A STUDY OF THE POLYPEPTIDES OF PORCINE SERUM LOW DENSITY LIPOPROTEINS

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Biochemistry

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

VLDL - very low density lipoproteins.

LDL - low density lipoproteins

HDL - high density lipoproteins

apoVLDL - protein moiety of VLDL

apoLDL - protein moiety of LDL

apoHDL - protein moiety of HDL

apo-ala, apoLP-ala, R-ala - apoprotein with COOH-terminal alanine

apo-glu, apoLP-glu, R-glu - apoprotein with COOH-terminal glutamic acid

apo-ser, apoLP-ser, R-ser - apoprotein with COOH-terminal serine

Alcohol D.H. - alcohol dehydrogenase (yeast)

CD - circular dichroism

ESR - electron spin resonance

Hb - haemoglobin

IR - infra red

ORD - optical rotary dispersion

NMR - nuclear mass resonance

R_m - relative mobility

SDS - sodium dodecyl sulphate

S_f - flotation coefficient in Svedbergs in salt solution density 1.063 g/cm^3, 26°C.

Chapter 1

INTRODUCTION

1.1 Introduction

The problem of transporting nonpolar lipids in the blood has been overcome in mammals by association of the lipid with specific proteins, to form water soluble lipoproteins. This association occurs in a reproducible fixed ratio of lipid to protein and was first demonstrated by Macheboeuf (1929). More recently the role played by the proteins in lipid transport has been the subject of intensive investigation.

The circulating lipoproteins are divided into classes. Two operational systems of classification are used, one based on density and the other on electrophoretic mobility. The lipoproteins floating in a centrifugal field at a density of less than 1.006 g/cm^3 are classified as VLDL. These have a flotation coefficient, S_f, of between 20 and 400 and move in the pre-β band on paper electrophoresis. Those floating at density 1.006 - 1.063 g/cm^3 (S_f = 0 - 20) are classified as LDL and run as the β band on electrophoresis. HDL float at density 1.063 - 1.21 g/cm^3 and run as the α_1 band on electrophoresis, and the chylomicrons have an S_f of greater than 400 and remain at the origin in electrophoretic separations, Figure 1.

