HETEROGENEITY OF HUMAN SERUM VERY LOW DENSITY LIPOPROTEINS AS DEMONSTRATED BY DEAE-CELLULOSE CHROMATOGRAPHY

W. K. K. HO
Radioisotope Unit, University of Hong Kong, Hong Kong

Received 23 November 1974

1. Introduction

The human serum very low density lipoproteins (VLDL*) are heterogenous with respect to size, density and chemical composition [1]. Recent studies have shown that the protein moiety of this class of lipoprotein is composed of several apoproteins with the major ones belonging to the LP-B and LP-C families [2–4]. In view of the heterogenous nature of the VLDL protein, to draw any meaningful conclusions on the structure of VLDL, one must first consider the nature of the interaction between the constituent apoproteins and lipids.

Studies on the fractionation of VLDL protein indicate that different apoproteins have different affinity to bind DEAE-cellulose [2–4]. Based on this difference in affinity, selective fractionation of lipid-apoprotein complexes from native VLDL may be possible when this lipoprotein fraction is chromatographed on DEAE-cellulose under various eluting conditions. The components produced in this manner should provide insights to the structural organization of lipids and apoproteins within the VLDL molecule. The present report describes the fractionation of VLDL by DEAE-cellulose chromatography and the chemical characterization of the components produced by this procedure.

* *Abbreviations*: VLDL, very low density lipoprotein (d< 1.006 g/ml); LDL, low density lipoprotein (d 1.006–1.063 g/ml); DEAE-cellulose, diethylaminoethyl-cellulose; LP-B, LP-C, apolipoprotein families as defined in [4].

2. Materials and methods

2.1. *Isolation of lipoproteins*

Blood samples were obtained from healthy male subjects who had fasted for at least 14 hr. Serum was prepared as described previously [5]. VLDL was isolated from serum according to the ultracentrifugal procedure of Hatch et al [6] using a Type 65 rotor (Beckman, Palo Alto, Calif., U.S.A.). Purity of the VLDL preparation was confirmed by immunodiffusion assay and by agarose electrophoresis. 0.1 mg/ml sodium azide was added to all lipoprotein solutions to prevent bacterial growth.

2.2. *DEAE-cellulose chromatography*

Microgranular DEAD-cellulose (Whatman DE52, W. & R. Balston Ltd., Maidstone, UK) was washed and equilibrated in 0.01 M phosphate buffer, pH 7.1, containing 0.1 mg/ml EDTA according to the manufacturer's instructions. The equilibrated DEAE-cellulose was packed into a 1.6 × 30 cm column under a constant pressure of 5 p.s.i. VLDL samples were dialyzed exhaustively against the equilibrating buffer at 4°C before they were applied onto the column. Usually 3 ml of VLDL, containing approximately 5 mg of protein, were applied. Elution was first carried out with the equilibrating phosphate buffer, and when a total volume of 20 ml was eluted the ionic strength of the buffer was increased linearly over a gradient from 0 to 0.5M NaCl. Flow rate was maintained at 12 ml/hr by a peristaltic pump (LKB, Bromma, Sweden) and 4 ml fractions were collected. Fractions were monitored for absorption at 280 nm, conducti-

vity, protein and lipid. Recovery of VLDL protein and lipid was around 70 to 80%. No additional material could be eluted when a 0.5 M Tris–phosphate buffer, pH 4.6 was used. Fractions corresponding to the protein peaks were pooled, concentrated by ultrafiltration, dialyzed against a 0.01 M ammonium carbonate buffer, pH 10.2 and analyzed for apoprotein and lipid compositions as described below. All chromatographic operations were performed at 18°C.

In orienting experiments, other elution conditions, such as steeper gradient and larger sample volume, were tried, however, these all proved to give inferior resolution than the conditions described above.

2.3. *Lipid and protein analysis*

Protein concentration was determined by a modified method of Lowry et al. [7] using human serum albumin as standard (Sigma, St. Louis, Mo.). An equal volume of 2% sodium deoxycholate (Sigma, St. Louis, Mo.) solution was added to clarify samples of high lipid concentration. Lipid concentration was determined by a phosphovanillin procedure described by Frings et al. [8] using olive oil (Sigma, St. Louis, Mo.) as standard. Lipids were extracted by a modified method of Sperry and Brand [9]. The extracted lipids were fractionated by thin layer chromatography on silica gel using a solvent system of hexane/diethyl ether/acetic acid (60/40/1). Lipid fractions were visualized by exposure to I_2 vapor. The spots corresponding to cholesteryl ester, triglyceride, free cholesterol and phospholipid were scrapped and eluted with a chloroform/methanol (1/1) mixture for further analysis. Concentrations of triglyceride, cholesterol (both free and esterified) and phospholipids were determined colorimetrically according to the procedures of Fletcher [10], Leffler [11] and Dryer et al. [12], respectively. Lipoprotein fractions were delipidated by the method of Shore and Shore [3] and their apoprotein patterns were determined by polyacrylamide gel electrophoresis in 8 M urea [4].

