COMPARATIVE PHYSICOCHEMICAL PROPERTIES OF PREPARATIONS OF TROPHOBLASTIC β_1-GLYCOPROTEIN ISOLATED FROM RETROPLACENTAL BLOOD

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Trophoblastic β_1-glycoprotein (TBG) was described in 1970 as a specific β_1-globulin of pregnancy [7]. TBG is now widely used as an indicator for the early diagnosis of pregnancy, and for screening and monitoring pathology of pregnancy and trophoblastic tumors [5, 7, 8, 10].

Serum or plasma from pregnant women, placental extracts, and retroplacental blood serum are used as the original material for the isolation and purification of TBG, using the basic methods of preparative biochemistry. A method of isolation of TBG exists, which consists of precipitation of proteins with rivanol, ion-exchange chromatography, gel-filtration, and preparative electrophoresis [11]. Another method of isolation of TBG also is known, consisting of salt fractionation, hydrophobic chromatography, and negative antibody-affinity chromatography [14]. Later, TBG was isolated from blood serum from pregnant women by chromatography on hydroxyapatite and immunosorbents [12].

However, all the known methods of isolation and purification of TBG consist of many stages, they are laborious, and they cannot be used on an industrial scale. Moreover, the TBG preparations obtained contain a number of contaminating proteins, which are difficult to eliminate.

The aim of the present investigation was to develop an original method of isolation and purification of TBG and to compare its physicochemical properties with those of a preparation obtained previously [11].

EXPERIMENTAL METHOD

Serum from retroplacental blood, precipitated with ammonium sulfate at 30% saturation, was used as the material for isolation of TBG. After incubation for 18 h at 4°C the mixture was centrifuged for 30 min at 6000 rpm. The residue was dialyzed for 48 h in distilled water and 24 h in 0.05 M NaCl. The dialyzed sample was then subjected to hydrophobic chromatography on hexylsepharose. The TBG was eluted from the sorbent with 1 M NaCl. The eluate, measuring 250 ml, was concentrated in an Amicon system to 100 ml and dialyzed against acetate buffer, then treated by affinity chromatography on a sorbent with immobilized lectin (con A). Elution was carried out with 0.5 M glucose solution. The fraction containing TBG was dialyzed and lyophilized. The resulting preparation was called TBG-1.

The second TBG preparation was obtained by the method described previously [11] and described as TBG-2.

Antiserum to TBG was obtained by immunizing rabbits with a semipurified preparation and then precipitating the nonspecific antibodies with lyophilized normal human blood plasma [1].

The TBG content was monitored at all stages of purification by immunodiffusion titration against a standard test system [9].

Disk electrophoresis (Reanal apparatus, Hungary) was carried out in 7.5% polyacrylamide gel with a current of 4 mA applied to the tube [2].

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TABLE 1. Scheme of Isolation and Purification of TBG

Stages of isolation and purification	Purity of preparation, %	Yield of preparation
Retroplacental blood serum	0	100
Perecip. with ammonium sulfate, 30% saturation	30	85
Chromatog. on hexylsepharose	87	60
Chromatog. on Con A-sepharose	96	20
Dialysis and luophilization	96	20

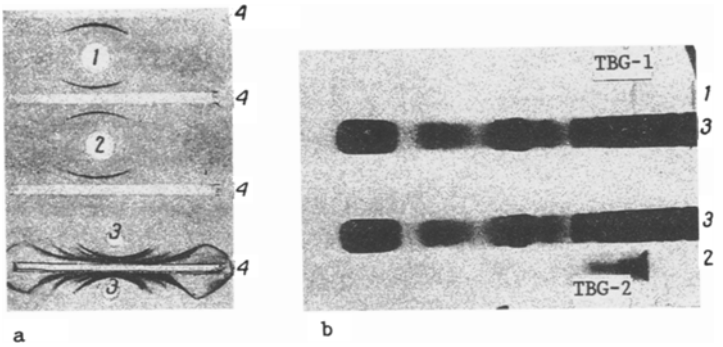

Fig. 1. Immunoelectrophoresis and disk electrophoresis of TBG-1 and TBG-2 preparations. 1) TBG-1 (a, b); 2) TBG-2 (a, b); 3) normal human blood serum (a), retroplacental blood serum (b); 4) antiserum against TBG (a); 5) antiserum against normal human blood serum proteins (a).

TABLE 2. Physicochemical Properties of TBG-1 and TBG-2 (M ± m)

Properties	TBG-1	TBG-2
Mol. wt., kD		
Gel-filtration	100±7	100±5
Electrophoresis	90±4	82±2
Electrophoretic mobility		β_1-globulin
Thermolability, °C	85	85
Precip. by ammonium sulfate, 25-50% saturation		Precipitated
Precipitation by ethanol		«—»
Precipitation by ethanol		Not precipitated
Interaction with lectin con A		Bound

The molecular weight of the TBG was determined by gel-filtration on a 100 × 1.25 column with Sephadex G-150, equilibrated with 0.1 M Tris-HCl buffer, pH 9.0, and also by polyacrylamide gel electrophoresis with dodecylsulfate [13]. A set of proteins of known molecular weight (Pharmacia Fine Chemicals, Sweden) was used as the standard. One-dimensional immunoelectrophoresis was carried out in agar gel (Difco), in Veronal-lyedinal buffer pH 8.5, ionic strength 0.05, with a voltage of 140 V and a current of 40 mA for 2 h [4].