Following the ingestion of a triglyceride rich meal

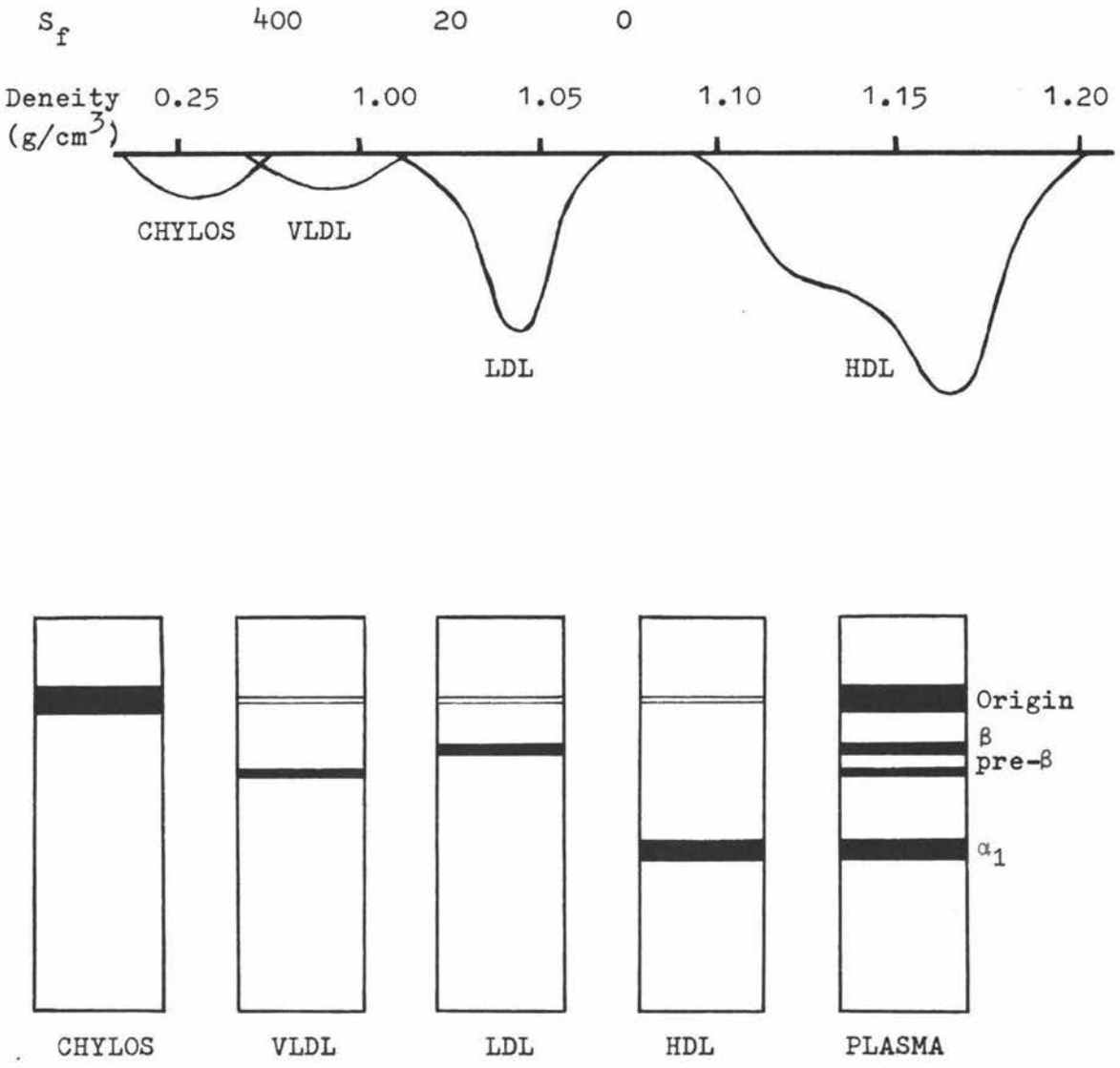

Figure 1. Classification of plasma lipoproteins: (i) by density (Fredrickson, Lux & Herbert, 1973); (ii) by paper electrophoresis (Levy, Lees & Fredrickson, 1966).

the lipid is transported from the intestine mainly as triglyceride in the chylomicrons. Fatty acids synthesized in the liver from carbohydrate and protein are secreted into the plasma as triglycerides bound to VLDL produced in the liver. VLDL is also produced in the intestine (Windmueller, 1968; Ockner et al., 1969), and possibly in other organs as well.

The rate of VLDL release is influenced by the requirement for triglyceride transport from the liver, which is dependent on the rate of fatty acid synthesis in the liver and the level of exogenous medium chain length fatty acids reaching the liver via the hepatic portal vein.

VLDL is thought to be catabolized in the capillary walls of the muscle and adipose tissue by lipoprotein lipase, to release the lipid and form HDL and LDL. Scott & Hurley (1970) have shown, using I^{125} tracer techniques, that LDL is then broken down in the liver, skin and capillary walls.

1.2 Metabolic Relationship Between Lipoprotein Classes

Since it became apparent that the same polypeptides were present in different lipoprotein classes and that VLDL was the major means of transport for esterified lipid, investigation turned to determining the metabolic relationship between the lipoprotein classes.

Evidence now exists for a precursor-product relationship

between VLDL and LDL and at least its partial control by lipoprotein lipase. Fredrickson, Levy & Lees (1967) have shown that in patients whose catabolism of VLDL is slow due to low lipoprotein lipase activity (Type I hyperlipoproteinemia) the plasma LDL concentration is also low. This relationship is also suggested by the results of experiments by Nichols et al. (1968) and Nichols (1969) who showed that heparin, due to its activation of lipoprotein lipase, causes a drop in concentration of plasma VLDL and a concomitant increase in concentration of plasma LDL.

