IMMUNOCHEMICAL IDENTIFICATION OF β-GLOBULIN IN HUMAN SEMINAL PLASMA

Yu. S. Tatarinov, A. K. Tagirova, and A. A. Terent'ev

UDC 616.69-008.939.624-097-078.33

KEY WORDS: affinity chromatography, seminal plasma, globulin

The search for new proteins in human placental extracts and seminal plasma, conducted during the last two decades with the aid of monospecific polyclonal antisera, has led to the discovery of more than 25 individual antigenic components [13]. However, only certain of these have so far been identified as specific proteins of the human reproductive system, namely: trophoblastic β_1-glycoprotein [6, 10], placenta-specific α_1-microglobulin [4, 12], fertility α_2-microglobulin [3, 15], placental protein-5 [11] and, evidently also, chorionic prealbumin [7]. According to expect evaluation by the World Health Organization, of more than 100 antigenic determinants discovered in the human trophoblast and sperm with the aid of monoclonal antibodies, only five epitopes could be included in the group of specific determinants characteristic of the human reproductive system [9]. We consider the identification, isolation, and purification of specific proteins of the reproductive system to be an urgent task, with the aim of studying the role of these proteins both in the molecular mechanisms of fertility and for the preparation of contraceptive vaccines based on them.

This paper gives the results of immunochemical identification of seminal β-globulin (β-SG), some of its physicochemical properties, and the results of its immunodiffusion analysis in human tissues and biological fluids.

EXPERIMENTAL METHOD

Semipurified preparations of β-SG were obtained by affinity chromatography of seminal plasma obtained from normal donors on 17β-estradioltriazine-sepharose, by the method described by the writers previously [1]. Proteins nonspecifically bound were removed from the adsorbent with the aid of 0.1M NaCl solutions (pH 6.5), under optical density control. Elution was carried out with 2M NaCl (pH 6.5), to yield protein fraction 1. Fraction 2 of proteins was eluted with 0.2M Tris-glycine buffer containing 0.2M butanol (pH 8.6). Proteins of fraction 2 were used as antigen to immunize rabbits. The immunization schedule consisted of five subcutaneous injections (single dose 100 μg protein) mixed with Freund's complete adjuvant, at intervals of 7, 14, 28, and 42 days. The rabbits were reimmunized 30 days after the last injection by a single injection of 100 μg of fraction 2 protein. Three batches of antisera were obtained and their specificity was tested by immunoelectrophoresis. Immunoelectrophoresis was carried out in 1% agarose gel ("Pharmacia") in harbiturate buffer with ionic st strength of 0.02 (pH 8.6). The fractional composition of the eluted protein preparations was studied by disk electrophoresis in a concentration gradient of 8-15% polyacrylamide gel, with or without the addition of sodium dodecylsulfate (SDS) by the method described previously [14].

Immunodiffusion semiquantitative analysis of β-SG in the various tissue extracts and biological fluids was carried out with a standard test system [8], when the β-SG protein (fraction 2), isolated from seminal plasma, and monospecific antiserum against β-SG were used as the antigen. Altogether 80 individual samples of seminal plasma, 30 of normal human blood serum, 30 of saliva, 28 of urine, five of human milk, and 82 extracts of definitive and embryonic human tissues were analyzed.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that fraction 2, isolated from seminal plasma, differed from fraction 1 in relative homogeneity. Antisera to fraction 2, exhausted with dry human

Department of Biochemistry and Problem Laboratory for Immunochemistry of Malignant and Embryonic Tissues, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 11, pp. 469-471, November, 1990. Original article submitted July 6, 1989.

