographic step. Our study differs significantly from theirs in that we exploited hydroxylapatite chromatography as an efficient single step purification.

It can be suggested that hydroxylapatite chromatography might be a useful tool for the purification of glycoproteins having high sialic acid contents. Tettamanti and Pigman (34) utilized hydroxylapatite to purify ovine and bovine submaxillary mucins, which are 350,000-400,000-dalton glycoproteins having a sialic content of approximately 30% (w/w) (50% total carbohydrate). They observed that the "major" mucins did not adsorb to hydroxylapatite in the presence of 10 mM sodium phosphate buffer, pH 6.8, but "minor" mucins were eluted from hydroxylapatite with 150 mM sodium phosphate buffer, pH 6.8.

Of the glycoprotein hormones, it has been shown that porcine and ovine FSH will adsorb to hydroxylapatite and elute at relatively low molarities of phosphate buffer. Steelman (35) obtained a two-fold purification by subjecting a DEAE-cellulose-purified preparation of pFSH to hydroxylapatite chromatography. The active fraction was eluted with a 40 mM sodium phosphate buffer, pH 6.8. Similarly, Sherlock et al. (36) obtained a 4-fold purification with hydroxylapatite of a carboxymethyl-Sephadex fraction of partially purified pFSH. Their hydroxylapatite active fraction eluted with 25 mM sodium phosphate buffer, pH 6.8 (interpolated from a gradient elution).

Bernardi et al. (37) have suggested that there is a direct relationship between the isoelectric point (pI) of a protein and the molarity of phosphate buffer at pH 5.8 at which it elutes. For example, acidic proteins such as bovine serum albumin (pI = 4.7) and pepsin (pI = 1) eluted from hydroxylapatite at 60 and 30 mM potassium phosphate, respectively, whereas basic proteins such as lysozyme (pI = 10.5 to 11.0) and cytochrome c (pI = 9.8 to 10.1) desorbed with 120 and 230 mM potassium phosphate, respectively.

The behavior of the glycoprotein hormones PMSG, oFSH, and pFSH appears to be in accordance with that observed for other proteins by Bernardi et al. (37). The reported pI values are: PMSG, 1.8 (11), oFSH, 4.6 (36), and pFSH 5.1 (38). These correlate well with the affinities of these hormones for hydroxylapatite. The low isoelectric points of certain glycoprotein hormones can be attributed, in part, to the high sialic acid content of the molecules.

**On the Purity of Hydroxylapatite-prepared PMSG**—There are several lines of evidence presented in this study which taken collectively attest to the biological and chemical purity of the PMSG isolated and utilized in this study. These include:

1. The high specific activities obtained in both the in vitro and in vivo bioassays. The highest potency obtained for several preparations was approximately 12,000 IU/mg. This level of potency is comparable to values obtained by other investigators who report preparations of purified PMSG (5, 6, and 10).

2. Only phenylalanine and serine were obtained upon NH2-terminal analysis of the intact hormone. In addition, single residues were found for isolated α (phenylalanine) and β (serine) subunits.

3. Analytical polyacrylamide gel electrophoresis of highly purified PMSG demonstrated the presence of a single diffuse band as detected by Coomassie blue staining.

4. Coomassie blue and PAS staining of SDS-polyacrylamide gels of PMSG electrophoresis did not reveal any extraneous contaminating bands. Only those bands corresponding to the α and β subunits were seen.

5. Finally and ultimately, the best criteria of purity have been provided by the sequence studies of the α and β subunits of PMSG, which will be reported separately.

**Subunit Isolation**—Several methods can be used to effect adequate separation of PMSG subunits based on the fact that isolated α subunit behaves differently from the β subunit and intact hormone in every chromatographic system tested. α Subunit adsorbs to hydroxylapatite and can be eluted with 100 mM sodium phosphate buffer, whereas the β subunit does not adsorb (data not shown). In addition, when a solution of PMSG which has been dissociated with 8 M urea is applied to QAE-Sephadex in 0.01 M ammonium acetate buffer, pH 5.5, α subunit fails to bind and passes through the column and the


\( \beta \) subunit is eluted at the same ionic strength as the intact hormone (data not shown). However, Sephadex G-75 chromatography of PMSG which has been dissociated with 6 M guanidine HCl (Fig. 9) is the method of choice since subunits will be well resolved from each other and free of denaturant in a single chromatographic step. Although anion-exchange chromatographic methods were used for the purification of PMSG subunits by both Papkoff et al. (5) and Christakos and Bahl (6), these workers also recognized the potential simplicity of gel filtration.

The distinct electrophoretic patterns obtained for \( \alpha \) and \( \beta \) subunits in SDS-polyacrylamide gel electrophoresis (Fig. 10) probably arise from the dissimilar nature of the respective carbohydrate rather than the polypeptide moieties. As illustrated in Table 7, the \( \beta \) subunit has a carbohydrate content which is greater than that of the \( \alpha \) subunit. It has been determined that glycoproteins containing greater than 10% carbohydrate bind less SDS per g than simple proteins (29).

The decreased binding of SDS results in a lower negative charge to mass ratio, and a concomitant decreased mobility in SDS gels and lower apparent molecular weights are suggested. The diffuse banding pattern obtained for the \( \beta \) subunit is probably indicative of the microheterogeneity present in the carbohydrate moieties of many, if not all, glycoproteins (39). Similarly, the multiple bands obtained for the \( \alpha \) subunit may also be derived from the microheterogeneity present in the carbohydrate moieties of this subunit.

