CHEMICAL AND BIOLOGICAL PROPERTIES OF THE SUBUNITS OF PREGNANT MARE SERUM GONADOTROPIN

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SUMMARY: The two subunits (α and β) of pregnant mare serum gonadotropin have been dissociated and partially characterized. Recombination of the biologically inactive subunits results in the restoration of both the follicle stimulating and leuteinizing activities of pregnant mare serum gonadotropin. In addition, the α subunit of pregnant mare serum gonadotropin can be combined with the β subunit of either ovine luteinizing hormone, human chorionic gonadotropin, or follicle stimulating hormone with generation of the specific activity expected of the β subunit.

Pregnant mare serum gonadotropin (PMSG) is a glycoprotein hormone possessing both follicle stimulating hormone (FSH) and luteinizing hormone (LH, ICSH) activity. Simple procedures for its isolation from serum and crude commercial preparations have been described and some chemical and physical properties determined (1-3). Insofar as all of the pituitary glycoprotein hormones as well as human chorionic gonadotropin (hCG) have been convincingly shown to consist of two nonidentical subunits (4-6), it was considered likely that PMSG would also be so constituted. Indeed, terminal group analysis (2) and studies by sodium dodecyl sulphate polyacrylamide electrophoresis (3) suggest this to be the case. The results reported here show that PMSG can be dissociated and its subunits isolated by techniques previously found applicable to hCG (7, 8). The PMSG subunits have been

Abbreviations: PMSG, pregnant mare serum gonadotropin; FSH, follicle stimulating hormone; LH, luteinizing hormone; hCG, human chorionic gonadotropin; OAAD, ovarian ascorbic acid depletion.

partially characterized chemically and an assessment made of their biological activities by specific tests for FSH and LH activity.

EXPERIMENTAL RESULTS

Preparation of PMSG Subunits. The methodology employed for the dissociation and isolation of the PMSG subunits was essentially that described by Morgan and Canfield (8). Highly purified PMSG (20 mg) prepared as previously reported (2) was dissolved in 0.4 ml of 10 M urea, pH 4.5, and incubated at 37° C for 1 hour. Following incubation, 0.1 ml of 0.03 M glycine and 0.5 ml of 8 M urea-0.03 M glycine buffer, pH 7.5, was added and the solution applied to a column of DEAE-Sephadex A-25 (1 x 15 cm) previously equilibrated with the same urea-glycine buffer. Upon development with this buffer, an unadsorbed peak was obtained (later designated PMSG-α), and following a step-wise switch to 0.5 M Tris-HCl buffer of pH 7.5, another peak was eluted (designated PMSG-β). These results are seen in Figure 1. Following dialysis against 1% acetic acid and distilled H_2O, the pools comprising each peak were lyophilized. Approximately equal amounts of

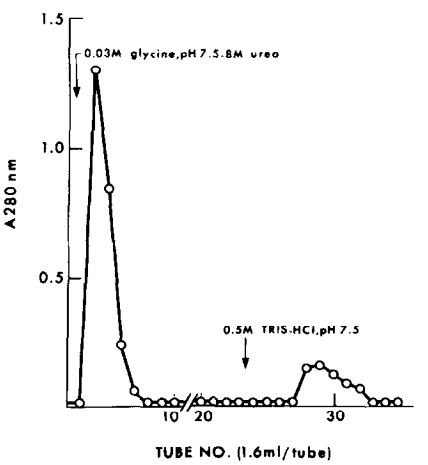

Figure 1: Separation of urea-dissociated pregnant mare serum gonadotropin by chromatography on DEAE-Sephadex employing conditions indicated in the figure.

PMSG-α and PMSG-β were obtained with an overall recovery of about 60% of the starting material.

Characterization Studies of PMSG-α and PMSG-β. The PMSG subunits have been analyzed for the presence of amino terminal amino acids by the dansyl technique (9). These results showed PMSG-α to possess phenylalanine as the major N-terminal amino acid. Traces of aspartic acid, glycine, and serine were also detected. With PMSG-β, the major N-terminal amino acids determined were glycine and serine. Only minute traces of phenylalanine were seen.

Amino acid analysis were done by the method of Spackman et al. (10) in a Beckman model 120B analyser. The results of these analyses are shown in Table I, where they are compared with the results previously obtained with intact PMSG (2). Examination of the results leaves little doubt that the composition of PMSG-α differs significantly from PMSG-β. Thus, PMSG-α possesses a much higher content of lysine, tyrosine, aspartic and glutamic acids, whereas PMSG-β is rich in serine, proline, and alanine. Spectrophotometric measurements indicate that PMSG-α and PMSG-β each contain tryptophan.

