CHEMISTRY OF HIGH DENSITY LIPOPROTEINS

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Received on June 20, 1977; Revised on August 11, 1977

ABSTRACT

Human serum lipoproteins have been classified into five major classes namely, chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL) and very high density lipoproteins (VHDL). While the structure and function of these lipoproteins have been reviewed elsewhere, no effort has been made to review the progress made in the understanding of the structure of serum HDL obtained from various mammalian and non-mammalian species. In this review an attempt has been made to describe the physical and chemical characteristics of serum and egg yolk high density lipoproteins.

Key Words: High density lipoproteins, Lipovitellins.

INTRODUCTION

Lipoproteins can be classified into two major types, the structural lipoproteins and the soluble lipoproteins. Examples of structural lipoproteins are lipoproteins of membranes, such as those of plasmalemma, mitochondria, chloroplast, myelin and bacterial plasma membrane. The soluble lipoproteins may be classified into three groups namely, milk lipoproteins, serum lipoproteins and egg yolk lipoproteins.

1. MILK LIPOPROTEINS

Lipoprotein of cow's milk occur almost exclusively as constituents of the fat soluble membrane, a complex lipid-protein system oriented at the fat/plasma interface. Hayashi and Smith [1] have reported the isolation of a high density lipoprotein from cream globule by treatment with detergents or by mechanical disruption. Not much structural studies have been carried out on these lipoproteins.

2. SERUM LIPOPROTEINS

Human serum lipoproteins

Most of the work done in the field of serum lipoproteins concern predominantly with human serum. However, studies of serum lipoproteins

on species other than human have also been reported in recent times. A number of reviews have been published concerning the structure and function of serum lipoproteins [2–4]. Human serum lipoproteins have been classified into five classes based on electrophoretic mobility or by their rate of ultracentrifugal sedimentation or flotation in salt solutions [5]. They are chylomicrons, very low density lipoproteins (VLDL) or pre β-lipoproteins, low density lipoproteins (LDL) or β-lipoproteins, high density lipoproteins (HDL) or α-lipoproteins and very high density lipoproteins (VHDL).

Human serum high density lipoproteins

HDL are the smallest of the lipoprotein particles with a diameter range of 90–120 Å [6]. Human serum HDL has been classified into three subclasses: HDL_1, HDL_2 and HDL_3. HDL_1 was originally isolated between densities 1·050 and 1·063 gm/ml [7]. HDL_2 is isolated in between densities 1·063 and 1·12 gm/ml and HDL_3 between densities 1·12 and 1·21 gm/ml. However, HDL_1 has been shown to be similar to HDL_2 by electrophoretic and immunochemical properties [7]. While HDL_2 and HDL_3 have been fractionated into several subspecies by differential and rate/zonal ultracentrifugation and by analytical and preparative gel electrofocusing [8–13] not much studies have been carried out on these subspecies. In normal male subjects the concentration of HDL_2 is about 70 mg/100 ml serum while that of HDL_3 is 230 mg/100 ml serum. In females concentration of HDL_2 is about 219 mg/100 ml serum and HDL_3 is 238 mg/100 ml [14–16]. The reason for the sex-dependent differential distribution is not clearly known. However, a relation between HDL_2 and estrogen levels could exist as this class of HDL is three times more in premenopausal women than in men [14–16].

The physical characteristics and protein components of HDL_2 and HDL_3 [17]are given in Table I while the chemical characteristics of human HDL are compared with HDL of other animal systems in Table II. Human serum HDL contains approximately 50% protein and 50% lipid. Phospholipids, cholesterylester and triglycerides are the main lipid components. Phosphatidyl choline constitutes 70–80% of the phospholipids and sphingomyelin 12–14%. Phosphatidyl serine and phosphatidyl inositol are minor components. Linoleic acid is the predominant fatty acid of the cholesteryl ester [2].

Very high density lipoproteins

VHDL is isolated between densities 1·21 and 1·25 gm/ml. It is present at a concentration of 20 mg per 100 ml serum. The physical characteristics

are compared with those of HDL_2 and HDL_3 in Table I. VHDL is approximately 60% protein and 40% lipid. Phospholipids are the major components of lipids and cholesteryl ester is a minor component [14].

Apoprotein structure of human serum HDL

The protein components of HDL are soluble in water and hence they have been very well characterised. About 90% of the protein moiety of HDL is composed of two major apoproteins which are designated as apoA–I

TABLE I

Physical characteristics and protein components of human serum HDL, HDL_2, HDL_3 and VHDL

Lipoproteins	HDL	HDL_2	HDL_3	VHDL
Density (gm/ml)	1·063–1·21	1·063–1·12	1·12–1·21	1·21-1·25
Flotation rate	F 1·2–0·20	F 1·2 3·5-9·0	F 1·20-3·5	..
$S_{20,w}$	..	4·79	5·0	..
Molecular Weight	170,000 360,000	360,000	170,000	154,000
Size[1] (Å)	80–100	100	80	74
Apoproteins	ApoA–I (70%) ApoA–II (18%)	ApoA–I (68%) ApoA–II (20%)	ApoA–I (74%) ApoA–II (16%)	
	ApoC–I ApoC–II ApoC–III (12%)	ApoC–I ApoC–II ApoC–III (12%)	ApoC–I ApoC–II ApoC–III (10%)	
	Thin line protein and Arginine rich protein			

[1]. As determined by Electron microscopy.
Data obtained from references [2, 14, 17].

