STUDIES ON THE INTERACTION OF CONCANAVALIN A WITH MAJOR DENSITY CLASSES OF HUMAN PLASMA LIPOPROTEINS. EVIDENCE FOR THE SPECIFIC BINDING OF LIPOPROTEIN B IN ITS ASSOCIATED AND FREE FORMS

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1. Introduction

Normal human plasma lipoprotein system consists of four distinct lipoprotein families referred to as LP-A,* LP-B, LP-C, and LP-D [1–4]. In the density above 1.019 g/ml, the lipoprotein families occur mainly as separate physicochemical entities [3, 5]. However, in the density below 1.019 g/ml the lipoprotein families B and C and, if present, lipoprotein families A and D form an association complex(es) [2, 5]. In a continuing effort to develop simpler procedures for the isolation and separation of free and associated forms of lipoprotein families, we have systematically studied the interaction of concanavalin A with the major lipoprotein density classes and with individual apolipoproteins. While these studies were in progress, Shore and Shore reported that most VLDL particles applied to a column of concanavalin A–Sepharose were adsorbed [6].

Our report describes the fractionation of normal human plasma VLDL, LDL_1, LDL_2 and HDL by affinity chromatography on concanavalin A–Sepharose and presents evidence for a selective binding of concanavalin A to LP-B. Results show that affinity chromatography of plasma lipoproteins on concanavalin A–Sepharose can be used as a simple procedure for the isolation of free and associated forms of LP-B.

* Abbreviations: VLDL, very low density lipoproteins ($d < 1.006$ g/ml); LDL_1, subclass of low density lipoproteins (d 1.006–1.019 g/ml); LDL_2, subclass of low density lipoproteins (d 1.019–1.063 g/ml); HDL, high density lipoproteins (d 1.063–1.21 g/ml); HDL_2, subclass of high density lipoproteins (d 1.063–1.125 g/ml); HDL_3, subclass of high density lipoproteins (d 1.125–1.21 g/ml); LP-A, lipoprotein A, family of lipoproteins characterized by the presence of apolipoprotein A; A-I and A-II, constitutive polypeptides of apolipoprotein A; LP-B, lipoprotein B, family of lipoproteins characterized by the presence of apolipoprotein B; LP-C, lipoprotein C, family of lipoproteins characterized by the presence of apolipoprotein C; C-I, C-II and C-III, constitutive polypeptides of apolipoprotein C; LP-D, lipoprotein D, family of lipoproteins characterized by the presence of apolipoprotein D [4]. The ABC-nomenclature used in this paper has been described previously [1].

2. Materials and methods

2.1. *Isolation of lipoproteins and apolipoproteins*

Plasma samples obtained from healthy young men and women by plasmapheresis were characterized by normal lipid levels. The VLDL, LDL_1, LDL_2 and HDL were separated by sequential preparative ultracentrifugation [2]. After two consecutive recentrifugations at the appropriate solution densities, all lipoprotein preparations were free of albumin, as determined by double diffusion analyses. The A-I and A-II polypeptides of apolipoprotein A, the C-I, C-II and C-III polypeptides of apolipoprotein C, and LP-B

were isolated as previously described [2]. Apolipoprotein D ('thin-line' polypeptide) was isolated according to a recently described procedure [4].

2.2. *Affinity chromatography on concanavalin A–Sepharose*

Concanavalin A–Sepharose 4B was purchased from Pharmacia Fine Chemicals, Inc., Piscataway, New Jersey. Affinity chromatography of lipoprotein density classes was carried out according to a modified procedure described by Avrameas and Guilbert [7]. The columns (2 × 30 cm) of concanavalin A–Sepharose 4B were equilibrated at room temperature with an equilibration buffer containing 0.05 M Tris, 1.0 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $MnCl_2$, pH 7.0. Each lipoprotein density class isolated from 500 ml of plasma was dialyzed against this buffer and applied to the column. The unretained fraction of lipoproteins was eluted with the same buffer. The retained lipoproteins were eluted with the equilibration buffer made 0.2 M with methyl α-D-glucopyranoside (Sigma, St. Louis, Mo.). The elution pattern was monitored by adsorbance at 280 nm with an Ultraviolet Absorption Meter (Gilson Medical Electronics, Middleton, Wisc.). The fractions of unretained and retained lipoproteins were combined separately and concentrated by ultracentrifugation at the solution density of the elution buffer or at the appropriate density of the lipoprotein density class applied to the column. The concentrated fractions were dialyzed against 0.15 M NaCl, pH 7.2, and tested by double diffusion and 7% polyacrylamide gel electrophoresis.

2.3. *Immunochemical and analytical methods*

Double diffusion and immunoelectrophoresis of lipoproteins, and the preparation and characterization of antisera to human HDL_3, LP-B, LP-C, A-I, and A-II polypeptides, respectively, were carried out as previously described [2]. The preparation of a monospecific antiserum to apolipoprotein D was made as reported in a recent publication [4].