3. Results

Fig.1 shows a typical experiment in which VLDL was chromatographed on a DEAE-cellulose column using a linear NaCl gradient. The fractions collected were assayed for protein and lipid content as well as for absorption at 280 nm. As indicated, no material was eluted until the NaCl concentration of the eluting buffer was increased to 0.05 M. The elution profiles determined by absorption at 280 nm and by assay of lipid content were fairly similar with major and minor components occuring around fraction numbers 26 and 38, respectively. (figs.1 A and C). The large absorption value at 280 nm around fraction 26 was probably due to the light scattering property of lipid-rich particles. On the other hand, when the fractions were assayed for protein content, three distinct peaks

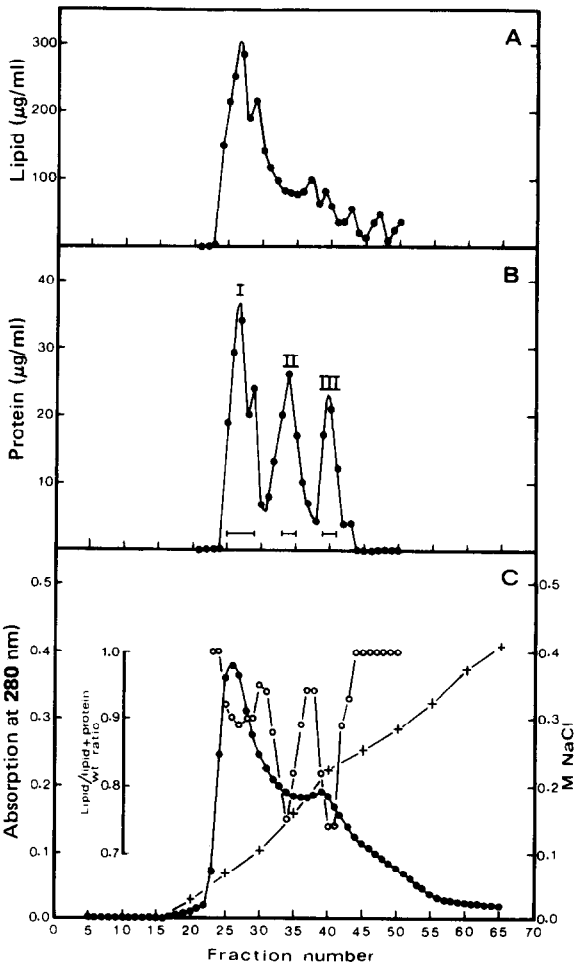

Fig.1. Elution profile of VLDL applied to DEAE-cellulose column. Individual points represent values at the corresponding 4 ml fraction. A, lipid concentration; B, protein concentration; C, (●–●–●), absorption at 280 nm; (○–○–○), lipid/lipid plus protein weight ratio; (+++), NaCl concentration.

(designated as I, II and III) were observed at the regions corresponding to fraction numbers 26, 34 and 40 (fig.1B). The positions of protein peaks I and III were roughly identical to those of the major and minor components of the profile obtained by absorption at 280 nm.

When the ratio of lipid to lipid plus protein weight was determined, fractions corresponding to protein peak I had a ratio of approximately 0.9 while those corresponding to protein peaks II and III had a ratio of around 0.75 (fig. 1 C). The former ratio was very similar to that of the parent VLDL and the latter was closer to that of LDL.

In view of the difference in lipid to lipid plus protein weight ratio, we analyzed the apoprotein and lipid compositions of the three protein peaks. The results are summarized in fig. 2 and table 1. When native VLDL protein was subjected to polyacrylamide gel electrophoresis in 8 M urea, patterns containing approximately ten bands were usually obtained (fig.2, pattern A). Compared with the apoprotein pattern of native VLDL, patterns of the three major protein peaks all lacked some of the bands. Bands b, c, d, e, h and j were absent in the peak I pattern, bands a and j were absent in the peak II pattern and bands a, c, d, f, h and j were absent in the peak III pattern. Although bands g and i were present in all of the protein peaks, judging from the relative intensity of stain uptake, the amount of material corresponding to these bands appeared to be highest in protein peak I and lowest in protein peak III.

Lipid analysis on protein peak I indicated it had a lipid composition very similar to that of native VLDL. On the other hand, when compared with VLDL, the lipid compositions of protein peaks II and III showed an increased proportion of triglyceride and a decreased proportion of phospholipid and unesterified cholesterol. This change was more pronounced in peak III than peak II.