0007-4888/90/0011-1477$12.50

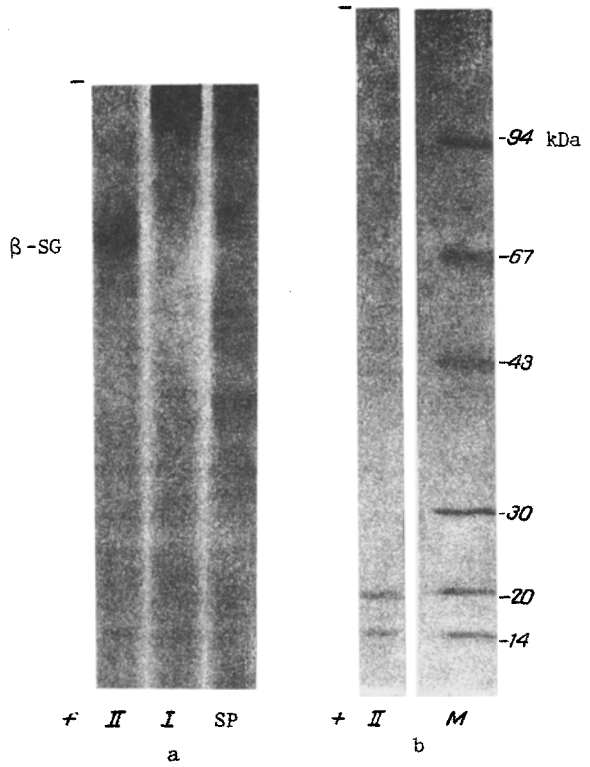

Fig. 1. Characteristics of proteins from seminal plasma with affinity for estradioltriazine-sepharose. Staining with Coomassie blue R-250. a) Without SDS, in 7% polyacrylamide gel (PAG); b) in presence of SDS and in 8-15% PAG. 1) Eluate of seminal plasma (fraction 1); 2) eluate of seminal plasma (fraction 2). SP) Seminal plasma; M) standard markers with indicated molecular mass (in kDa).

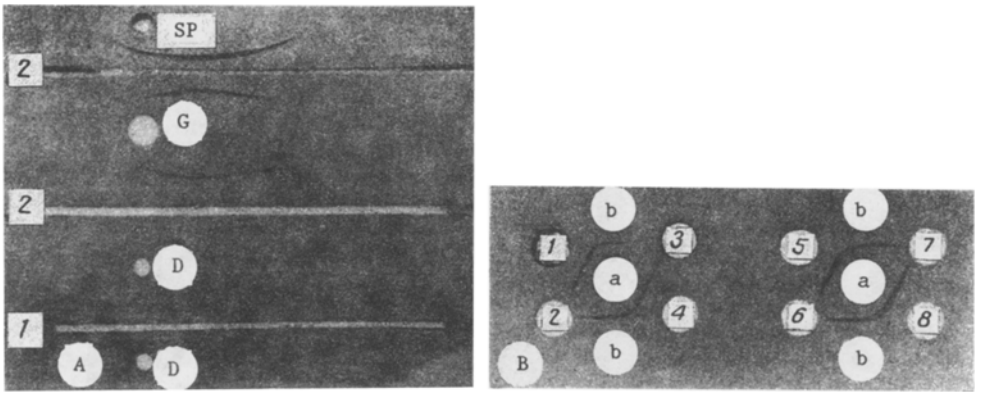

Fig. 2. Immunoelectrophoretic characteristics of β-SG in 1% agarose, pH 8.6 (a), comparative immunochemical analysis using a standard test system for β-SG (b). A: 1) Antiserum to human serum proteins, 2) monospecific antiserum to β-SG. D) Serum from normal donor; SP) seminal plasma; G) β-SG. B: a) Antiserum to β-SG; b) β-SG preparation. 1) Undiluted saliva; 2) placenta α_1-microglobulin; 3) fertility α_2-microglobulin; 4) seminal plasma, diluted 64 times; 5) eluate of seminal plasma (fraction 1); 6) prostatic β-globulin; 7) trophoblastic β_1-globulin; 8) eluate of seminal plasma (fraction 2).

plasma, revealed one antigenic component both in seminal plasma and also in fractions obtained from seminal plasma by affinity chromatography (Fig. 2). The relative electrophoretic mobility of the identified component was 0.39 ± 0.15, corresponding to mobility of the β_1-globulins. The test system for β-SG was compared on immunodiffusion analysis with known seminal and placental antigens, including trophoblastic β_1-glycoprotein [6, 10], placenta-specific α_1-microglobulin [4, 10], fertility α_2-microglobulin [3, 15], chorionic prealbumin [7], thermostable seminal antigen [5], prostatic β-globulin [2], and lactoferrin. No crossed reactions of β-SG with the above-mentioned proteins could be detected (see Fig. 2b).

