PRELIMINARY ASSIGNMENT OF THE INHERITED Hl 1 ANTIGEN TO THE apoA-I POLYPEPTIDE OF RABBIT HIGH DENSITY LIPOPROTEIN

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1. Introduction

The first genetic polymorphism of serum high density lipoprotein (HDL) in any species was found by Berg et al. [1] in the rabbit. The presence or absence of an antigen designated Hl 1 appeared to be controlled by autosomal dominant inheritance.

In human serum the protein moiety of HDL is known to contain at least five polypeptide chains [2, 3]. The two main polypeptides are called ApoA-I and ApoA-II according to the nomenclature of Kostner and Alaupovic [4]. The other three polypeptides ApoC-I, ApoC-II, and ApoC-III, constituting about 10% of the HDL apoprotein are also the major components of the protein moiety of very low density lipoprotein (VLDL) [5].

In this report we present data which indicate that also rabbit HDL is composed of several polypeptides with a pattern resembling that of man, and that the Hl 1 antigen resides in one of the two major polypeptides.

2. Materials and methods

2.1. *Isolation of rabbit HDL*

The solute density of a pool of 120 ml serum from three rabbits possessing the Hl 1 antigen was adjusted to 1.063 g/ml by adding solid KBr. The solution was submitted to ultracentrifugation at 105,000 *g* for 24 hr, at 8°. The top fraction containing the low density lipoprotein (LDL) and VLDL was removed by a tube slicing technique. The density of the remaining fluid was adjusted to 1.21 g/ml with solid KBr, and the solution was centrifuged for 48 hr. The HDL fraction recovered from the top layer was washed by recentrifugation under identical conditions, and dialyzed against 0.85% (w/v) saline solution containing 5 mg/l Na_2EDTA.

2.2. *Preparation of HDL polypeptides*

Rabbit HDL was delipidated by extraction with ethanol–diethylether (3:2) at −10° according to Scanu [6].

A sample of the delipidated HDL (30 mg in 3 ml) was dialyzed for 48 hr against a Tris-HCl buffer of pH 8.6 containing 0.01 M Tris, 0.001 M Na_2EDTA, and 8 M urea, and submitted to gel filtration on a Sephadex G-200 column (2.5 × 90 cm) as described by Scanu et al. [3]. The effluent fluid was collected in fractions of 5 ml and their absorbance was measured at 280 nm. The fractions corresponding to each protein peak were pooled and concentrated by ultrafiltration, using Diaflo filters UM-10 and UM-2 (Amicon Corp., Lexington, Mass.). Urea was removed from the protein preparations by extensive dialysis against a Tris-HCl buffer of pH 8.6 containing 0.01 M Tris and 0.001 M Na_2EDTA.

2.3. *Immunological analysis*

Agar purum (Behringwerke AG, Marburg) was used for gel diffusion tests with specific antiserum (R 56) to the Hl 1 antigen [1].

3. Results

The delipidated rabbit HDL was separated by gel filtration on Sephadex G-200 in 8 M urea into five fractions (fig. 1). These were numbered I–V according

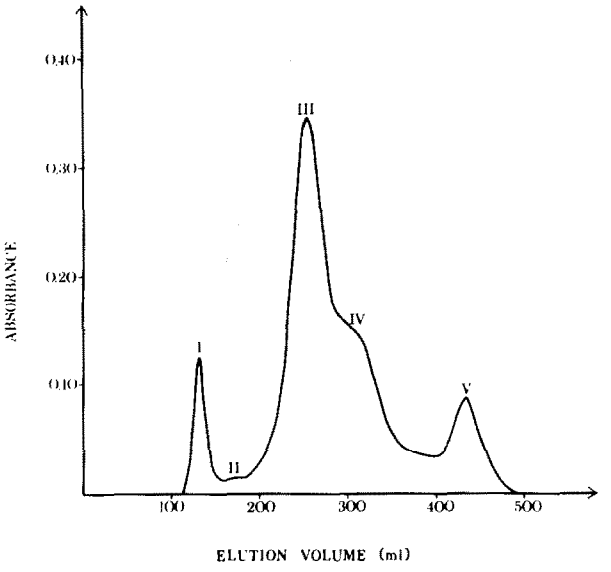

Fig. 1. Protein profile obtained upon gel filtration on Sephadex G-200 in 8 M urea of delipidated rabbit HDL, showing a separation into five fractions which have been numbered according to Scanu et al. [3].