TABLE II

Chemical composition of serum HDL from various animals

Sl. No.	Animals	Types of HDL	Protein	Lipid	Lipid composition* (percentage by weight)						References
					PL	CE	UC	TG	FA	Miscel-laneous	
1	2	3	4	5	6	7	8	9	10	11	12
Mammals											
1.	Human	HDL	50·0	50·0	48·0	32·0	8·0	10·0	2·0	..	4, 11
		HDL_2	43·0	57·0	50·9	30·0	8·7	8·7	1·7	..	4, 11
		HDL_3	56·0	44·0	52·2	27·2	9·0	7·0	4·5	..	
2.	Chimpanzee	HDL_2	41·8	58·2	42·2	42·8	11·2	3·8	..	..	76
		HDL_3	57·7	42·3	45·3	40·2	8·0	7·0	..	..	..
3.	Rhesus monkey	HDL_2	50·0	47·5	50·5	33·2	9·0	6·9	..	..	77
		HDL_3	53·0	46·0	57·6	28·2	8·0	6·0	..	..	
4.	Patas monkey	HDL	39·7	60·3	70·3	29·3	NR	0·7	..	..	81
5.	(*a*) Bovine	HDL	32·0	68·0	27·0	37·0	6·0	29·0	..	..	80
	(*b*) Bull (Hereford)	HDL	30·0	70·0	24·6	47·0**	..	27·0	1·4	..	83

6.	Bison	HDL	NR	NR	22·0	48·0	8·0	13·0	3·0	H—3, MG—1, DG—2	85
7.	Horse	HDL	NR	NR	50·0	30·0**	..	10·0	10·0	..	86
8.	Camel	HDL	30·0	70·0	20·0	52·5	14·5	11·0	..	..	87
9.	Pig	HDL_2	27·4	62·6	51·3	37·7	5·6	5·3	..	..	128
		HDL_3	42·6	57·4	57·5	33·4	3·8	5·3	..	..	
10.	Dog	HDL	41·5	58·5	47·3	41·5	7·8	NR	2·4	G–1·1	129
11.	Sheep	HDL_1	32·0	68·0	26·4	54·7	8·0	4·3	1·3	..	95
		HDL	49·0	51·0	36·5	53·2	5·5	1·0	3·8	..	
		VHDL	NR	NR	33·4	13·2	9·8	0·5	43·1	..	
12.	Rat	HDL	32·8	67·2	41·8	50·0	5·3	2·6	..	..	87
13.	Rabbit	HDL	51·9	48·1	34·3	43·3	5·2	17·0	..	..	87
14.	Guinea pig										
	(*a*) normal diet	HDL	39·0	61·0	41·0	41·0	14·7	3·3	..	..	99
	(*b*) cholesterol rich diet	HDL	27·6	72·4	42·5	21·0	33·8	2·7	..	..	99

TABLE II—(*Contd.*)

Sl. No.	Animals	Types of HDL	Protein	Lipid	Lipid composition* (percentage by weight)						References
					PL	CE	UC	TG	FA	Miscellaneous	
1	2	3	4	5	6	7	8	9	10	11	12
15.	Hedge Hog	HDL	40·7	59·3	41·2	44·5	10·6	3·7	..	..	87
16.	Whale	HDL	NR	NR	34·6	56·7	5·7	1·9	1·2	..	100
Aves											
17.	Chi ken	HDL	43·9	56·1	51·1	28·5	9·0	11·4	..	..	130
18.	Goose	HDL	52·6	47·4	37·2	40·0	4·2	18·5	..	..	87
19.	Pigeon	HDL	46·8	53·2	48·2	34·7	4·8	12·3	..	..	87
Reptiles											
20.	Grass snake	HDL	40·0	60·0	41·2	40·6	12·2	5·8	..	..	87
21.	*Natrix piscator*	HDL	37·1	62·9	34·3	34·8	23·5	7.5	..	..	87
22.	Water monitor	HDL	63·5	36·5	36·3	39·1	8·5	16·1	..	..	87
23.	Tortoise	HDL	28·9	71·1	20·0	56·8	17·0	6·2	..	..	87

Amphibians										
24. Frog	HDL	51·0	49·0	26·7	45·7	21·0	6·3	..	..	87
Fish										
25. Salmon	HDL	NR	NR	49·9	30·5	5·3	11·4	2·7	..	105
26. Pacific sardine	HDL	NR	NR	55·0	22·0	15·0***	10·0	..	..	106
27. Dog fish	HDL	NR	NR	20·8	33·2	20·0	2·6	..	..	87
28. Hag fish	HDL	NR	NR	58·8	0·9	26·4	13·2	..	..	87
Invertebrates (insects)										
29. American silk moth	HDL	52·0	48·0	18·0	4·1	5·4	6.0	2·7	DG—56·6 MG—2·1 H—5·0	107

* Lipid composition of some of the animals do not add up to 100%. They have been presented as reported.

** Includes unesterified cholesterol and cholesteryl ester.

*** Includes fatty acids and diglycerides.

Abbreviations

PL = Phospholipids
UL = Unesterified cholesterol
CE = Cholesterylester
TG = Triglyceride
FA = Fatty acids
DG = Diglycerides
MG = Monoglyceride
H = Hydrocarbons
NR = Not reported
G = Glycerides

and apoA–II. The remaining 10% is mainly composed of apoC–I, apoC–II and apoC–III proteins [18]. The ratio of apoA–I and apoA–II varies in HDL_2 and HDL_3. HDL also contains minor constituents like apoA–III or thin line protein [19, 21] and "arginine-rich" protein [22–24]. A brief description of the apoproteins are given here. The amino acid sequence of apoA–I, apo–II, apoC–I and apoC–III have been determined [25–35]. Chemical synthesis of a protein similar to apoC–I has also been achieved [36].

