PURIFICATION AND PHYSICOCHEMICAL CHARACTERISTICS OF CHORIONIC α_1-MICROGLOBULIN

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In 1977 we identified [2] an organ-specific antigen in the placenta which differs immunochemically from those hitherto known. This antigen is a specific protein with molecular weight of about 20,000 daltons and with the electrophoretic mobility of α_1-globulins [3]; it was accordingly named chorionic α_1-globulin (CAG-1).

CAG-1 is present in the largest amounts in amniotic fluid during the first half of pregnancy. Chorionic tissue extracts contain much less CAG-1, and it cannot be detected at all in maternal blood serum within the limits of sensitivity of immunodiffusion analysis [2]. In some cases of pregnancy complicated by late toxemia, however, this protein can be found by immunodiffusion in the blood serum. To assess the diagnostic importance of determination of CAG-1 in maternal blood highly sensitive methods are thus required, and highly purified preparations of this protein are necessary for their development. The biological role of CAG-1 is unknown.

The object of this investigation was to purify CAG-1 and to determine its physicochemical properties.

EXPERIMENTAL METHOD

The method of obtaining monospecific antisera against CAG-1 was described by the writers previously [2]. Amniotic fluid between the 16th and 25th weeks of pregnancy was used as the source for isolation of this protein. The amniotic fluid was treated with 10% lanthanum chloride solution in the ration of 20:1 so that its concentration was 0.5%. The precipitate thus formed was centrifuged for 30 min at 8000 rpm and dissolved in a saturated solution of disodium hydrogen phosphate. The resulting solution was fractionated with ammonium sulfate at 50% saturation and the precipitate was dissolved in distilled water. In both cases the volume of the dissolved precipitated fractions was made up to the original volume of amniotic fluid fractionated. The fraction thus obtained was then reprecipitated with lithium sulfate at 90% saturation and the residue was dissolved in a small volume of distilled water (usually 1/20 of the volume of the original material) and dialyzed against distilled water. For further purification, the impurities were twice absorbed with calcium pyrophosphate, by adding an equal volume of the wet absorbent to the protein solution.

The total protein content during isolation was determined spectrophotometrically at 280 nm. The concentration of CAG-1 was calculated from the results of titration with a standard test system [5], the sensitivity of which was about 2 μg/ml. Immunoelectrophoresis was carried out by the method of Grabar and Williams [7], relative electrophoretic mobility was determined by Uriel's method [4], and molecular weight was determined by thin-layer gel-filtration on Sephadexes G-100 and G-200 [1] with immunodevelopment [3]. Rocket immunoelectrophoresis was carried out by Laurel's method [8]. Concanavalin A, alone or immobilized on Sepharose (Pharmacia, Sweden) were used. To determine the carbohydrate component tests also were carried out with neuraminidase (Calbiochem, USA) and hyaluronidase (100 i.u. enzyme to 0.3 mg CAG-1). The resistance of CAG-1 to the action of papain was studied. The action of

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TABLE 1. Scheme of Purification of Chorionic α_1-Microglobulin

Stage of purification	Purity, %	Yield, %
Amniotic fluid 16-25 weeks	4	100
Precipitation with 0.5% lanthanum chloride	25	90
Precipitation with ammonium sulfate at 50% saturation	35	70
Precipitation with lithium sulfate at 60% saturation	60	60
Adsorption chromatography on calcium pyrophosphate	90	30