Further support for this hypothesis comes from the results of experiments with radioactive tracers. Fidge & Foxman (1971) working with rats, and Bilheimer et al. (1971) with humans, both isolated VLDL and labelled the apoproteins with I^{125}. The radioactive label was found by Bilheimer et al. (1971) to be distributed in the VLDL apoproteins, before injection, in the proportion: apo-LDL, 45%; apo-ala plus apo-glu, 25%. 10 minutes after intravenous injection of the apoproteins-I^{125}, apo-ala-I^{125} and apo-glu-I^{125} had exchanged between VLDL and HDL, but all the apo-LDL-I^{125} remained in the VLDL. Radioactive LDL appeared subsequently in a fraction of density 1.006 - 1.019 g/cm^3 which reached a maximum after 6 - 12 hours followed by a rapid decline, and then in a fraction of density 1.019 - 1.063 g/cm^3 which reached a maximum after 24 hours and was followed by a slow decline. This process was accelerated by heparin and slowed in patients with Type I hyperlipoproteinemia. Langer (1970) showed in similar experiments with I^{125} labelled VLDL apoproteins, that VLDL is not metabolized to higher density fractions and is not apparently recycled to new VLDL.

Rubenstein & Rubenstein (1972) used radioactive phospholipid and protein to study VLDL metabolism in rats. They found a free two way exchange of tritiated protein and phospholipid-P^{32} between VLDL and HDL. Tritiated protein in VLDL exchanged within 20 minutes with HDL, but not with LDL. Using gel electrophoresis they found exchange between 3 of the 6 bands in apoVLDL and apoHDL.

These results appear to support the hypothesis that LDL is a product of the action of lipoprotein lipase on VLDL and that its formation is to some extent dependent on the rate of lipase activity. Whether all circulating LDL derives from VLDL is not known, nor is it known if LDL has a specific function.

The relationship between HDL and VLDL is less clear. The free exchange of components between them could suggest that some or all HDL apoproteins were precursors to VLDL formation, but further investigation will be required before definite conclusions can be drawn.

1.3 Cofactor Activity of Lipoproteins

Korn (1955) showed that lipoprotein lipase could hydrolyse a triglyceride emulsion only after activation by serum lipoproteins and suggested a possible role of lipoproteins in the control of lipid transport. Although evidence has been presented involving VLDL, LDL and HDL as activators (Bier &

Havel, 1970), it is generally considered that HDL is the activator for human lipoprotein lipase. However, current information does not permit definite conclusions to be drawn on the mechanism, nature and specificity of cofactors in lipoprotein lipase activity. The most serious shortcoming is that most experiments are carried out with crude enzyme preparations and the results may well be influenced by the nature and amount of impurities. Another problem is that the polypeptide preparations being tested for cofactor activity have not always been well characterized and in some cases different amino acid compositions have been reported for peptides with the same COOH-terminal groups.

LaRosa et al. (1970) found that peptides with glutamic acid and alanine COOH-terminal groups (apoLP-glu and apoLP-ala) had cofactor activity with crude lipoprotein lipase preparations. In contrast to this Brown & Baginsky (1972) showed that purified apoLP-ala (of VLDL) inhibits lipoprotein lipase at the level of 2% of the substrate. Bier & Havel (1970) reported activation by HDL and also by VLDL at 0.2% of substrate level. They suggest that either of the polypeptides apoLP-thr and apoLP-glu, being common to both classes, could be the activator.

1.4 Composition of LDL

The composition of the lipoprotein classes is given in Table 1.

Table 1. Composition of Lipoprotein Classes

Composition % by weight	Chylomicrons	VLDL	LDL	HDL	LP-X
Protein	2	5-12	20-22	50	6
Carbohydrate			3-5		
Total Lipid	98	88-95	75-78	50	94
Cholesterol ester	4	10-13	35-41	20	2
Phospholipid	4	13-20	21-23	24	66
Cholesterol	2	3-5	8-9	2	23
Triglyceride	88	50-60	6-8	4	3

LDL also contains traces of lysolecithin, glycolipids, and other unidentified lipids (Skipski et al., 1967). The saturated fatty acids are mainly 16:0 and the unsaturated mainly 18:1 and 18:2. LDL also contains 50% of serum tocopherol (McCormick et al., 1960) and 75% of serum β-carotene and lycopene (Krinsky et al., 1958).

LDL has been found to have a polysaccharide component containing galactose, mannose, glucosamine, fructose and sialic acid. Evidence exists for covalent bonding of protein and carbohydrate and for the terminal position of sialic acid on the polysaccharide chain (Margolis & Langdon, 1966a).

The predominant lipoprotein in obstructive jaundice, LP-X, has a significantly different composition from LDL.