(i) *ApoA–I*

This is a major apoprotein constituting about 68% of apoHDL. It has a molecular weight of 28,300 and is a single polypeptide chain of 245 amino acid residues. Aspartic acid is the N-terminal amino acid and glutamine is the C-terminal amino acid. It does not contain cysteine, cystine or isoleucine. ApoA–I occurs in two polymorphic forms [37], apoA–I–1 and apoA–I–2 which have slight differences in amino acid composition and electrophoretic mobility. ApoA–I contains about 60–70% α-helix, 37% random coil and 8% antiparallel β-structure [38]. It has a physiological function of activating an enzyme known as lecithin: cholesterol acyl transferase (LCAT) [39, 40]. ApoA–I may also have a function in regulating the content of membrane lipids [41–43] and maintaining proper membrane fluidity [44]. The term apoA–I is generally referred to a serum HDL–apoprotein which has a molecular weight of about 28,000.

(ii) *ApoA–II*

This protein constitutes about 20% of HDL proteins. It contains two identical monomers of 77 amino acid residues which are linked through disulfide bridge at residue 6. The N-terminal amino acid is pyrrolidone carboxylic acid and the C-terminal amino acid is glutamine. ApoA–II does not contain carbohydrates, histidine, arginine or trytophan. It contains 35% α-helix, 13% β-structure and 52% disordered structure [38]. The term apoA–II is referred to a serum HDL–apoprotein which has a molecular weight of about 8,500.

(iii) *ApoC–I*

While apoC–proteins are the major protein components of VLDL, it forms about 12% of HDL apoproteins. ApoC–I is a single polypeptide chain of 57 amino acid residues. The N and C-terminal amino acids are threonine and serine respectively. It does not contain histidine, tyrosine,

cysteine or cystine. It activates two lipoprotein enzymes namely, LCAT [47] and lipoprotein lipase purified from human post-heparin plasma [48, 49].

(iv) *ApoC– II*

ApoC–II contains about 100 amino acid residues with a molecular weight of 12,500 [50]. It is a potent activator of lipoprotein lipase from human and rat post-heparin plasma and cow's milk [51–54].

(v) *ApoC–III*

ApoC–III is a major component of VLDL containing 79 amino acid residues. However, it is present as a minor component in HDL. ApoC-III does not contain isoleucine, cystine or cysteine. The N and C-terminal amino acids are serine and alanine respectively. At Threonine–74 a carbohydrate moiety is attached by *o*-glycosidic linkage which contains one residue each of galactose, galactosamine and either 0, 1 or 2 residues of sialic acid per mole of the polypeptide [50]. This corresponds to three polymorphic forms, namely, apoC–III–0, apoC–III–1 and apoC–III–2. ApoC–III is an inhibitor of lipoprotein lipase at a concentration of 2% (W/W) [55]. This inhibition is not reversed by apoC–II which is an activator of this enzyme. Phospholipid binding with four fragments of apoC–III corresponding to the sequence 41–79, 48–79, 55–79 and 61–79 showed that sequence 48–79 binds significant quantities of phosphatidyl choline [56]. Trypsin digestion of apoC–III–PC complex suggested that hydrolysis proceeds in a facile manner at the N-terminal half, while the C-terminal half is protected from cleavage [57]. Based on these observations, the C-terminal region is found to be the phospholipid binding site.

Models of human serum lipoproteins

Any model describing the structure of serum lipoproteins should consider the problem concerning the solubility of serum lipoproteins in water. Hence models describing the relative location of lipid and proteins are largely influenced by the existing knowledge about the structure of miscells. The lipid-rich lipoproteins like chylomicrons and VLDL are known to contain a lipid-core structure made of cholesteryl ester and triglycerides. Cholesterol, protein and phospholipids form the outer surface [58, 59]. In the case of LDL, two models have been proposed concerning the relative location of lipid and proteins. Based on small angle X-ray data, a phospholipid bilayer model consisting of cholesteryl ester, cholesterol and triglycerides has been suggsted.

This bilayer surrounds a central core of proteins [60]. A slightly different model consisting of a trilayer structure has been proposed based on NMR spectroscopic studies [61].

Models of human serum HDL

Earlier models of HDL are based on electron microscopic studies [62.] Since the publication of small angle X-ray data of HDL, considerable progress has been made in the understanding of the structure of human serum HDL. Small angle X-ray data revealed the existence of two distinct regions in HDL namely, the electron-deficient central core which has a diameter of 86 Å and an outer electron-rich region which has a radius of 14 Å [63]. Phospholipids can be accommodated in this region since the phospholipid head groups, measure about 11 Å wide. Proteins are also located on the outer surface and the evidence for the surface location of proteins and phospholipids comes from a large number of experimental data. Almost all the proteins and phospholipids are accessible for enzymatic hydrolysis [64, 65]. More than 90% of the ϵ-amino groups of lysine are available for succinylation reaction [66]. More than 90% of apoA-I is detected by radioimmunoassay [67]. The central core of HDL consists of cholesteryl ester and triglycerides.