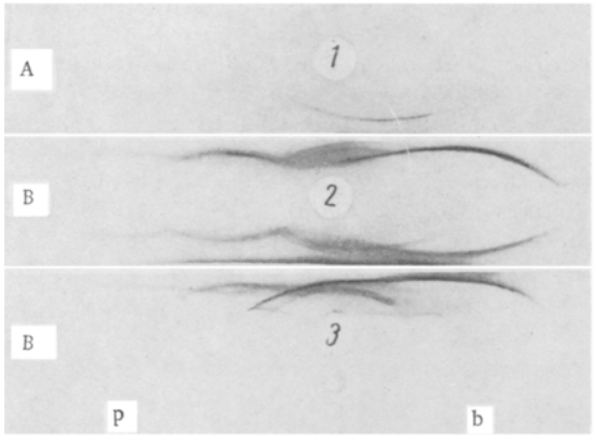

Fig. 1

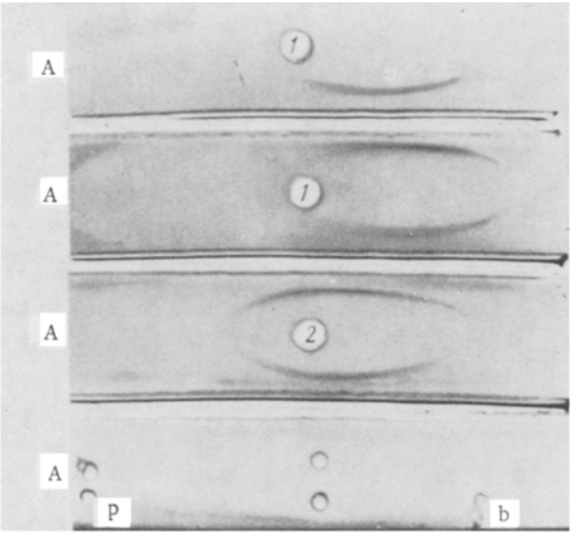

Fig. 2

Fig. 1. Immunoelectrophoretic analysis of CAG-1. 1) The CAG-1 preparation, 2) amniotic fluid, 3) donor's blood serum; A) monospecific antiserum against CAG-1, B) unexhausted antiserum against amniotic fluid. Reference substances for immunoelectrophoresis: p) pyronine, b) Evans' blue.

Fig. 2. Effect of protamine sulfate on electrophoretic mobility of GAG-1. 1) Amniotic fluid, 2) amniotic fluid incubated with protamine sulfate. Remainder of legend as in Fig. 1.

the enzymes was assessed from changes in titer or electrophoretic mobility of protein. Monospecific antisera against trophoblast-specific α_1-globulin, α_1-globulin of the "pregnancy zone," "α_1-fetoprotein," and chorionic α_1-globulin, prepared in the writers' laboratory, were used.

EXPERIMENTAL RESULTS

CAG-1 was isolated from amniotic fluid at the 16th-25th week of pregnancy by the scheme shown in Table 1. This protein is practically completely precipitated by lanthanum and lithium salts, so that it can be effectively isolated from protein mixtures. As Table 1 shows, as a result of a combination of salting out with lanthanum chloride, ammonium sulfate, and lithium sulfate a semipurified preparation of CAG-1 (about 60% purity) was obtained; the yield of this protein was 60%. It was freed from salts by dialysis. Much of the protein impurities, unlike CAG-1, is adsorbed on calcium pyrophosphate. By repeated adsorption a preparation with a purity of 90% could be obtained.

The preparation thus obtained was immunochemically pure and the CAG-1 preserved its immunologic properties fully. On immunoelectrophoresis it formed one precipitation peak in

the α_1-globulin zone (Fig. 1) and its relative electrophoretic mobility with respect to albumin was 0.83 ± 0.02. The molecular weight of the CAG-1 was 20,000 ± 2000. This protein was destroyed by trypsin and papain but preserved its antigenic properties and electrophoretic mobility fully after treatment with neuraminidase and hyaluronidase. On incubation of the CAG-1 preparation with concanavalin A, alone or immobilized on Sepharose, and also with cytohemagglutinin, no change took place in the titer of this protein and there was no change in the height of peaks compared with the controls in rocket immunoelectrophoresis. These results indicate that CAG-1 does not bind with the above-mentioned lectins [6].

As a result of contact with protamine sulfate a sharp decrease took place in anodal electrophoretic mobility of the CAG-1 (Fig. 2). In control experiments there was no change in the electrophoretic mobility of trophoblast-specific β_1-globulin, α_1-globulin of the pregnancy zone, α-fetoprotein, or chorionic α_2-microglobulin after their incubation with protamine sulfate, indicating that the binding of the latter with CAG-1 exhibits definite selectivity. This may not only be evidence in support of the acid properties of this protein, but it may also be of definite importance for metabolism.

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