Studies on a Glycoprotein of Fetal Calf Serum That Protects Nucleic Acids from Acid Precipitation (35492)

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In an attempt to stabilize a dilute nuclease by keeping it in a protein-rich environment, it was accidentally noted that small amounts of fetal calf serum prevented precipitation of DNA by trichloroacetic acid (TCA). It was at first thought that this activity was due to a DNase present in the serum that hydrolyzed DNA to acid-soluble products. However, evidence is presented in this communication which shows that the fetal serum component responsible for the inhibition of precipitation of DNA by acid is a glycoprotein possessing protective rather than catalytic properties.

Materials and Methods. Fetal calf serum was purchased from Flow Laboratories Ltd.; Sephadex G-100 and G-200 were products of Pharmacia A. B. Electrophoresis in polyacrylamide gels was carried out in a shandon disk electrophoresis apparatus. Calf thymus DNA was prepared according to the method of Kay *et al.* (1). Commercial yeast RNA from BDH was precipitated by 5% TCA and the acid-insoluble products were dialyzed against water. Protein was determined by the method of Kingsley (2) or Lowry *et al.* (3) and carbohydrates by the methods of Dische *et al.* (4) and Dubois (5). The protective activity was assayed by adding 1.5 ml of 10% (w/v) TCA to 1.5 ml of a solution containing 0.5 mg of heat-denatured DNA and 0.15–1.5 units of activity. Following centrifugation at 17500*g* for 10 min, the absorbancy of the supernatant solution was determined in a Beckman DU spectrophotometer at 260 nm. A unit of activity is defined as the amount of the protecting agent that retains sufficient DNA in solution under the assay conditions to give a reading of 1.00 at A_{260}. Specific activity or potency is defined as units per milligram of protein.

Results. Purification of protecting agent.

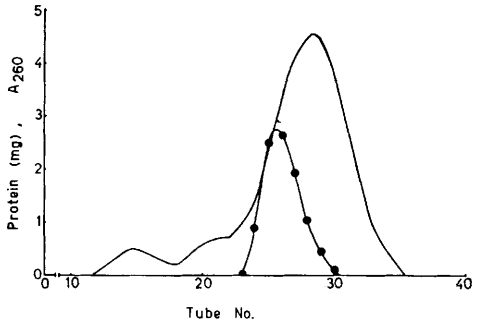

FIG. 1. Gel filtration of fetal calf serum through a 2.2 × 40-cm column of Sephadex G-200: filtration medium, 0.14 *M* NaCl; fraction size, 10 ml; (—) protein (mg/tube); (●—) protective activity (units/tube).

Three ml of fetal calf serum are filtered in the cold room through a 2.2 × 40 cm column of Sephadex G-200 in 0.14 *M* NaCl. Three-ml fractions are collected and assayed for protein and protecting activity. Figure 1 is the filtration profile of one such experiment. The active fractions are pooled and, to the resulting solution, 1.2 vol of acetone are added at 0°. The precipitate formed is discarded and the supernatant solution is rendered 63% with respect to acetone. The precipitate is collected by centrifugation and dissolved in 2 ml of H_2O. After rendering the solution 5% with respect to sucrose, the preparation is layered in 0.5-ml portions over 0.8 × 12.5 cm 7.5% polyacrylamide gels prepared in 0.37 *M* Tris–HCl buffer, pH 8.6. Electrophoresis is carried out in 0.05 *M* Tris–glycine buffer, pH 8.6, for 4 hr at 5 mA/gel. One gel is stained with 1% amido black in 7% acetic acid. The remaining gels are sliced in pieces of 1 cm and extracted with 0.01 *M* Tris–HCl buffer, pH 8.0, overnight. Activity is associated with the main band of the stained gel (Fig. 2). This prepa-

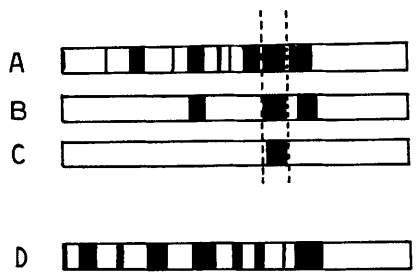

FIG. 2. Polyacrylamide gel electrophoresis at pH 8.6 of: (A) fetal calf serum; (B) 67% acetone precipitate following gel filtration of fetal calf serum through Sephadex G-200; (C) active fraction following preparative gel electrophoresis; (D) calf serum. Areas between broken lines indicate active fractions.

ration was used for all further experiments because (a) gel filtration of the active component through Sephadex G-100 column both in the presence and absence of 6 *M* urea resulted in the appearance of one protein peak that was slightly retained on the column, with constant potency in all fractions; and (b) gel electrophoresis of the active preparation either at pH 8.6 or 6.5 resulted in the appearance of a single band. The active component comprises approximately 15% of the total fetal serum protein.