The primary sequence of HDL-apoproteins seems to have some specificity which permit them to bind lipids. ApoA proteins bind significant amount of neutral lipids only in the presence of phospholipds. They also exhibit a significant increase in helical content upon binding phospholipids [68]. Hence the interaction of phospholipids and proteins is of primary importance. Assuming helical structure of proteins are important for binding lipids, Segrest *et al.* [69] have suggested that these proteins have amphipathic helical regions, that is, one side of the helix is polar and the other side of the helix is non-polar. Such an arrangement facilitates the interaction between non-polar region of the internal core and the external aqueous medium simultaneously. Prediction analysis of apoA-I suggests that the occurrence of at least nine helical regions of approximately equal engths in the central and C-terminal portion of the chain, separated by helix breaking sections containing proline, glycine or both [90]. Each helical region contains 15–20 amino acid residues. This arrangement also causes the helix to be amphipathic [90]. Helical areas between residues 11–30 of apoA-II (70) and residues 7–14, 18–29 and 33–53 of apoC-I can also give raise to amphipathic areas [41]. The polar face of the amphipathic helix of apoC-I contains a highly unusual topographical distribution of charged

amino acid residues. Glutamic and aspartic acids are localised at the centre of the polar face, while positively charged lysine and arginine residues are oriented towards the lateral edges at the interface between the polar and non-polar faces. This arrangement leads to a suggestion that there may be electrostatic interaction between the positively charged choline groups of the phospholipid with glutamic or aspartic acid side chains and between the phosphates and the lysine or arginine residues. The importance of lysine in the binding of lipid is demonstrated by the abolition of lipid binding by maleylated apoA-II[72]. ApoA-I binds very little HDL lipids. However, in presence of apoA-II, apoA-I binds significant amount of total HDL lipids[73], suggesting that portein-protein interaction is also necessary for binding lipids. Based on these results two models have been proposed for HDL.(Fig. 1). In the model of Jackson *et al.* [74], the long axis of fatty acyl

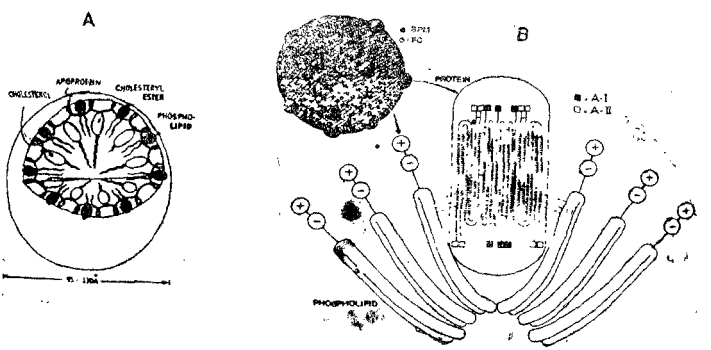

FIG. 1. Schematic representation of human serum high density lipoproteins.
A: HDL model of Jackson *et al.* [74]
B: HDL model of Assmann and Brewer [70].

hain is perpendicular to the helical protein which is surrounding the surface. Assmann *et al.* [70] have proposed a lipid mosaic model similar to hat of membranes proposed by Singer [75]. According to this model roteins are like "ice-bergs" floating in a sea of lipids and phospholipid cyl chains are parallel to *a*-helical portions of the protein, which is in contrast to the previous model. Both these models, however, permit the interction of carbon atoms 2-4 of the fatty acyl chain of phospholipid with the ydrophobic side of the helix and possibly with the hydrophobic region f lysine and arginine residues.

Mammalian serum HDL

Chimpanzee serum HDL

Chimpanzee serum HDL has been fractionated into HDL_2 and HDL_3 and they differ significantly in chemical composition [76]. Chimpanzee apo–HDL differs from the human apo– HDL by a significant decrease in the realative content of apoA–II. Amino acid composition of chimpanzee apoA–I is very similar to human apoA–I except for glutamic acid, leucine and aspartic acid (Table III). Chimpanzee apoA–II is similar to human apoA–II except for histidine and arginine (Table IV). These differences are attributed to small contamination by apoA–I. One of the interesting features of chimpanzee apoA–II is the presence of cystine. So far cystine containing apoA–II has not been isolated from any species except human and chimpanzee.

Rhesus monkey HDL

Rhesus monkey HDL_2 and HDL_3 are similar to their human counterpart in terms of hydrodynamic, spectroscopic and morphological criteria [77]. The ratio of HDL_2 to HDL_3 is 2:1 in rhesus monkey as compared to 1:3 ratio in human serum. The chemical composition of HDL_2 and HDL_3 are described in Table II. Rhesus monkey apoA–I is very similar to human apoA–I in amino acid composition except for glutamic acid and arginine (Table-3). It has 9 residues more of glutamic acid and 4 residues less of arginine when compared to human apoA-I(78). Monkey apoA-II shows some differences with human apoA-II (Table-IV). The former has been partially sequenced at both N and C-terminal ends and the sequence is similar to human apoA-II except for the substitution of serine for cysteine at position 6, and glutamic acid for lysine at position 3(79).

Patas Monkey serum HDL

Patas monkey apoA–I is the major component of apoHDL while apoA-II is a minor one. ApoA–I is strikingly similar to human apoA–I in amino acid composition except for lysine and serine (Table III). Partial sequence of 20 amino acid residues from the N-terminal end shows that it is identical to that of human apoA–I with the following substitutions: monkey-6-threonine to human 6-serine, monkey-15-valine to human 15-alanine and monkey-2-glutamic acid to human-2-aspartic acid. ApoA–II appears to be similar to the monomeric form of human and rhesus monkey apoA–II in amino acid composition. Patas monkey apoA–II does not contain histidine, cysteine isoleucine and tryptophan.

