BIOPHYSICS AND BIOCHEMISTRY

Placental Seminal β_2-Globulin: Immunochemical Identification, Physicochemical Characteristics, and Localization in the Reproductive System

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More than 20 individual human placental antigens are known at present. Three protein groups may be singled out among them: 1) ogran-specific: chorionic gonadotropin, and placental lactogen, trophoblastic β-globulin [2,4,6]; 2) interorgan proteins occurring not only in the placenta but in other tissues as well [5]; 3) placental seminal proteins synthesized both by placental cells and by cells of the male seminal duct. Belonging to this third group is placental α-microglobulin-2, identified in early placental tissue and later found in male seminal plasma [1]. We hope that further search for and study of such antigens will help determine the role of individual proteins of the reproductive system in the mechanisms of fertilization and early embryogenesis. This report presents the results of immunochemical identification of placental seminal β_2-globulin (PSBG) and of a study of its physicochemical characteristics and localization in the reproductive system.

TABLE 1. Physicochemical Characteristics of PSBG

Characteristic	Values and results
Molecular weight, kD	20±2
Relative electrophoretic mobility	0.3±0.03
Staining for glycoproteins	negative
Ammonium sulfate sedimentation, % saturation	0–55
0.4% rivanol sedimentation	sedimented
50% ethanol sedimentation	not sedimented

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MATERIALS AND METHODS

Antiserum to PSBG was prepared by immunizing rabbits with extract of early chorion isolated from abortion material obtained from aborted pregnancies (up to 12 weeks). Chorionic fragments washed in cold normal saline were homogenized in an equal volume of 50 mM tris-glycine buffer, pH 8.0, three times frozen at -20°C and defrosted, and centrifuged; the supernatant was used for immunization.

The rabbits were immunized 5 times, each animal subcutaneously injected 100 mg protein with complete Freund's adjuvant at 5-day intervals. Sixty days after the last injection, reimmunization was performed with the same protein dose without adjuvant. Blood was collected from the marginal vein of the ear on days 7, 10, and 13 postimmuniza-

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TABLE 2. Immunodiffusion Analysis of PSBG in Tissues of 12-Week Fetuses and Adults

Sample	Number of samples tested	PSBG content, mg/liter
Fetal kidney	12	5
Adult kidney	18	–
Fetal stomach	12	5
Adult stomach	8	–
Fetal testicle	12	5
Adult testicle	9	5
Early (12-week) chorion	10	1
Terminal placenta (12-week)	8	–

TABLE 3. Immunodiffusion Analysis of PSBG in Biological Fluids

Sample	Number of samples tested		PSBG content, mg/liter
	total	% PSBG positive	
Adult CSF	12	20	1-5
Preterm infant CSF	14	55	1-10
Seminal plasma	16	100	20-120
Amniotic fluid	12	20	1-5
Prostatic fluid	8	–	–

tion. The resultant antiserum was adsorbed with dry donor plasma, liver and spleen extracts, and breast milk.

Immunodiffusion analysis was carried out in 1.5% agar gel (Difco, USA) prepared on 50 mM tris-veronal buffer, pH 8.6, with 2.5% NaCl, using monospecific PSBG test system with a sensitivity of 5 mg/liter [3].

Immunoelectrophoresis was carried out in 1% agarose gel (Sigma, USA) in 50 mM tris-veronal buffer, pH 8.6, on 9×6 cm glass at 9 mA current and 100 V voltage. Bromophenol blue conjugated with albumin was used as the anode mobility marker, while cathode mobility was assessed with pyronine. The markers were added directly to the wells with the test samples. The resistance of PSBG contained in seminal plasma to various chemicals was assessed.

RESULTS

After specific absorption, rabbit antiserum to early chorionic proteins exhibited three antigenic components in the preparation for immunization. Immunodiffusion analysis showed one of them to be identical to trophoblastic β-globulin and another to placental lactogen; the third antigenic component was not identical to any of the known placental proteins.