Nature and properties of the protecting component. The active component is a glycoprotein with an absorption maximum at 277 nm and a molecular weight of approximately 70,000 since it appears slightly ahead of albumin from a Sephadex G-200 column (Fig. 1). It contains 14% carbohydrate, expressed as mannose equivalents. It completely protects DNA against the precipitating action of 2.5% TCA. As the TCA concentration increases, the protection gradually decreases although, even in 20% TCA, 30% of DNA remains in solution under the assay conditions described above. The glycoprotein effectively protects DNA from other acids also, such as hydrochloric and perchloric, even though not to the same extent as from TCA. Furthermore it can protect RNA from the precipitating action of TCA. With impure preparations, the protective activity is evident only when single-stranded DNA is employed. As the purification proceeds, however, double-stranded native DNA is also protected. With the pure glycoprotein, activity increases as the protein concentration increases until a plateau is reached at a protein to DNA ratio of 1:2 (w/w). With the less pure preparations, however, a drop in activity is observed as the protein concentration increases but this is due to the antagonistic action of inactive serum proteins. Indeed when 25 μg of crystalline serum albumin were added to the assay mixture, precipitation of DNA did occur in spite of the presence of sufficient pure glycoprotein to keep DNA in solution. Bivalent metals strikingly suppress the glycoprotein protective activity (Fig. 3). Co^{2+} is the most potent in this respect. Neither extremes in pH or temperature have any deleterious effect on the activity of the purified glycoprotein. Thus, the purified preparation remains in solutions of pH 2 to 12 for 15 min as well as in a boiling waterbath at pH 7 for 30 min without detectable changes in its protective activity. However, proteolytic digestion, whether by trypsin or pepsin, tends to destroy this activity.

Discussion. Tunis and Weinfeld reported, a decade ago, that purified human serum glycoproteins were able to keep nucleic acids in

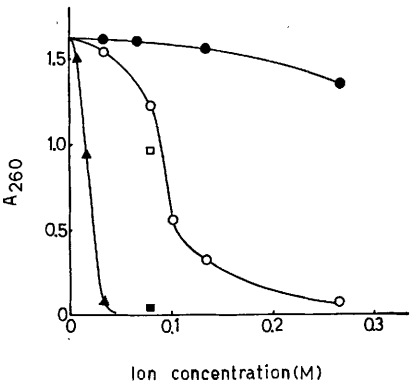

FIG. 3. Effect of Na^+ and bivalent cations on the ability of purified glycoprotein of calf serum to protect DNA from the precipitating action of 5% TCA. To 1.5 ml of an aqueous solution containing 300 μg of DNA, 100 μg of purified glycoprotein of fetal calf serum and Na^+ or bivalent metal as the chloride salt of the indicated final molarities, 1.5 ml of 10% TCA was added. Following centrifugation, the absorbancy of the supernatant solution was determined at 260 nm: (●) Na^+; (○) Mg^{2+}; (▲) Co^{2+}; (■) Mn^{2+}; and (□) Ca^{2+}.

solution in the presence of TCA (6). In the present communication it is shown that fetal calf serum contains large amounts of one such glycoprotein that is not present in detectable amounts in calf serum. We feel that with the widespread use of fetal calf serum as a source of growth factors in tissue cultures, one should be aware of the presence of this nucleic acid-interacting component.

Summary. Fetal calf serum contains large amounts of a glycoprotein that inhibits the acid precipitation of nucleic acids. The glycoprotein has been purified by gel filtration, acetone precipitation, and polyacrylamide gel electrophoresis. Its protective activity is insensitive to extremes in pH and temperature, but sensitive to proteolytic digestion and low concentration of bivalent metals.

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