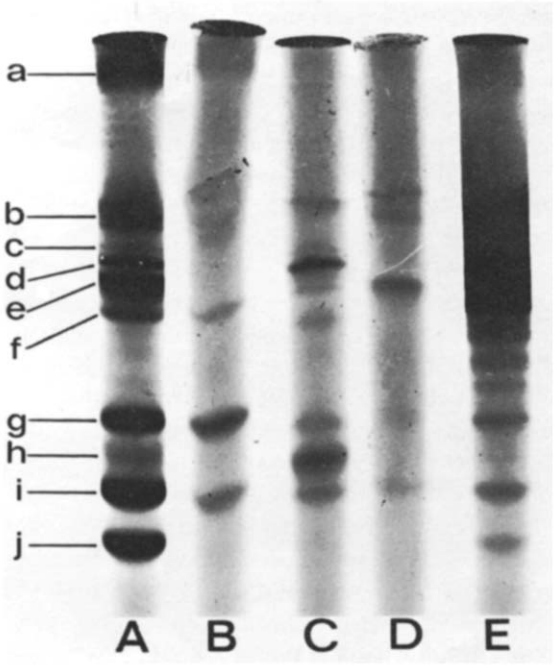

Fig.2. Apoprotein patterns of VLDL components from DEAE-cellulose chromatography. A, VLDL; B, protein peak I; C, protein peak II; D, protein peak III; E, HDL. Band b is composed of several minor components.

4. Discussion

The utilization of chemical and physical procedures, such as partial organic solvent extraction [13,14] and sonication [15], to disrupt human serum lipoprotein

Table 1
Lipid composition of VLDL components from DEAE-cellulose chromatography

Lipoprotein fraction	% Total lipid component weight			
	Free cholesterol	Cholesteryl ester	Triglyceride	Phospholipid
VLDL	15	10	49	26
Protein peak I	14	11	48	28
Protein peak II	11	11	59	20
Protein peak III	8	11	69	12

has yielded pertinent information on the structural organization of lipid and protein within these macromolecules. In the present study, we demonstrated that human serum VLDL could be fractionated into three distinctive protein components by chromatography in DEAE-cellulose under increasing ionic strength. In contrast to earlier studies using organic solvent to disrupt the lipid moiety of VLDL [13,14], the present procedure presumably fractionates VLDL according to the affinities of its constituent apoproteins or apoprotein–lipid complexes for DEAE-cellulose. The elution of different components at different ionic strengths provides suggestive evidence that ionic interaction probably plays an apparent role in mediating protein–protein as well as protein–lipid interaction within the VLDL molecule.

When human serum VLDL is fractionated by either molecular sieving chromatography [16] or ultracentrifugal flotation analysis [1], it was shown that 1) lipoprotein fractions having a higher proportion of triglyceride generally have lower proportions phospholipid and free cholesterol, 2) increased triglyceride content is associated with increased size and flotation rate and increased lipid/protein ratio. Similar to the results of these studies, the lipid compositions of the three VLDL components we presently obtained showed reciprocal relationships for triglyceride and phospholipid and for triglyceride and free cholesterol. On the other hand, compared with protein peak I, proteins peaks II and III had higher proportions of triglyceride (59 and 69% vs. 48%) but smaller lipid to lipid plus protein weight ratio (0.75 vs. 0.90). This discrepancy between our results and those obtained by molecular sieving chromatography and ultracentrifugal flotation analysis may be due to differences in the nature of the fractionation techniques. Thus, DEAE-cellulose chromatography fractionates VLDL by charge while molecular sieving chromatography and ultracentrifugal flotation fractionate VLDL by size and shape.

Recent experimental data on the primary structure of some of the VLDL apoproteins indicate that the association between these apoproteins and phospholipid may involve the ionic interaction between adjacent pairs of oppositely charged residues on the protein chain and the zwitterionic polar group of the phospholipid molecule [17]. In the present study, we observed that protein peaks having higher content of phospholipid were eluted at a lower ionic strength than those having lower phospholipid content. If the negatively charged side groups of the VLDL apoproteins were involved in the binding of phospholipid, these groups would be less available to interact with the cationic groups of the DEAE-cellulose. As a result, VLDL components of higher phospholipid content were eluted at lower ionic strength. Even though the above theory may explain what we observed, substantiation of this proposal requires further investigation.

Compared with the apoprotein pattern of native VLDL, the apoprotein pattern of protein peak I contained only three of the ten major bands. In spite of this, the lipid composition as well as the lipid to lipid plus protein weight ratio of this peak is very similar to that of native VLDL. Whether the apoproteins corresponding to these three bands possess the ability to bind the full complement of VLDL-lipid should be of great interest in the study of lipid–protein interaction.

Acknowledgements

I thank Mr W. Y. Chan for his skillful technical assistance and Miss Susan Thai for taking blood samples. Financial support from the World Health Foundation (Hong Kong), University of Hong Kong and Mr F. S. Ko is gratefully acknowledged.

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