TABLE III

Variations in apoA–I of serum HDL

Animals	Amino acids absent	Variation in amino acids, when compared to human apoA-I		References
		Deficient	Excess*	
1. Human	Isoleucine Cystine	..	..	3
2. Chimpanzee	Isoleucine Cysteine	Glutamic acid Leucine	Aspartic acid	76
3. Rhesus monkey	Isoleucine Cysteine	Arginine	Glutamic acid	78
4. Patas monkey	Isolecuine Cysteine	Lysine	Serine	81
5. Bovine	Cysteine	Methionine	Alanine Isoleucine	83
6. Canine	Cysteine	Lysine Threonine	Glycine Isoleucine Alanine Serine	129
7. Rat	..	..	Isoleucine	22
8. Guinea pig	Cysteine Proline	Leucine Methionine	Threonine Isoleucine Alanine Aspartic acid	131
9. Pig	Cysteine	Glycine	Isoleucine Alanine	89
10. Chicken	Cysteine	Aspartic acid Glycine Alanine Phenylalanine Histidine	Threonine	102
11. Salmon	Tryptophan	Aspartic acid Leucine	Lysine Methionine Tyrosine Alanine Isoleucine	105

* Amino acids absent in human apoA–II but present in other animals are considered excess.

TABLE IV

Variations in apoA–II of serum HDL

Sl. No.	Animals	Amino acids absent	Variation in the amino acids, when compared to human apoA-I		References
			Deficient	Excess*	
1.	Human	Histidine Arginine Tryptophan	..	..	3
2.	Chimpanzee	..	..	Histidine Arginine	76
3.	Rhesus monkey	Histidine Isoleucine Tryptophan Cysteine	Glycine	Arginine	78
4.	Patas monkey	Histidine Isoleucine Tryptophan Cysteine	..	Arginine Valine	81
5.	Rat	Histidine Tryptophan Cysteine	Valine Lysine	Arginine Alanine Aspartic acid	97
6.	Salmon	Cysteine	Threonine Serine Glutamic acid Phenylalanine	Histidine Arginine Aspartic acid Alanine Methionine Isoleucine Glycine	105

* Amino acids absent in human apoA–I, but present in other animals are considered excess.

Baboon serum HDL

Though the chemical composition of baboon serum HDL has not been reported, apoA–I and apoA–II has been studied [78]. While apoA–II does not contain cystine or cysteine, apoA–I is reported to be similar to human apoA–I, though its amino acid composition is not given [78].

Bovine serum HDL

Bovine serum HDL is the predominant lipoprotein which constitutes 80% of the total lipoprotein by weight [80] and it resembles human HDL_2. Bovine HDL has a significantly large amount of triglycerides (Table II) [81]. Bovine apoA–I constitutes 88% by weight of the apoprotein [82]. The remaining 12% is constituted by two polypeptides of molecular weight 11,000 and 13,000. They resemble human apoC proteins in molecular weight and elution position during sephadex gel filtration. Bovine apoA–I is similar to human apoA–I in amino acid composition except for alanine and isoleucine which are slightly more and methionine being little less. Cysteine is absent.

Hereford bull serum HDL has a single component as shown by paper electrophoresis and analytical ultracentrifuge methods [83]. It resembles cow serum HDL in having a large amount of triglycerides (Table II). Bull serum apoA–I is partially sequenced from the N-terminal region [84] and the sequence is similar to that of human apoA–I. The exceptions are, as compared to human apoA–I, glutamic acid in place of proline at position 4 and phenylalanine in place of aspartic acid at position 13. Leucine is absent at position 14 [84].

Bison serum HDL

HDL is the major component and it comprises 64% of the total serum lipids [85]. Only the lipid composition of HDL has been reported. An interesting feature of this composition is the presence of hydrocarbons and a large amount of sterol esters (Table II).

Horse serum HDL

HDL is the major component of horse serum [86]. Presence of large amounts of free fatty acid appears striking (Table II) and possibly requires confirmation.

Camel serum HDL

HDL occurs at a concentration of 11 mg/100 ml serum [87]. It contains about 30% protein and 70% lipid (Table II). No report on protein fractionation has appeared.

Pig serum HDL

Porcine HDL_3 differs considerably from HDL_3 in chemical composition [88]. ApoA–I is the major component of pig apo–HDL and apoA–II appears to be absent [89]. Pig apoA–I has 261 amino acid residues as compared to 245 residues in human apoA–I. The amino acid compositions of porcine and human apoA–I are similar except that pig apoA–I contains isoleucine (Table III). Like human apoA–I, pig apo–I also activates LCAT. The structure of porcine HDL studied by several physical methods like small angle X-ray diffraction, NMR and ESR spectroscopy, circular dichorism and differential scanning calorimetric methods show that most of the polar groups of phospholipid and protein are located at the surface [90, 91].

Dog serum HDL

HDL is the major lipoprotein of canine serum lipoproteins. It has a HDL fraction termed HDL_1 which has a density lower than 1·063 gm/ml and it has been shown to be similar to high density lipoprotein by immunochemical reactivity, electrophoretic mobility and apoprotein content [92]. HDL_1 does not resemble any high density fraction of human serum. An interesting feature of the lipid and phospholipid composition of canine HDL is that glycerides are present at very low concentrations (Table II) and phosphatidyl choline is the major component of phospholipids. ApoA-I of canine HDL constitutes 90% of apo–HDL, it has 243 amino acid residues as compared to 245 residues of human apoA–I. They are similar in amino acid composition with the exception of isoleucine, alanine and histidine [93]. Canine apoA–I has been partially sequenced from the N-terminal end and the sequence is similar to that of human apoA–I. The only exception being the absence of proline at position 4 [94]. Canine apo-HDL has a polypeptide of molecular weight 8,000 which appears to be analogous to human apoC proteins [93].

