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# IMMUNOCHEMICAL IDENTIFICATION AND PHYSICOCHEMICAL STUDY OF $\alpha_2$-GLYCOPROTEIN FROM THE ATHEROSCLEROTIC AORTIC WALL

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The principal changes in the cardiovascular system and other organs in atherosclerosis are due, it is generally agreed, to morphological and functional changes in the vascular wall. The intimate mechanism of the genesis of the atherosclerotic plaque in the wall of the aorta and other arteries remains a crucial problem in the pathogenesis of atherosclerosis that still awaits solution [1, 3, 6].

The study of metabolism in the atherosclerotically changed vascular wall and, in particular, the study of its protein composition, is particularly interesting on account of the discovery of the so-called "atherocalcin" [4], a specific protein contained in the atherosclerotic plaque.

The aim of this investigation was an immunochemical study of the antigenic composition of the atherosclerotic aortic wall and to isolate specific components contained mainly in the affected zone of the aortic wall.

## EXEPRIMENTAL METHOD

Human aortic tissue with well-marked atherosclerotic plaques was taken for investigation. The plaques (weighing about 50 g) were excised inside their visible boundaries, washed with physiological saline to remove blood, homogenized in a "Biomix" homogenizer (Hungary) at 10,000 rpm, and delipidized with a mixture of chloroform and methanol (3:1) for 24 h at 0-4°C. The residue was freeze-dried and dissolved in the minimal volume of distilled water. The resulting preparation was used for primary immunization of chinchilla rabbits by the usual method. The antiserum obtained was adsorbed with freeze-dried extracts of human liver and spleen and also with dried human blood plasma.

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TABLE 1. Physicochemical Properties of $\alpha_2$-Glycoprotein from Atherosclerotic Aortic Wall

| Property of protein | Result of test |
|---|---|
| Electrophoretic mobility | $\alpha_2$-Globulin |
| Relative mol. wt. | 80 ± 10 kD |
| Isoelectric point | 4.3 |
| Interation with lectins: | |
| Lentil-lectin | 70% |
| Concanavalin A | 40% |
| Wheat germ | Not bound |

Purified fractions were obtained by treating the original preparation for the subsequent study of their chemical properties and for use in repeated immunization and preparation of monospecific antiserum. The extract of atherosclerotic aortic wall was subjected to exchange chromatography on cellulose DEAE-52 (Whatman, England). Elution was carried out with 0.5M Na-phosphate buffer, pH 7.0, containing NaCl in a gradient of 0 to 1 M. The eluent in the range from 0.12 to 0.2 M NaCl was used for further investigation. The fraction contained in this range was freeze-dried and subjected to gel-filtration on Sephadex G-150 (Pharmacia, Sweden) in 0.05M Na-phsophate buffer pH 7.0. The protein fraction with mol. wt. of 80 ± 10 kilodaltons (kD) was withdrawn and subjected to affinity chromatography on Sepharose lentil-lectin 4B (Pharmacia). This fraction was eluted with a 2.5% solution of α-methyl-D-glucopyranoside, made up in 0.05 M Na-phosphate buffer, pH 7.0, containing $MnCl_2$, $MgCl_2$, and $CaCl_2$ (1 mM of each).

The preparation obtained after affinity chromatography contained 91% of the protein to be used for obtaining monospecific antisera and for the study of its physicochemical properties. The amino-acid composition was determined after incubation of the sample in 6 N HCl for 12 h at 105°C on a Durrum DS-500 amino-acid analyzer (USA).

Antisera and preparations containing the various components of the atherosclerotic aortic wall were standardized by immuniduffusion analysis with standard test systems [2, 5].

## EXPERIMENTAL RESULTS

By immunochemical study of the extract from the atherosclerocic aortic wall an antigen with mobility of $\alpha_2$-glubulins was identified. By the immunodiffusion method it was found only in atherosclerotically changed tissues of the arterial wall and was not found in extracts of human organs and tissues (altogether 28 extracts were tested); this test protein likewise could not be found in blood serum from healthy subjects under 30 years of age (Fig. 1).

