The Isolation and Identification of the P-Component of Normal Human Plasma Proteins

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A normal human plasma protein called the P-component, which has a reaction of identity with the pentagonal structure found in amyloid-laden organs, has been isolated and identified with a recently characterized protein, the 9.5S α_1 -glycoprotein.

Two proteins have been identified in amyloid-laden organs by electron microscopy: the fibril and the pentagonal structure (Cohen & Calkins, 1959; Bladen *et al.*, 1966). The fibril, because of its abundance, characteristic ultrastructure and Congo Red-binding, is the component associated with the presence of amyloid. The pentagonal structure, by contrast, is found in only small amounts, does not bind Congo Red and has an amino acid and carbohydrate composition different from that of the fibril (Binette *et al.*, 1971). In 1967 the pentagonal structure was found to have a reaction of identity with a normal plasma protein (Cathcart *et al.*, 1967*a*), which was called P-component (Cathcart *et al.*, 1967*b*). The isolation of this plasma protein and its identification with a newly described glycoprotein are reported in the present paper.

Experimental

Materials. Normal human plasma was obtained from the hospital blood bank. It was clotted by the addition of thrombin and the clot was centrifuged. The Bio-Gel and the acrylamide for electrophoresis were purchased from Bio-Rad Laboratories, Richmond, Calif., U.S.A., and the Gradipore gels from Ortec Inc., Oak Ridge, Tenn., U.S.A.

Methods. Dialysis of plasma against distilled water provided an easy and convenient first step in the purification of P-component, as it is precipitated out of solution under these conditions. The precipitate was dissolved in salt solutions (usually 0.5M-Tris-EDTA-boric acid, pH8.4) and the solution was passed through a Bio-Gel P-300 column equipped with an upward-flow adaptor. Further purification was achieved by electrophoresis of the concentrated peak containing the P-component on a formalated cellulose support in 0.5M-Tris-EDTA-boric acid solution, pH8.4 (LKB column 3340-C), with Bromophenol Blue serving as a marker. Final purification was achieved by preparative polyacrylamide-gel electrophoresis in the Uniphor apparatus under the following conditions: 5% polyacrylamide gel, 0.4% *NN'*-methylenebisacrylamide in 0.068M-Tris-glycine buffer, pH8.4, in the gel and 0.05M,

pH8.1, in the electrode vessels. The column was 10cm high. The sample was dialysed, freeze-dried and dissolved in 1–2ml of 0.01M-Tris-glycine buffer, and allowed to enter the gel with voltage set at 200V and 75 pulses/s to minimize thermal convection. The voltage was eventually advanced to 450V and the pulses/s were gradually increased to 300, providing a current of 10mA (Ortec 4100 pulsed constant power supply). Elution was carried out at 12ml/h with 0.1M-Tris-glycine solution. The electrophoretic run lasted approx. 27h.

Identification was achieved throughout the isolation procedure from the reaction of identity displayed by the pentagonal structure and the P-component on immunodiffusion against an antiserum to the pentagonal structure. Analytical polyacrylamide-gel electrophoresis was performed on a Gradipore gel varying in concentration from 4 to 26%. For electron microscopy, solutions of the purified proteins were dialysed against distilled water and freeze-dried. A 1mg sample of protein was mixed with 0.1ml of a 2% (w/v) solution of phosphotungstic acid adjusted to pH5.4 with NaOH solution and allowed to stand at room temperature for 10min. The carbon-coated grids were floated on a drop of the mixture, washed with boiled distilled water and examined immediately in the electron microscope.

Results

The peak containing the P-component was slightly retarded on the Bio-Gel P-300 column and had an R_F value of 0.78 with the use of Blue Dextran as marker, suggesting a molecular weight of approx. 300000. Gel filtration served to purify P-component further and allowed electrophoresis on formalated cellulose by removing most of the residual lipoproteins, thus avoiding precipitation on the column. P-component has the mobility of an α_1 -globulin on the cellulose column and was the first peak to be eluted. By contrast, and in spite of its charge, the P-component was the last protein eluted from the preparative 5% polyacrylamide gel, again suggesting a large molecule retarded by the sieve effect of the gel. Analytical electrophoresis on the Gradipore gel

of this concentrated protein peak showed a single band, and immunodiffusion against an antiserum to the pentagonal structure showed a reaction of identity with the latter.