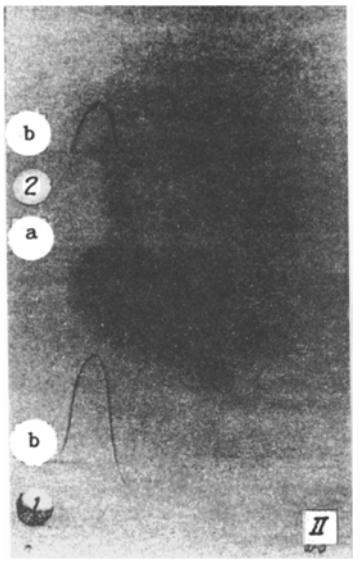

Fig. 2. Crossed immunoelectrophoresis of TBG preparations (I) and densitometry of TBG-1 and TBG-2 (II). 1) TBG-1, 2) TBG-2. a) Agarose without additions, b) agarose with 4S monospecific antiserum against TBG.

Low-voltage crossed immunoelectrophoresis was carried out in 1% agarose gel (Merck, West Germany), made up in 0.05 M Veronal-Medinal buffer, pH 8.5, in the first direction for 1 h at a voltage of 40 V. Electrophoresis in the second direction was carried out in agarose gel containing antiserum against TBG for 18 h, with a voltage gradient of 2 V/cm.

The TBG preparations were subjected to densitometry by a scanner (Helena, France) [2].

EXPERIMENTAL RESULTS

The TBG-1 preparation was isolated by the scheme illustrated in Table 1. This scheme shows that hydrophobic chromatography on hexyl sepharose and affinity chromatography on con A-sepharose constituted the main stages of purification. As a result of the use of these stages, TBG protein with sufficiently high purity could be obtained in a yield of about 20%.

Immunoelectrophoresis showed (Fig. 1a) that preparations of TBG-1 and TBG-2 isolated by the method of Bohn et al. [11] form clear precipitates in the β_1-globulin region and possess identical electrophoretic mobility.

In crossed immunoelectrophoresis (Fig. 2) the two isolated preparations formed complete precipitates in the anodal region, but the precipitate of TBG-1 had a higher "rocket" than TBG-2.

The physicochemical property of the antigens are given in Table 2. For instance, the molecular weight according to the results of gel-filtration was 100 kD, whereas according to electrophoresis it was between 82 and 90 kD, in agreement with results obtained by other workers [3, 6, 11]. The reaction of the proteins to inorganic and organic reagents indicates that the two antigens have similar properties.

Thus preparations of trophoblastic a β_1-glycoprotein, isolated by different methods, preserve their physicochemical and immunochemical property. The TBG-2 preparation obtained by a multistage method of isolation contains contaminating proteins, and is not completely free from residues of rivanol, which interfere with its subsequent study, whereas the TBG-1 preparation, obtained by a sufficiently simple and easily reproducible method, has a yield of 20% and its purity is sufficient, as shown by disk-electrophoresis and densitometry.

The TBG preparation thus isolated can be recommended for obtaining monospecific antisera and immunodiagnostic kits and for studying biological activity in an in vitro system.

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HDL_2-DEPENDENT BILE ACID SYNTHESIS IN RABBIT HEPATOCYTE CULTURE: EFFECTS OF OXIDIZED CHOLESTEROL DERIVATIVES

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Preparations of cholesterol kept for a long time at room temperature undergo autooxidation, the principal products of which are 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, 3,5-cholestadien-7-one, 3β,5α,6β-cholestanetriol, and 25-hydroxycholesterol [13]. Feeding rabbits with cholesterol in this form accelerates the development of high hypercholesterolemia by 1-1.5 months compared with rabbits kept on a diet with purified cholesterol [1]. Massive deposition of lipid inclusions, consisting of cholesterol and its esters, is observed under these circumstances in the hepatocytes [7]. Since excretion of cholesterol is determined mainly by its oxidation into bile acids [12], the writers postulated previously [7] that depression of bile acid synthesis under the influence of oxidized cholesterol derivatives is one of the principal mechanisms of more rapid development of hypercholesterolemia. Support for this hypothesis is given by data obtained on liver microsomes of rats, showing that 7β-hydroxycholesterol and 7-ketocholesterol are competitive inhibitors of cholesterol-7α-hydroxylase [11]. Meanwhile there are no data directly relating to the effect of oxidized sterols on bile acid synthesis in the literature at the present time.

The aim of this investigation was to study the effect of oxidized cholesterol derivatives in bile acid synthesis by cultures of rabbit hepatocytes.

EXPERIMENTAL METHOD

Male chinchilla rabbits weighing 2.5-3 kg were used. Hepatocytes were isolated by perfusion of the liver with collagenase solution [7]. The cells were cultured in Eagle's minimal medium containing 10% fetal calf serum, 100 μg/ml of kanamycin, 1 mM essential amino acids and L-glutamine, at 38°C in an atmosphere of 95% air and 5% CO_2. Fraction 2 of high-density lipopro

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