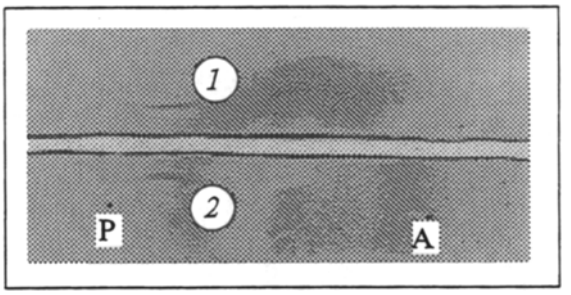

Fig. 1. PSBG electrophoresis in 1% agarose gel. a) antiserum to PSBG; 1) early chorion extract; 2) 8-fold diluted seminal plasma; P: pyronine; A: albumin.

The physicochemical characteristics of PSBG were studied by electrophoresis in agarose gel, by gel filtration in Sephadex G-200, fractionation with salt and organic solvents, and specific staining for carbohydrates (Schiff-iodine reaction). This protein was characterized by β_2-globulin mobility (Fig. 1), was eluted in gel filtration in volumes corresponding to a molecular weight of 20 kD, precipitated at 55% ammonium sulfate saturation, and reacted negatively to staining for carbohydrates (Table 1). With the use of a monospecific test system for PSBG this protein was analyzed in fetal and adult human tissue extracts and in biological body fluids. At the level of sensitivity of immunodiffusion analysis (5 mg/liter) this protein was detected in fetal renal and gastric extracts and in fetal and adult testicular extracts. If the sensitivity of the method was increased to 1 mg/liter PSBG could be detected in early chorionic tissue (Table 2). Testing of biological fluids for this protein revealed it in adult seminal plasma, cerebrospinal fluid (CSF) of preterm babies and adults, and in amniotic fluid. PSBG was found in 100% of seminal plasma samples, in 55% of preterm infant CSF samples, in 20% of adult CSF samples, and in 20% of amniotic fluid samples. The protein concentrations were highest in seminal plasma (up to 120 mg/liter), in preterm infant CSF up to 10 mg/liter, and in adult CSF and amniotic fluid samples less than 5 mg/liter (Table 3). Immunodiffusion analysis did not detect PSBG in extracts

TABLE 4. Immunodiffusion Analysis of PSBG in Male Reproductive Organs

Sample	Number of samples tested	PSBG content, mg/liter
Testicle	10	1-5
Testicular appendage	10	–
Seminiferous duct	10	–
Seminal vesicles	10	–
Prostate	10	–
Spermatozoa	16	–

of fetal and adult liver, adult kidney, fetal and adult lung, spleen, intestine, ovary, pituitary, or adult stomach, or in samples of donor, pregnant women's or fetal blood serum, pregnant women's and donor urine, breast milk, or saliva.

For location of the site of PSBG synthesis in the male seminal duct, extracts of the semen-producting and semen-ejaculating organs were examined, as were spermatozoa isolated from the seminal plasma. PSBG was detected only in testicular extract but not in extracts of the other male reproductive organs (Table 4).

The data suggest that a protein characterized by β_2-globulin mobility and a molecular weight of 20 kD is present in early placental, fetal renal, and fetal and adult testicular tissue and is secreted into the amniotic fluid, seminal plasma, and cerebrospinal fluid. Placental cells appear to secrete it into the amniotic fluid, and the placenta seems to be responsible for its presence in the fetal stomach, where this protein might enter with the amniotic fluid. Testicular cells may secrete PSBG into the seminal plasma. The presence of this protein in the cerebrospinal fluid merits more detailed study.

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Effect of Lipoproteins Modified by Lipid Peroxidation on Platelet Aggregation

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Platelets play an important role in the injury to the vascular wall during the development of atherosclerosis. In a number of studies the platelets of patients with hypercholesterolemia have been shown to be hyperactive [3]. It has been suggested that lipoproteins (LP) markedly contribute to the changes in platelet aggregation. Low-density LP (LDL) from the blood of patients with atherosclerosis have been found to enhance the collagen-induced platelet aggregation to a greater degree then LDL from the plasma of healthy donors [10]. Similar results have been obtained for LP from the blood of patients with hypercholesterolemia and hyperglycerolemia. *In vitro* experiments have demonstrated that LDL enhance the platelet response to various aggregation inducers [2,8], as well as being able by themselves to stimulate platelet aggregation [8].

The development of atherosclerosis is attended by the activation of lipid peroxidation (LPO). Oxidized LP have been found in the vascular wall and in the blood of patients with atherosclerosis

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