Note

Hydrophobic Components of Hen's Egg Yolk Low Density Lipoprotein

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Previously we reported the isolation of LDL from hen's egg yolk by affinity chromatography with stearyl-Sepharose 4B (C 18-Sepharose 4B).¹⁾ To investigate the hydrophobic properties of LDL, we examined the hydrophobic components of apo LDL. In the present paper we describe the chemical composition, amino acid composition and molecular weight of the partially delipidated LDL soluble in 10 mM Tris buffer of pH 8 (pd-LDL).

LDL was prepared from the yolk of fresh eggs obtained from High sex chickens according to Martin *et al.*²⁾ Yolk was diluted with an equal volume of 0.16M NaCl and centrifuged at $45,000 \times g$ for 30 min at 4°C. The NaCl concentration of the supernatant liquid was adjusted to 1M and then it was centrifuged at $90,000 \times g$ for 16 hr at 4°C. The floating LDL was dialyzed against 0.05M Tris-HCl, pH 8.0, containing 1 mM EDTA. The lyophilized LDL was delipidated with *n*-heptane as previously reported.³⁾ SDS polyacrylamide gel electrophoresis was done according to the method of Shapiro *et al.*,⁴⁾ using 5% polyacrylamide gel and a current of 8 mA per tube. The gels were stained for protein with Coomassie brilliant blue R 250 and for carbohydrate with periodic acid-Schiff reagent. Hexose was determined by the phenol-sulfuric acid reaction as described by Dubois *et al.*⁵⁾ Hexosamine was determined by the method of Cessi and Piliego,⁶⁾ using glucosamine as a standard. Sialic acid was determined by the thiobarbituric acid assay of Warren.⁷⁾ Amino acid

analysis was carried out with a Hitachi 835 automatic amino acid analyzer. Quantitative analysis of individual lipids was performed by silicic acid chromatography.⁸⁾

Delipidation of LDL with chloroform-methanol (2:1) results in the formation of insoluble apoproteins. But partial delipidation of LDL with ether or *n*-heptane results

TABLE II. AMINO ACID COMPOSITION OF WHOLE APO LDL AND pd-LDL

	Whole apo LDL (mol%)	pd-LDL (mol%)
Asp	11.7	12.4
Thr	6.4	6.9
Ser	6.8	5.6
Glu	11.6	13.1
Pro	3.1	2.8
Gly	4.5	2.1
Ala	8.1	4.9
1/2 Cys	0.7	0.2
Val	6.6	8.8
Met	0.3	1.1
Ile	6.9	4.9
Leu	11.7	13.1
Tyr	4.2	4.6
Phe	4.0	4.1
Lys	7.2	7.9
His	1.0	0.4
Arg	5.4	7.2

FIG. 1. SDS Polyacrylamide Gel Electrophoresis of Whole Apo LDL and pd-LDL.

(A), whole apo LDL stained with Coomassie brilliant blue; (B), pd-LDL stained with Coomassie brilliant blue.

TABLE I. CARBOHYDRATE COMPOSITION OF APO LDL

Fraction	Hexose (%)	Hexosamine (%)	Sialic acid (%)
Whole apo LDL	2.23	0.70	0.40
pd-LDL	1.40	0.36	0.31

in a soluble phospholipid-protein residue.⁹⁻¹¹⁾ We also prepared the partially delipidated LDL soluble in Tris buffer (pd-LDL) and found that 22.3% of the partially delipidated LDL was solubilized in 10 mM Tris buffer of pH 8. pd-LDL contained 81.3% of lipid. 91.7% of the lipid content was phospholipid. The carbohydrate composition of whole apo LDL and that of the protein portion of pd-LDL are shown in Table I. The contents of hexose, hexosamine and sialic acid of whole apo LDL were somewhat different from those reported by Abraham *et al.*¹²⁾ The protein portion of pd-LDL contained 1.40% of hexose, 0.36% of hexosamine and 0.31% of sialic acid. These values were lower than those for the whole apo LDL. But the protein portion of pd-LDL contained more hydrophobic amino acids such as valine, leucine and methionine, as shown in Table II. pd-LDL was fractionated by electrophoresis in SDS gel into 4 major components, as shown in Fig. 1. The 4 major components have apparent molecular weights of 135,000 (Band 1), 97,000 (Band 2), 70,000 (Band 3) and 60,000 (Band 4). These bands were also stained with periodic acid-Schiff reagent. Apolipoproteins in pd-LDL were rich in smaller molecular weight proteins. It was found that the protein portion which combined with phospholipid in LDL was rich in hydrophobic amino acids.

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