The binding capacity of concanavalin A (Mann Research Laboratories, New York, N.Y.) for major lipoprotein density classes and for individual apolipoproteins was tested qualitatively by its ability to form precipitates in double diffusion experiments in 1% agarose [8]. The 7% polyacrylamide gel electrophoresis was carried out as previously described [2].

3. Results and discussion

Double diffusion analyses of concanavalin A with lipoprotein density classes and with individual polypeptides, when tested at the concentration of 1 mg/ml, showed positive precipitin lines only with VLDL, LDL_1, LDL_2 and HDL_2, and with LP-B; concanavalin A failed to react with HDL_3, and with A-I, A-II, C-I, C-II, C-III, and apolipoprotein D. Most of these reactions are shown in fig. 1. Serial dilutions of concanavalin A (3.52; 0.352; 0.0352; 0.00352 mg/ml) with the equilibration buffer revealed no positive reactions with any of the individual polypeptides, except LP-B. These results strongly suggest a selective binding of concanavalin A to LP-B. The precipitin lines seen in the reaction between concanavalin A and VLDL, LDL_1, LDL_2 and HDL_2 reflect and confirm the previously established occurrence of LP-B in these density classes [2, 3, 5]. Electrophoresis of LDL_2 and HDL_2 followed by subsequent reaction with concanavalin A demonstrated precipitin arcs of LP-B in the β-globulin position (fig. 1).

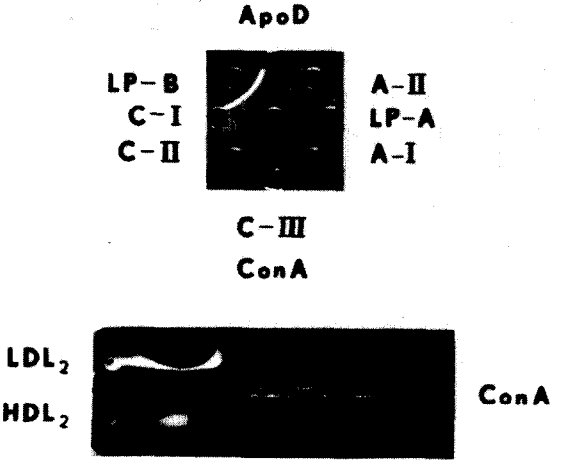

Fig. 1. Double diffusion (upper pattern) and electrophoretic analyses (lower pattern) in 1% agarose of human plasma lipoproteins and polypeptides tested against concanavalin A (ConA).

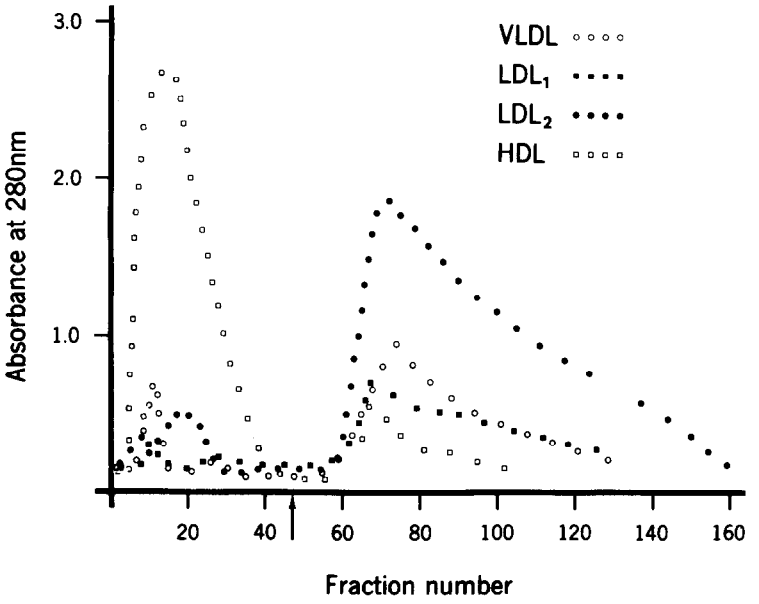

Fig. 2. Elution profile of isolated density classes applied to concanavalin A–Sepharose 4B column. Arrow indicates the application of 0.2 M methyl α-D-glucopyranoside.