The combination of these characteristics, high molecular weight, rapid electrophoretic mobility and reaction of identity with the pentagonal structure, suggested a new plasma protein. However, Haupt *et al.* (1972) have reported the characterization of a plasma protein that they called '9.5S α_1 -glycoprotein', and the properties of this newly described protein and the P-component appeared to be closely similar. Samples of purified 9.5S α_1 -glycoprotein and its antiserum were sent to us by Dr. Heimbürger and tested by immunodiffusion and Gradipore-gel electrophoresis. The three proteins gave reactions of identity against antisera to the pentagonal structure and the 9.5S α_1 -glycoprotein (Plate 1), and all three displayed the same mobility on Gradipore-gel electrophoresis (Plate 2). Electron microscopy of the 9.5S α_1 -glycoprotein revealed a pentagonal structure with the dimensions of the pentagon isolated from amyloid-laden organs and the P-component isolated from plasma.

Discussion

We conclude from these experiments that the same protein through a series of independent investigations had received three names: 'pentagonal structure', because of its appearance in the electron microscope; 'P-component', because of its presence in plasma; and, finally, '9.5S α_1 -glycoprotein', on the basis of its physicochemical characteristics.

The reportedly high carbohydrate concentration of the 9.5S α_1 -glycoprotein, double that of P-component, had been difficult to reconcile with the identical mobility of the proteins on Gradipore-gel electrophoresis. In particular, the strong negative charge conferred upon the glycoprotein by sialic acid and its presumed terminal position on the

carbohydrate chain should have been reflected by a difference in the electrophoretic mobility, particularly in a high-resolution system such as Gradipore-gel electrophoresis. However, direct comparison in our laboratory of the sialic acid content of the 9.5S α_1 -glycoprotein and the P-component gave identical results (Warren, 1959), thus adding further evidence for the identity of the two proteins, which now includes: immunochemical identity, the same sialic acid content, identical mobility on Gradipore-gel electrophoresis and the appearance of a pentagon by electron microscopy.

No physiological role has yet been ascribed to the P-component, but the reported ability of the 9.5S α_1 -glycoprotein to bind to polyanions and metal ions suggests an explanation for the presence of P-component in amyloid-laden organs (binding to fibrils) and a possible role in the body's economy (calcium and heparin binding). The low concentration of P-component in plasma would suggest more binding specificity than capacity.

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- Binette, P., Matsuzaki, M., Calkins, E., Alper, R. & Winzler, R. J. (1971) *Proc. Soc. Exp. Biol. Med.* **137**, 165-167
- Bladen, H. A., Nysten, M. U. & Glenner, G. G. (1966) *J. Ultrastruct. Res.* **14**, 449-459
- Cathcart, E. S., Shirahama, T. & Cohen, A. S. (1967a) *Biochim. Biophys. Acta* **147**, 392-393
- Cathcart, E. S., Wollheim, F. A. & Cohen, A. S. (1967b) *J. Immunol.* **99**, 376-385
- Cohen, A. S. & Calkins, E. (1959) *Nature (London)* **183**, 1202-1203
- Haupt, H., Heimbürger, N. & Baudner, S. (1972) *Hoppe-Seyler's Z. Physiol. Chem.* **353**, 1841-1849
- Warren, L. (1959) *J. Biol. Chem.* **234**, 1971-1975

EXPLANATION OF PLATE I

Immunodiffusion analysis showing reaction of identity between 9.5S α_1 -glycoprotein (1), pentagonal structure (2) and P-component (6), with antiserum to pentagonal structure in the centre well

EXPLANATION OF PLATE 2

Gradipore-gel electrophoresis (4–26%) of P-component (1), 9.5S α_1 -glycoprotein (2), pentagonal structure (3), normal human serum (4) and a mixture of the first three (5)