Sheep serum HDL

Sheep serum HDL_1 has been isolated between densities 1·063 and 1·075 gm/ml and HDL_2 between densities 1·075–1·20 gm/ml and VHDL at

a density greater than 1·21 gm/ml(95). HDL_1 constitutes 5% of total serum lipoproteins and HDL is characterised by a large amount of cholesteryl ester (Table II).

Rat serum HDL

Rat apoA–I is the major component of apo-HDL and constitutes about 62% by weight of HDL(96). The amino acid composition of rat apoA–I is similar to human apoA–I (Table III) except isoleucine (22). Rat apoA–II is similar to human apoA–II, with both having pyrrolidone carboxylic acid at amino terminus, both lacking histidine and tryptophan and both being particularly rich in glutamine and/or glutamic acid. Rat apoC–I is insoluble in acidic buffers. It lacks tyrosine and cysteine but contains histidine and is rich in lysine. It does not contain sialic acid or hexosamine. Rat apoC–II contains histidine but does not contain sialic acid or hexosamine. It is similar to human apoC–II in amino acid composition. Like human apoC–II, rat apoC–II activates lipoprotein lipase [97]. Rat apoC–III is found in at least two polymorphic forms that differ only in their carbohydrate content. One of the two polymorphic forms of rat apoC–III is designated as rat apoC–III–0 which does not contain sialic acid or hexosamine, while the other major polymorphic form, apoC–III–3, contains one residue of galactosamine and three residues of sialic acid. No trisialylated apoC–III has been described among human apolipoproteins. An apoC–III–0 which does not contain sialic acid has been identified in human serum VLDL. It is, however, not determined whether it also lacks hexosamine. It is also significant that both rat apoC–III and human apoC–III could not be distinguished from rat apoC–II on alkaline polyacrylamide gel electrophoresis. Other significant compositional differences between rat and human apoC–III include high glycine content, presence of isoleucine and absence of histidine in the former. Rat apoC–III is unique in containing C-terminal proline [97].

Rabbit serum HDL

It occurs at a concentration of 160 mg/100 ml serum and cholesteryl ester is the major component of lipids [87].

Guinea Pig serum HDL

HDL is found in small amounts with LDL being the major component. The distribution of these lipoproteins is highly dependent on the fat content of the animal diet. When this is low, the principal lipoprotein

has an hydrated density close to 1·063 gm/ml. But at higher levels the hydrated density falls less than 1·03 gm/ml. The decrease in density is accompanied by an increase in molecular weight, particle diameter and in the ratio of lipid to protein [98]. In animals fed with normal diet, the concentration of HDL is low while with cholesterol rich diet, the levels of HDL is high. Chemical composition of HDL obtained from animals fed on normal diet was 39% protein and 61% lipid. In cholesterol-rich diet animals, the protein constituted 27% and lipid 72% [99]. The lipid composition of the two HDLs are given in Table II. Guinea pig apoHDL has been fractionated into 6 fractions [100]. One of these fractions resembles human apoA-I but differs in not having proline but containing isoleucine. It contains less of leucine and large amount of alanine. Guinea pig apoC-I differs from human apoC-I in not having proline but it contains smaller amounts of histidine and tyrosine, and larger amounts of glycine and alanine [98].

Hegde hog serum HDL

HDL is present at a concentration of about 430 mg/100 ml serum and it has 41% protein and 59% lipid [87].

Dolphin serum HDL

HDL is the major lipoprotein present in the serum of pinnipeds (seals) and bottle-nose dolphins. An interesting feature of HDL is that phospholipid is the major component of lipids in pinnipeds whereas cholesteryl ester is the major lipid in dolphins [99].

Whale serum HDL

Killer whale HDL constitutes 50–70% of the serum lipoproteins and cholesteryl ester is the major component of lipids [100].

Avian serum HDL

Chicken serum HDL

Avian HDL is isolated between the same densities (1·063–1·21 gm/ml) as human serum HDL. Rooster serum has significantly more HDL than chicken serum. Immunochemically three distinct lipoproteins have been distinguished. They are VLDL and LDL, HDL and lipovitellins [101]. HDL is the only serum lipoprotein component which shows similarity to the human serum HDL. Chemical composition of rooster HDL resembles

that of human HDL_2 and physical properties of rooster HDL such as partial specific volume, frictional ratio, hydrated or unhydrated density and molecular weight resemble that of human HDL_3.

Chicken apo-HDL consists of about 85% of apoA–I and the rest of them are apoC proteins [102]. Apo–II is absent. Chicken apoA–I contains 234 amino acid residues and it is similar to human apoA–I in amino acid composition with the exception of isoleucine, histidine, leucine and glycine. N-terminal region of chicken apoA–I has been sequenced [82, 102] and it differs considerably from human apoA–I. A striking feature of chicken apoA–I is that the protein contains a large amount helical content (about 90%) [102]. The helical content of human apoA–I is about 65%.

Other avian serum HDL

HDL of duck, guinea-hen, pheasant and qail serum have been used for immunochemical studies [103, 104]. There is no report on the physical or chemical characteristics of these lipoproteins or their protein components.

Chemical composition of HDL of pigeon and goose have been reported. The interesting feature of HDL in these two birds is that it is present in the serum at a high concentration. Goose serum has 761 mg HDL/100 ml serum and pigeon has 1352 mg/100 ml serum [87].

Reptilean and amphibian serum HDL

HDL of four species of reptiles and one species of amphibian have been isolated and their chemical compositions are given in Table II [87]. Fractionation of their protein components have not been reported.