The physicochemical properties of the protein and the amino-acid composition of its molecule were studied.

It will be clear from Table 1 that the test protein has a relative mol. wt. of 80 ± 10 kD, determined by the gel-filtration method. The isoelectric point, found by isochromatic focusing, was 4.3. The high affinity of the preparation for lectin will be noted, evidence of a

TABLE 2. Amino-Acid Composition of $\alpha_2$-Glycoprotein of Atherosclerotic Aortic Wall

| Amino acid | Content, % of 100 amino acids) | Amino acid | Content, % (of 100 amino acids) |
|---|---|---|---|
| Alanine | 8,8 | Aspartic acid | 7,63 |
| Valine | 8,6 | Threonine | 3,4 |
| Methionine | 1,9 | Serine | 3,8 |
| Isoleucine | 2,5 | Glutamic acid | 13,6 |
| Leucine | 6,92 | Proline | 12,1 |
| Tyrosine | 0,4 | Glycine | 10,82 |
| Phenylalanine | 2,44 | Lysine | 3,65 |
| Histidine | 1,1 | Arginine | 2,19 |
| Cysteine ($O_3H$) | 1,51 | | |

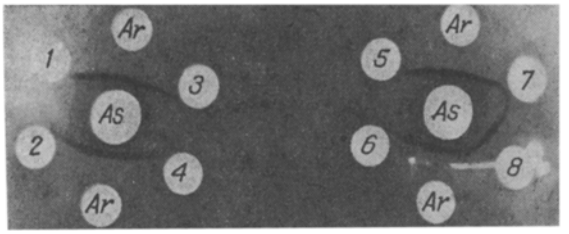


Fig. 1

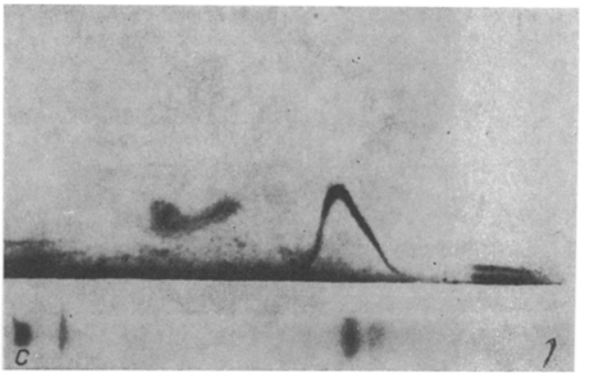


Fig. 2

Fig. 1. Immunodiffusion assay of $\alpha_2$-glycoprotein in atherosclerotic aortic wall and in various human organs and tissues. 1) Tissue from normal human aorta; 2) liver tissue extract; 3) splenic tissue extract; 4) lung tissue extract; 5) skin tissue extract; 6) cerebrospinal fluid; 7) normal human blood plasma from donors under 30 years old; 8) extract of atherosclerotic aortic wall. Ar) Purified extract of atherosclerotic aortic wall. As) Antiserum.

Fig. 2. Purified $\alpha_2$-glycoprotein from atherosclerotic aortic wall immunodeveloped with monospecific antiserum.

carbohydrate component in its structure. On the basis of these data, the protein was identifield as the $\alpha_2$-glycoprotein of the atherosclerotic aortic wall (Fig. 2).

Analysis of the amino acid composition of the $\alpha_2$-glycoprotein demonstrated predominance of glutamic acid and proline in the protein part of its molecule (Table 2).

It can thus be concluded from these investigations that extract from the atherosclerotic aortic wall contains an identified protein, which is an $\alpha_2$-glucoprotein, cannot be detected in other human tissues and organs, and is isolated from the blood serum of patients with atherosclerosis.

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