Preliminary carbohydrate analyses also indicate PMSG-α to differ from PMSG-β with respect to content of neutral sugars (α, 8.4%; β, 15.8%), hexosamines (α, 5.7%; β, 18.2%), and sialic acid (α, 4.5%; β, 21.3%)

Biological Studies. Since PMSG possesses both LH and FSH activity, it was of interest to examine the subunits and recombinations of them for both of these activities. The ovarian ascorbic acid depletion (OAAD) assay (11) was used to measure LH activity and the hCG augmentation assay (12) for FSH. The results are summarized in Tables II and III. With respect to LH activity (Table II), it is seen that PMSG-α and PMSG-β possess little LH

Table I

Amino Acid Content[a] of PMSG and its Subunits

Amino Acid	PMSG[b]	PMSH-α	PMSG-β
Lysine	5.4	6.0	2.7
Histidine	2.5	3.0	1.2
Arginine	6.1	5.5	5.0
Aspartic	5.5	7.7	4.6
Threonine	8.6	7.4	7.4
Serine	8.0	5.4	10.5
Glutamic	8.4	11.0	7.7
Proline	12.5	8.7	14.9
Glycine	5.1	6.9	6.7
Alanine	7.5	5.9	9.9
Half-cystine	7.5	8.2	6.1
Valine	4.6	4.5	4.9
Methionine	1.7	1.7	2.0
Isoleucine	4.8	3.8	4.7
Leucine	6.3	5.7	7.2
Tyrosine	2.4	3.8	1.5
Phenylalanine	4.0	4.7	3.0

[a] 20 hr. hydrolysis; results calculated as residues/100 amino acid residues analyzed.
[b] Taken from Schams and Papkoff (2).

activity relative to the intact PMSG (3.6 and 6.2%, respectively). Upon recombination of the subunits (1 : 1 by weight of each, pH 7.0 phosphate, 37° C, 20 hours incubation), a considerable regeneration of the LH activity

Table II

LH Activity[a] of PMSG Subunits and Subunit Recombinations[c]

Preparation	Potency (%)[b]
PMSG-α	3.6 (2.0 - 5.9)
PMSG-β	6.2 (4.1 - 8.5)
PMSG-α + PMSG-β	31.5 (26.5 - 40.9)
	23.3 (15.0 - 33.1)
PMSG-α + LH-β	20.6 (13.9 - 28.2)
LH-α + PMSG-β	7.0 (not significant)
PMSG-α + hCG-β	33.2 (21.1 - 52.0)
hCG-α + PMSG-β	5.1 (not significant)

[a] Assayed by the OAAD test (11).

[b] Expressed in terms of PMSG (15,000 l.u./mg) taken as 100% and employed as assay standard.

[c] Recombination conditions: 100 μg of each subunit in 50 λ of pH 7.0, 0.1 μ phosphate incubated at 37° C for 20 hours before dilution for assay. Ovine LH subunits were used.

is achieved (23-32% of native PMSG). In addition, PMSG-α can be combined with both ovine LH-β or hCG-β with comparable regeneration of activity (21 and 33%, respectively). PMSG-β, however, does not readily recombine under these conditions with either ovine LH-α or hCG-α.

Table III shows the results obtained when FSH activity is assessed. Once again, PMSG-α and PMSG-β are of very low activity (2.5 - 5.0% of

Table III

FSH Activity[a] of PMSG Subunits and Subunit Recombinations[c]

Preparation	Potency (%)[b]
PMSG-α	2.5
PMSG-β	5.0
PMSG-α + PMSG-β	29.3 (22.9 - 38.8)
	27.8 (30.0 - 52.8)
	43.6 (26.9 -145.0)
PMSG-α + FSH-β	16.0 (11.0 - 20.4)

[a] Assayed by the hCG augmentation test (12).

[b] Expressed in terms of PMSG (15,000 i.u./mg) taken as 100% and employed as assay standard.

[c] Recombination conditions: as in Table II. Ovine FSH-β was used.

PMSG). Recombination of the subunits results in significant restoration of the FSH activity (28-44%) of PMSG. In addition, PMSG-α is shown to be capable of combining with ovine FSH-β, giving rise to a smaller, but significant, degree of FSH activity.

DISCUSSION

The results detailed above show that PMSG, like other glycoprotein hormones, consists of chemically dissimilar subunits. The fact that the hormone can be dissociated in concentrated solutions of urea and the subunits

separated by ion-exchange chromatography similar to methodology employed for hCG (7, 8) or hFSH (13) suggests similar binding forces between the PMSG subunits (i.e., non-covalent linkage). In addition, we found that the counter-current distribution system employed for the preparation of ovine LH subunits (14) can be applied to PMSG as well (unpublished results).

Like other glycoprotein hormones consisting of subunits, the individual PMSG subunits are of very low biological activity when measured in standard, in vivo biological tests. Recombination of the relatively inert subunits, however, was accompanied by an impressive and significant regain of both the LH and FSH activity expected of PMSG. Full biological activity was not recovered, and this may reflect a lack of knowledge of the optional conditions for subunit recombination, lack of optimal molar ratios of the subunits employed, and possible physical damage to the subunits incurred in the preparation. It is significant and noteworthy, however, that both the LH and FSH activity of PMSG is lost upon dissociation into subunits and regained upon recombination, suggesting that both activities are intrinsic to PMSG. The fact that PMSG-α can be combined with either hCG-β, ovine LH-β, or ovine FSH-β suggests that it is chemically similar to the α subunits of these hormones and that the hormone-specific subunit of PMSG (i.e., PMSG-β) contains the chemical parameters giving rise to both LH and FSH activity.

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