Fish serum HDL

Salmon serum HDL

HDL is the only lipoprotein present in the serum of late pre-spawning pink salmon. In contrast to this, the early pre-spawning salmon contains both LDL and HDL [105]. Salmon HDL is similar to human HDL in physical properties like flotation rate, hydrated density and CD spectra. Salmon apoHDL contains both apoA–I and apoA–II proteins. Both of them differ considerably from their human counterpart. Salmon apoA–I does not contain tryptophan but it has much less of aspartic acid and leucine, considerably more of lysine, methionine and tyrosine and much more of

alanine and isoleucine than human apoA–I. Salmon apoA–II does not contain cystine but contains much less of lysine, threonine, serine, glutamic acid and phenylalanine and considerably more of histine, arginine, aspartic acid, glycine, alanine and isoleucine than human apoA–II [105].

Sardine serum HDL

Three species of HDL have been isolated from pacific sardine serum namely, HDL_1 (between densities 1·04–1·09 gm/ml), HDL_2 (between densities 1·09–1·12 gm/ml) and HDL_3 (between densities 1·12–1·21 gm/ml) [106]. HDL_3 is more abundant than HDL_2. HDL has also been isolated from the serum of dog fish and hag fish [87]. An interesting feature of the lipid composition of sardine serum HDL (Table II) is the absence of long chain fatty acid, which was expected to be present as these animals feed mainly on plankton rich in wax esters.

Invertebrates

Insect hemolymph HDL

HDL is the major lipoprotein of american silk moth *Hyalophora cecropia*. It is isolated between densities 1·156–1·170 gm/ml and VHDL has been isolated at 1·26 gm/ml density [107]. Hemolymph HDL has also been isolated from other insects [108, 109]. In American silk moth, HDL occurs at a concentration of 470–560 mg/100 ml. It contains 52% protein and 48% lipid and VHDL contains 94% protein. Phosphatidyl ethanolamine and diglycerides are the major components of lipids. Diglycerides constitute 69% of the total neutral lipids. It is suggested that hemolymph HDL may be the carrier of diglycerides in insects. Disc gel electrophoresis of HDL shows the presence of two major protein components.

General remarks about serum lipoproteins

Some mammals like man possess at least two classes of lipoproteins, the low density and high density types, while others have only one type. Mammals like guinea pig, camel and rabbit have very little HDL [87, 97], while cow, bison, horse, dog, dolphin and whale have HDL as the main lipoprotein [80, 84, 85, 92, 99, 100]. Moreover the pattern of serum lipoproteins within a species may be influenced by strain or variety. This difference is seen in salmon, where in early pre-spawning pink salmon both HDL and LDL are present, whereas in late pre-spawning type only HDL is present. By immunochemical reactions it has been shown that there is no cross

reaction between the HDL of animal species belonging to different classes [103, 104].

Chemically, mammalian serum HDL does not differ much from that of man. The general feature of mammalian serum HDL is that it contains 50–70% lipids and 30–50% proteins. Cholesterol is present mostly in the esterified form and triglycerides form less than 10% of the lipids. Phosphatidyl choline is the major phospholipid. The major HDL-apoprotein namely, apoA–I, has a molecular weight of about 28,000. It has a helical content greater than 50% in the intact lipoprotein. It usually lacks cysteine. Glutamic acid comprises nearly 20% of the total amino acids. An interesting feature of apoA–I is that isoleucine is absent in human and primates, but is present in all other species. Isoleucine is present in human apoA–II but it is absent in rhesus and patas monkey. Mammalian apoA–I does not contain carbohydrates. ApoA–II lacks histidine and tryptophan with the exception of the chimpanzee. Cystine or cysteine is also absent in mammalian apoA–II with the exceptions of human and chimpanzee (Table IV).

HDL from non mammalian species also contain 50–60% lipid and most of the cholesterol exists in the form of esters. However, in a primitive form of fish, the hag fish, most of the cholesterol is found in the unesterified form. In an invertebrate, such as American silk moth unesterified cholesterol is somewhat more than esterified cholesterol. The striking feature in insect HDL is the presence of large amounts of diglycerides. Unlike few higher animals this insect has lipoprotein of density greater than 1·21 gm/ml.

3. Egg Yolk Lipoproteins

Egg yolk lipoproteins have been isolated from certain oviparious vertebrates [110–116] and a few invertebrates such as crustaceans [117–121]. Avian egg yolk contains two types of lipoproteins, the very low density lipoproteins (VLDL) or lipovitellinin and the high density lipoproteins or lipovitellins (Lv). The high and low density classes in the avian egg yolk can be separated on a solvent density between 1·00 and 1·2 gm/ml [1]. Egg yolk of frog and fishes do not contain well defined VLDL [122, 123]. Egg yolk of invertebrates do not contain VLDL.

Avian Egg Yolk

Hen's egg yolk granules

Egg yolk granules constitute 23% of yolk solids and they are composed of lipovitellins (Lv), phosvitin and a small amount of low density lipoprotein

(LDFG), which constitute about 4% of the total yolk lipids [124]. Lipovitellins consist of two components namely, α-and β-lipovitellins and each has a dimeric molecular weight of 380,000–400,000. Although fractionation of these two have been achieved by TEAE-cellulose column chromatography [125–126], they are similar in many respects. They have similar amino acid and lipid composition [127]. α-Lipovitellin has 17% and β-lipovitellin has 18·5% lipid [126]. The lipid composition of a α-lipovitellin is, 60% phospholipids, 36% triglycerides and 4% cholesterol [124], whereas β-lipovitellin has 62% phospholipids, 34% triglycerides and 4% cholesterol. Cholesterol is present mostly in unesterified form. Phospholipid composition of α- and β-lipovitellins are given below [124].