The binding of concanavalin A to LP-B was confirmed by the results of affinity chromatography of the major lipoprotein density classes on concanavalin A–Sepharose 4B. The VLDL, LDL_1 and LDL_2 isolated from fasting subjects with normal serum lipid levels were almost completely retained on the column (fig. 2). Based on the planimetric measurement of areas under the elution curves, the retained lipoproteins accounted for 90–95% of the eluted lipoproteins. None of the unretained fractions reacted with antibodies to LP-B. The unretained fractions of VLDL and LDL_1 gave no reaction with antibodies to LP-A or LP-C; the unretained fraction of LDL_2 gave a faint precipitin line with anti-LP-A serum. On the other hand, retained fractions of VLDL, LDL_1 and LDL_2 reacted positively with antisera to LP-B and LP-C. Fusion of the immunoprecipitin lines of retained fractions of VLDL and LDL_1 with antisera to LP-B and LP-C (fig. 3) confirmed the previously reported evidence [2, 5] for the existence of an association complex(es) of LP-B and LP-C in these two density classes. The presence of LP-C in the retained fraction of LDL_2 suggests that the association of LP-B and LP-C may actually extend into this density class.

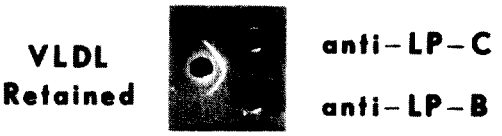

Fig. 3. Double diffusion analysis in 1% agarose of the retained VLDL fraction eluted from a concanavalin A–Sepharose 4B column.

In contrast to low density lipoprotein classes, affinity chromatography of HDL showed that the unretained fraction accounted for 85% of the eluted lipoproteins (fig. 2). The unretained fraction of HDL reacted positively with antibodies to A-I, A-II, HDL_3 and LP-C (fig. 4). Reaction of unretained fraction with anti-HDL_3 serum gave two precipitin lines characteristic of LP-A and LP-D ('thin-line' polypeptide). The retained fraction of HDL only reacted with antiserum to LP-B. In some cases, however, small quantities of LP-A, LP-C and LP-D were also retained. The retention of these lipoprotein families is currently being studied to establish whether it is caused by glycosylation of A-I and A-II polypeptides, by the presence of glycolipids, or the formation of an associa-

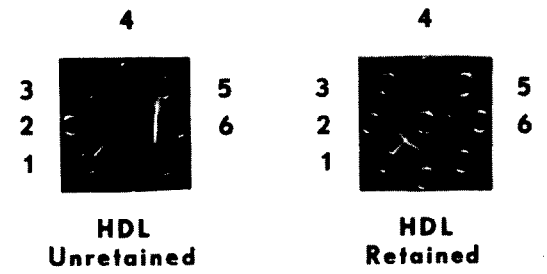

Fig. 4. Double diffusion analysis in 1% agarose of the unretained and retained HDL fractions eluted from a concanavalin A–Sepharose 4B column. Antisera were placed in outer wells, 1 = anti-LP-B; 2 = anti-albumin; 3 = anti-HDL_3; 4 = anti-A-II; 5 = anti-A-I; 6 = anti-LP-C.

tion complex with LP-B. Recent observations of neuraminidase treatment of LP-D (unpublished experiments) have demonstrated that removal of sialic acid from the carbohydrate moiety of apolipoprotein D led to the binding of neuraminidase-treated LP-D to concanavalin A–Sepharose.

The carbohydrate analyses of apolipoproteins or their constitutive polypeptides revealed the presence of a carbohydrate moiety only in apolipoproteins B [9, 10] and D (unpublished results), and in the C-III polypeptide of apolipoprotein C (11). The A-I [12], A-II [13] and C-I [11] polypeptides seem to lack a carbohydrate moiety. There is some uncertainty, however, regarding the possible presence of carbohydrate in the C-II polypeptide. Our results from double diffusion analyses and affinity chromatography suggest that, at least in the intact lipoprotein form, concanavalin A binds selectively to the glycoprotein moiety of LP-B. LP-C is retained only in the form of an association complex(es) with LP-B in VLDL and LDL_1. The free form of LP-C in HDL from normal subjects was not retained on concanavalin A–Sepharose columns. This finding was confirmed by studies which showed that lipoprotein *X* (LP-*X*), the protein moiety of which contains apolipoprotein C, was also not retained on concanavalin A–Sepharose. The LP-A family of lipoproteins did not bind to concanavalin A, except when possibly present in an association with LP-B. Although these findings are basically in agreement with those of Shore and Shore [6], there seems to be some discrepancy regarding the possible binding of C-II polypeptide (band F in the Shores' terminology). The clarification of these seemingly contradictory findings will obviously necessitate additional experiments including the carbohydrate analysis of the C-II polypeptide.

Specific binding of concanavalin A to LP-B renders the affinity chromatography of plasma lipoproteins on concanavalin A–Sepharose columns as an ideal preparative procedure for the selective isolation of LP-B in its free and/or associated forms. This procedure is simpler and milder than the use of a specific immunoadsorber [3, 15] because it does not require the use of monospecific antibodies to LP-B, and the elution of LP-B is carried out at neutral rather than at the usual low pH-values.

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