Data on the β-SG content in the various human biological fluids and tissue extracts are given in Table 1. The highest β-SG content was observed in seminal plasma and, correspondingly, in extracts of the seminal vesicles. An immunochemically similar antigenic component

TABLE 1. Results of Immunodiffusion on Determination of β-SG in Biological Fluids and Tissue Extracts

Tests objects	Number of individual tests	Results of determination of β-SG	
		number of positive tests	titer
Biological fluids			
sperm	80	80	1:256—1:1024
saliva	30	30	1:1—1:4
blood serum	30	0	0
urine	28	0	0
human milk	5	0	0
Organs of reproductive system			
seminal vesicles	3	3	1:2—1:6
testis, prostate	6	0	0
uterus, ovary	6	0	0
Somatic organs: lung, spleen, liver kidney, heart, stomach, adrenal, thymus	67	0	0

TABLE 2. Physicochemical Characteristics of β-SG (M ± m)

Parameter	Properties
Relative electrophoretic mobility in 1% agarose	0,39±0,15 β_1-Globulin
Molecular mass, determined in PAG with SDS, kDa	19.1±0.6 and 14.8±0.7
Salting out with ammonia sulfate, % saturation %	20—100
Precipitation by:	
picric acid 12%	Is precipitated
sulfosalicyclic acid 0.5 M	»
TCA, 10%	»
phosphotungstic acid 5%	»
perchloric acid 0.6 M	»
zinc sulfate 0.025M	»
copper sulfate 1%	»
Thermostability	Is destroyed on heating to 100°C
Resistance to the action of trypsin	Is inactivated

also was discovered, in a much lower concentration, in saliva.

No β-SG was found in other biological fluids or in tissue extracts both of the reproductive system and of somatic organs.

The physicochemical characteristics of β-SG are given in Table 2. The molecular mass of β-SG, determined in PAG in the presence of SDS, is represented by two components: 14.8 and 19.1 kDa. It is readily precipitated by various acids and salts, possesses thermolability, and is inactivated in the presence of trypsin. Its discovery in seminal plasma and saliva may indicate that β-SG is a secretory protein not only of the seminal vesicles, but also evidently of other excretory glands, including the salivary glands.

Thus the β-SG which we have isolated by affinity chromatography on an estradiol adsorbent from seminal plasma can be regarded as a relatively specific seminal protein.

LITERATURE CITED

1. V. I. Mis'kova, Z. M. Pchelkina, A. A. Terent'ev, and Yu. S. Tatarinov, Byull. Éksp. Biol. Med., No. 12, 732 (1985).
2. A. A. Nikolaev and N. I. Anshakova, Vopr. Med. Khim., No. 3, 128 (1985).
3. D. D. Petrunin, I. M. Gryaznova, Yu. A. Petrunina, and Yu. S. Tatarinov, Byull. Éksp. Biol. Med., No. 7, 803 (1976).
4. D. D. Petrunin, I. M. Gryaznova, Yu. A. Petrunina, and Yu. S. Tatarinov, Byull. Éksp. Biol. Med., No. 5, 600 (1978).
5. T. Ya. Pshenichnikova, O. P. Shevchenko, E. A. Obydena, et al., Akush. Ginekol., No. 9, 42 (1983).
6. Yu. S. Tatarinov and V. N. Masyukevich, Byull. Éksp. Biol. Med., No. 6, 66 (1970).
7. Yu. S. Tatarinov, Yu. A. Kalinin, and A. A. Terent'ev, Byull. Éksp. Biol. Med., No. 11, 552 (1988).
8. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).
9. D. J. Anderson, P. M. Johnson, N. J. Alexander, et al., J. Reprod. Fertil., 10, 231 (1987).
10. H. Bohn, Arch. Gynaek., 210, 440 (1971).
11. H. Bohn and W. Winckler, Artch. Gynaek., 223, 179 (1974).
12. H. Bohn and W. Kraus, Arch. Gynaek., 229, 229 (1980).
13. H. Bohn, Protections of the Placenta, W. Bischof and A. Klopper (ed.), Basel (1985), pp. 1-25.
14. G. King and U. K. Laemmli, J. Mol. Biol., 62, 465 (1971).
15. R. Sutcliffe, D. Brock, L. Nicholson, and E. Dunn, J. Reprod. Fertil., 54, 85 (1978).