USE OF WHEAT GERM LECTIN-SEPHAROSE TO ISOLATE SPECIFIC PREGNANCY PROTEIN

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The trophoblastic β_1-globulin of pregnancy (TPG) described previously, is a typical glycoprotein [2, 7]. The use of a serologic test based on determination of TPG in patients' blood for the diagnosis and monitoring of pregnancy and of trophoblastic tumors [3-5, 10] requires a rather large quantity of the purified preparation. The preparation is needed both for developing a method of radioimmunoassay and also in order to obtain antisera. Several methods of obtaining TPG from placental tissue and blood serum of pregnant women have now been described [6, 8, 9]. However, these methods as a rule are laborious, attended by considerable loss of protein, and yield a product which consists only partially of TPG protein molecules, which explains the heterogeneity of this antigen under the conditions used for isolation of the protein.

This paper describes a method of obtaining TPG with minimal loss of protein in the stages of isolation by methods of salt fractionation, affinity chromatography, gel filtration, and ion-exchange chromatography.

Scheme of Isolation of TPG

Retroplacental blood serum
↓
Precipitation of protein with ammonium sulfate at 50% saturation
↓
Precipitate
↓
Affinity chromatography on Wheat germ Lectin-Sepharose 6MB
↓
Gel-filtration on Sephadex G-200
↓
Hydrophobic chromatography on Phenyl-Sepharose CL-4B
↓
Ion-exchange chromatography on DEAE-Sephacel.

EXPERIMENTAL METHOD

The stages of isolation of TPG from retroplacental blood serum of women in labor are shown in the Scheme.

Retroplacental blood was fractionated with ammonium sulfate, which was added up to 50% saturation with constant stirring on a magnetic mixer. The mixture was incubated at room temperature for 1 h. The precipitate was separated by centrifugation at 6000g, dissolved in water, and dialyzed against tap water for 20 h, after which the protein was reprecipitated with ammonium sulfate at 50% saturation and the precipitate was dissolved in 0.05 M Na-phosphate buffer, pH 7.0, with the addition of 0.2 M NaCl and 0.02% of sodium azide.

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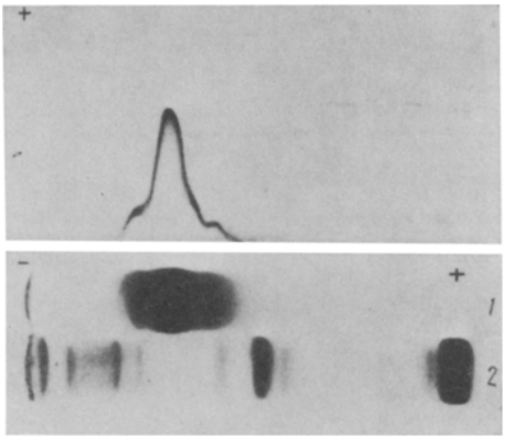

Fig. 1. Electrophoresis of TPG in 7.5% polyacrylamide gel. 1) TPG preparations developed by antiserum (50 μg applied); 2) retroplacental blood serum (1 μl applied). Gels stained with 1% Amido black 10 B made in 7% acetic acid.

The material obtained after salt fractionation was subjected to affinity chromatography on wheat germ Lectin-Sepharose 6MB (from Pharmacia, Sweden). Plastic columns with gel in a volume of 10 ml were used for affinity chromatography. The column was washed with 0.05 M Na-phosphate buffer, pH 7.0, with the addition of 0.2 M NaCl and 0.02% sodium azide, after which the material was applied. Unbound protein was thoroughly washed out with the start buffer and the TPG was eluted with a 10% solution of N-acetylglucosamine.

Gel filtration was carried out in column of 1.6 × 100 cm packed with Sephadex G-200 (Pharmacia) to a height of 80 cm, made up in 0.1 M Tris-HCl, pH 8.0, with the addition of 0.2 M NaCl; the flow was 10 ml/h. A 2-ml sample of protein, after affinity chromatography and preliminary concentration by precipitation with ammonium sulfate at 50% saturation, dissolved in the minimal volume of buffer for gel-filtration, was applied to the column. The protein fraction with relative molecular weight of 120,000 ± 10,000 daltons, containing TPG uncontaminated by α_2-macroglobulin, was selected.

Ammonium sulfate was added to the protein fraction obtained after gel-filtration up to 1.0 M and hydrophobic chromatography carried out on Phenyl-Sepharose CL-4B (Pharmacia). A column measuring 1.6 × 40 cm with 5 ml of gel equilibrated with 0.01 M Na-phosphate buffer, pH 6.8, with the addition of 1.0 M ammonium sulfate, was used. The flow rate was 80 ml/h. After gel-filtration the specimen was applied to the column in 1.0 M ammonium sulfate. Unadsorbed protein was washed off with the start buffer consisting of 1.0 M ammonium sulfate solution and 0.01 M Na-phosphate buffer, pH 6.8, after which the TPG was eluted with distilled water.

The protein fraction containing TPG, after hydrophobic chromatography, was subjected to ion-exchange chromatography on DEAE-Sephacel (Pharmacia). The anion-exchange resin was equilibrated with 0.001 M Tris-HCl, pH 8.0, in a column measuring 1.6 × 40 cm with gel up to a height of 5 cm. The flow rate was 80 ml/h. The sample was applied in the start buffer and the column was washed out with the same buffer before application of a linear gradient of NaCl in the start buffer from 0 to 0.5 M in the course of 4 h, by means of an Ultrograde (from LKB, Sweden). The fraction containing the purified TPG preparation was eluted within the 0.1-0.2 M NaCl range.

The purity of the TPG was tested at all stages of isolation by confluent immunoelectrophoresis [1], using antiserra against TPG and healthy human blood serum proteins, prepared in the writers' laboratory or obtained from Behringwerke (West Germany).

EXPERIMENTAL RESULTS

Immunochemical analysis of the TPG preparation revealed a high degree of purity and absence of contamination by the blood serum proteins of the original donor. Disk-electrophoresis of TPG in 7.5% polyacrylamide gel followed by immunodevelopment demonstrated the

molecular microheterogeneity of this protein. Besides the dominant protein zone in the region of haptoglobins there were two minor fractions with lower and higher anodal mobility, which preserved the immunochemical specificity of TPG (Fig. 1).

By immunizing animals with the TPG preparation, high-quality antisera were obtained and were used for successful development of immunoenzymic and radioimmune methods of assay.

The suggested method of obtaining TPG has advantages over those described previously. Protein losses are reduced to the minimum in the various stages of isolation. During one cycle about 2.0 mg of TPG can be isolated by the method, and no marked heterogeneity of the protein, which is an important shortcoming of methods described previously, can be detected during isolation.

The use of wheat germ lectin also is justified because on other lectins which specifically bind glycoproteins (concanavalin A, lentil lectin), besides TPG, an IgG similar in its physicochemical properties to TPG also is isolated, and their subsequent separation is difficult.

On the basis of these investigations the scheme of isolation described above can be recommended for obtaining a highly purified preparation of TPG.

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