to Scanu et al. [3]. Fraction II was not constantly present and therefore not studied further. The incomplete separation between fractions III and IV was improved by rechromatography, and this was done prior to immunological analysis. Fractions I, III, IV, and V were adjusted to have approximately the same protein concentration before such studies.

When fractions I, III, IV and V were tested in agar gel double diffusion experiments against antiserum to the Hl 1 antigen, a distinct precipitin band was formed only between the antiserum well and wells containing fraction III (fig. 2). In some experiments trace reactions could be detected also against wells containing fraction IV.

4. Discussion

The essential features of the protein profile obtained upon gel filtration of delipidated rabbit HDL are apparently the same as those observed with material of human extraction [3]. Thus, it is likely that also rabbit HDL contains several polypeptides. Provided that the similarity between rabbit and human

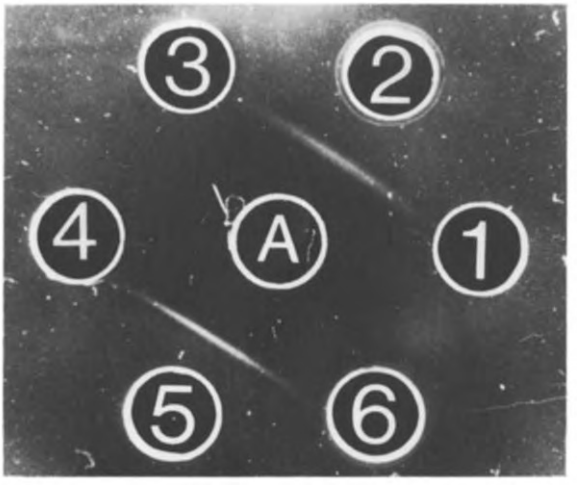

Fig. 2. Agar gel double diffusion experiment showing that the genetically determined Hl 1 antigen resides in fraction III (which corresponds to human apoA-I) of delipidated rabbit HDL. Central well: Antiserum R 56 (anti-Hl 1).
Well 1: Fraction I of delipidated rabbit HDL.
Well 2: Fraction III of delipidated rabbit HDL.
Well 3: Fraction IV of delipidated rabbit HDL.
Well 4: Fraction V of delipidated rabbit HDL.
Well 5: Pool of rabbit sera possessing the Hl 1 antigen.
Well 6: Rabbit serum lacking the Hl 1 antigen.
Note precipitin band between wells containing antiserum and fraction III (apoA-I), respectively.

HDL in apoprotein profile reflects a similar pattern of peptides, our fraction III should correspond to the apoA-I polypeptide and our fraction IV to the apoA-II polypeptide in man. Thus, the apoA-I polypeptide would appear to be the main polypeptide of rabbit as well of human [7] HDL.

The separation of the presumed polypeptides apoA-I and apoA-II appears to be somewhat more difficult to achieve with rabbit apoHDL than with human (fig. 1). However, the results of immunological tests conducted with apoA-I and apoA-II which had been rechromatographed, appear to permit the tentative conclusion that the Hl 1 antigen resides in the main apoA-I polypeptide of rabbit HDL, and that our antiserum R 56 discloses a genetic polymorphism within this polypeptide chain. The very weak reactions with preparations of apoA-II observed occasionally with antiserum R 56 were probably caused by trace amounts of apoA-I contaminating some batches of apoA-II. The apparently remote possibility that the Hl 1 antigenic variation resides in an as yet unidenti-

fied polypeptide contaminating the apoA-I preparations rather than in apoA-I itself, would require further investigations.

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