	Percentage of phospholipids	
	α-Lipovitellin	β-Lipovitellin
Phosphatidyl choline	75	76
Phosphatidyl ethanolamine	18	17
Lysolecithin Lysocephalin Sphingomyelin	7	6·6

Although lipovitellins have been characterised in a few species of birds, teleosts and crustaceans, their protein components are poorely understood. Franzen and Lee [133] reported the presence of two protein subunits of hen's egg yolk α- or β-lipovitellins, but detailed investigations were not carried out to characterise these two proteins. In view of limited information on these protein subunits, a detailed study of the protein components of hen's egg lipovitellins were undertaken in our laboratory [132] and a brief description is given here. The protein components of each of the two hen's egg lipovitellins were fractionated into two protein fractions. α-Lipovitellin was fractionated into α_I and α_{II} fractions and β-lipovitellin was fractionated into β_I and β_{II} fractions. α_{II} and β_{II} fractions were homogeneous each having a molecular weight of about 30,000. α_I and β_I fractions were heterogeneous containing more than one protein. All the four protein fractions contained neutral sugars, hexosamines, N-terminal lysine and phosphorus. Sialic acid was present only in α_{II} protein fraction. α_{II} and β_{II} fractions were rich in neutral sugars and phosphorus. α_I and β_I fractions were similar in amino acid composition while α_{II} and β_{II} were different in histidine, isoleucine and half-cystine.

Amphibian egg yolk lipovitellins

Yolk proteins of a few reptiles and fishes have been purified and characterised by TEAE–cellulose chromatography but their properties have not been reported [116]. Frog egg yolk platelets exist in a crystalline state and each platelet is made of two moles of phosvitin and one mole of lipovitellin [112, 113]. Lipovitellins of frog has only one component which contains 17·5% lipid, 81·5% protein and 0·45% protein phosphorus. It has a molecular weight of 430,000. Amino acid composition of frog apovitellin is similar to that of hen's egg yolk apovitellins with the exception of arginine and proline. Apovitellins of *Xenopus laevis* consists of two proteins in the ratio of 1 : 1. The molecular weight of the two proteins are 31,000 and 120,000. The smaller subunit has 2% phosphorus while the heavier subunit does not contain phosphorus.

Teleostean lipovitellins

Lipovitellins have been isolated from 13 species of teleosts [116] and they have been fractionated into α- and β-lipovitellins. β-lipovitellins occur in traces or totally absent in species like alewife, mudminnow, piperfish, sheepshed minnow, silver sides and butter fish. However, it is a major component in crappiei sauger and toad fish. Phosvitin is absent in many species. Lipid content of lipovitellins range from 15–29%. A feature of these lipovitellins is that α-lipovitellin is less phosphorylated than β-Lv. For example in toad fish, α- and β-lipovitellins contain 0·35% and 0·56% protein phosphorus respectively.

Crustacean lipovitellins

Several investigators have studied the lipovitellins of crustaceans. Wallace *et al* have studied the lipovitellins of six decapods [117]. They have an average molecular weight of 350,000 with a relatively large lipid content of 30%. Protein does not contain phosphorus. These lipovitellins do not undergo dissociation at alkaline pH but exhibit an intense and variable absorptivity in the visible spectra. These factors may be influenced by diet or environmental conditions. In contrast to decapod lipovitellins, an anostracan lipovitellin isolated from *Branchipus stagnalis* exhibited a slow and reversible association-dissociation phenomenon [121]. Dimerisation was observed at higher ionic strength. The molecular weight of an anostracan lipovitellin is 630,000. It contains 4 molecules of canthaxanthin as the carotenoid component and about 15% phospholipid of total dry weight, SDS–gel pattern of lipovitellin shows at least five major

bands [121]. Amino acid composition of anostracan lipovitellin is closely related to the amino acid composition of decapods and other invertebrates. Lipovitellins obtained from *Procambrium* species (cray fish) have a molecular weight of 500,000 [120]. It contains 35% lipid and it consists of a carotenoid, phosphatidyl choline and phosphatidyl ethanolamine. Aspartic and glutamic acids are the major amino acids of the protein. It exhibits a pH-dependent spectral shifts. At pH 7 it appears brown while at pH 8 it appears orange. The difference in the visible spectrum at these two pH are reversible [120]. In the case of marine invertebrates like *Cancer pagurus* and *Pecten maximus* the lipovitellins contain about 27% lipid of which two-thirds is phospholipids and 20–25% cholesterol [118]. The molecular weight of lipovitellins in the four species studied ranges from 170,000–630,000. Mannose is the major sugar consisting 2·5% of protein by dry weight. Cholesteryl ester is absent [118].

Several suggestions have been made on the role of carotenoids in these lipovitellins (sometimes referred to as carotenoproteins). One suggestion for the presence of carotenoids in lipovitellins is that they shield the developing embryo from solar radiations. Another suggestion is that they may be involved in the stabilization of lipid-protein interaction in the lipovitellins [118].

Zaglasky has studied the amino acid composition of various crustacean lipovitellins [119]. All of them have a high proportion of glutamic and aspartic acids. Lipovitellins of *Homarus gamarus* and *Plesionika edwardsi* do not contain cysteine or cystine. The similarities in composition become more apparent when the contents of groups of amino acids and their ratios considered. The values for the average hydrophobicity and percentage of non polar amino acids are similar to those found in globular proteins. It is unusual to have a high content of helix breaking amino acids like serine, threonine and proline. The low crysteine and helical content indicates random coil configuration for these proteins [119].

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