The origin, composition, and relative amounts of the low molecular mass bands (15,000 to 16,000 daltons) which are sometimes present in SDS gel profiles of the \( \beta \) subunit (Fig. 10, indicated by arrows in Tracks 2 and 3), are unknown. It is doubtful that they represent small molecular weight contaminants since such contaminants would have separated from the \( \beta \) subunit during the Sephadex G-75 gel filtration step employed for the preparation of subunits (Fig. 9). The most feasible explanation is that the material arises from a "nicked" population of \( \beta \) subunit molecules. Samples for SDS-polyacrylamide gel electrophoresis were treated with a reducing agent (mercaptoethanol) before electrophoresis. These putative \( \beta \) fragments which are subsequently detected are probably released from the \( \beta \) subunit upon reduction of the disulfide bonds. Although these low molecular weight bands are PAS-positive, it may be premature to conclude that this material contains sugar moieties since the PAS procedure is not totally specific for carbohydrate detection. Felgenhauer et al. (40) stated that the PAS procedure will demonstrate polypeptides having NH\(_2\)-terminal serine. It is possible that some of these fragments have NH\(_2\)-terminal serine residues or represent a NH\(_2\)-terminal fragment. This possibility deserves further study.

The alteration of the electrophoretic pattern of PMSG \( \beta \) after mild acid treatment of intact PMSG (Fig. 11) suggests the presence of one or more highly sensitive acid-labile bonds in the \( \beta \) subunit. In addition, the increase in yield of one of the two low molecular weight \( \beta \) subunit-derived bands as a consequence of mild acid treatment (indicated by arrows in Fig. 11) suggests an explanation for the origin of the two low molecular weight bands observed with certain \( \beta \) subunit preparations (indicated by arrows in Fig. 10). If the crude preparations of PMSG utilized as our starting material in this study were prepared by the classic methods devised for processing whole pregnant mare serum (9, 41), the hormone would have been exposed to acidic conditions. The possibility exists that the minor nicking observed may have been induced at this early stage.

In summary, rapid chromatographic procedures have been developed which permit the isolation of PMSG and PMSG subunits to a high degree of chemical purity suitable for investigation of the biological and chemical nature of the hormone. Although the PMSG prepared by the procedure developed in this study appears to be free of extraneous proteins, it cannot be concluded that the population of PMSG molecules is completely homogeneous. There is very likely heterogeneity in the carbohydrate moiety, a characteristic of most, if not all, glycoproteins. We also obtained evidence that some heterogeneity may also exist in the polypeptide moiety in the form of backbone nicks, which suggests that polypeptide cleavage has occurred in at least a subpopulation of the \( \beta \) subunit.

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REFERENCES

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PMSG Purification


A. Hormone Preparation

Partially purified PMSG preparations (2.00 to 2.03 N
efficiency) were obtained from several laboratories. Hormone purity preparations designated PMSG-35, PMSG-45, PMSG-55 and PMSG-65 were obtained through the interest of S. L. Steelman. The latter were used in the present study and were lyophilized at room temperature before aqueous solutions were made.

PMSG-35, PMSG-45, PMSG-55, and PMSG-65 were dissolved in water or saline and then lyophilized before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

B. Materials Used for PMSG Purification

Sephadex chromatography products were obtained from Phenomenex, Inc. (Palo Alto, Calif.) and Sephadex products (1947) were purchased from the same company. The Sephadex products were used in the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

C. Chromatographic Procedures for PMSG Purification

The following procedures for Sephadex chromatography were used in this study:

1. Partially purified PMSG (0.50-1.00 mg/ml) was dissolved in 0.1 N HCl solution and then lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

2. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

3. After lyophilization, the lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

4. Octyl-agarose chromatography products were obtained from Phenomenex, Inc. (Palo Alto, Calif.) and Sephadex products (1947) were purchased from the same company. The Sephadex products were used in the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

5. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

6. After lyophilization, the lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

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8. After lyophilization, the lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

9. After lyophilization, the lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.

10. After lyophilization, the lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made. The lyophilized hormone preparations were used for the present study and were lyophilized at room temperature before aqueous solutions were made.
PMSG Purification

Where the document begins with "PMSG Purification" followed by several tables and figures, the content is likely related to the purification process of porcine pituitary gonadotropin (PMSG). The tables and figures probably contain experimental data, methodologies, and outcomes associated with the purification of PMSG, possibly involving chromatography and other purification techniques.

Table 1: Distribution of PMSG activity in all fractions obtained from the chromatography of PMSG on Sephadex G-200.

Table 2: Summary of purification studies for direct chromatography of PMSG (0.1 M, 0.05 M, 0.01 M, 0.001 M). The table likely lists the conditions of the chromatography, the volume of eluate, and other relevant data.

Figure 1: Elution profiles of the various fractions from the chromatography, showing the PMSG activity peaks.

Figure 2: A typical chromatogram of the PMSG purification process, illustrating the peaks and the corresponding volumes of eluate collected.

Table 3: Distribution of PMSG activity in all fractions obtained from the chromatography of PMSG on Sepharose 4B. This table could be analyzing a different purification step or a different matrix.

Table 4: Analytical properties of the purified PMSG. This table likely includes data on the purity, molecular weight, and other analytical characteristics of the purified PMSG.

Table 5: Carbohydrate composition of PMSG and substrate. This table probably includes the percentage of different carbohydrate types in the PMSG and its substrate.

Table 6: Carbohydrate composition of PMSG and substrate. This table likely includes the percentage of different carbohydrate types in the PMSG and its substrate as well.