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DISCLAIMER

ORIGINS AND DETERMINANTS OF HDL POPULATIONS AND THEIR SUBPOPULATIONS

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Introduction

Small apolipoprotein AI (AI)-containing HDL particles are generally considered primary acceptors of cellular cholesterol and thereby crucial participants in reverse cholesterol transport (1). While the potential importance of small HDL particles in cholesterol redistribution is well appreciated, our understanding of compositional determinants of their structure and function is still rudimentary. Small particles fall within the 7.2-7.8 nm (HDL_{3c}) size interval or are smaller; molecular weights are approx. 75-115 kDa (HDL_{3c}) and lower, respectively (2). Small particles have been described in HDL preparations from various sources including (a) products of *in vitro* interconversion of plasma HDL, (b) nascent HDL from plasma of LCAT-deficient subjects and conditioned medium of human hepatoblastoma-derived cells (e.g., HepG2), and (c) particles from normal human plasma exhibiting pre-β electrophoretic mobility. The protein moiety of small HDL particles consists predominantly of AI; although some small *in vitro* interconversion products include AI-containing particles with (w) and without (w/o) apolipoprotein AII (AII). The number of AI molecules per particle within small AIw/oAII species is generally considered to be 2-1. At present, it is unclear whether small HDL particles of different origin share common functional and metabolic properties.

Small HDL Particles and their Subspecies

Based on available data, small HDL particles can be assigned to 3 groups according to their core lipid content. Group I particles (Table 1) exhibit the highest core lipid content with values that range from 10 to 22% (wt%). These particles are formed during *in vitro* interconversion of plasma HDL (primarily HDL_3) as mediated by factors including lipid transfer proteins (LTP) and hepatic lipase (3,4,5,6) (Figure 1). Group II particles (Table 2) exhibit a relatively low core lipid content (2-5%) and are found in plasma of lecithin:cholesterol acyltransferase

(LCAT)-deficient subjects (7) and in HepG2 conditioned medium (8) (Figure 2). HDL particles observed in plasma of fish-eye disease subjects are also enriched in small HDL particles (in HDL_{3c} interval) and are abnormally low in cholesteryl ester (CE) (total HDL core lipid content is approx. 7.5%) (9). AI-containing lipoproteins that exhibit a very low core lipid content (approx. 1.8%), together with an extremely low surface lipid content (phospholipid + unesterified cholesterol (UC) < 10%), have been isolated from human plasma. The molecular weight of these particles, estimated from gel permeation chromatography, is 80 kDa; by electrophoresis, these particles migrate with pre-β mobility (10). Group III particles (Table 3) contain essentially no core lipid and are represented primarily by pre-β1 HDL particles detected in normal human plasma (11). These particles are enriched in surface lipids (approx. 52%) and exhibit a molecular weight of 71 kDa by gradient gel electrophoresis (GGE). The chemical composition of pre-β1 is similar to that of small discoidal complexes of higher molecular weight (designated UC-class 1; approx. 123 kDa) formed during *in vitro* reassembly of phosphatidylcholine (PC) and UC with AI (12). Still larger particles with pre-β mobility (designated pre-β2 HDL; molecular weight 325 kDa) are also detected in normal human plasma. The chemical composition and molecular weight of these particles is comparable to those of reassembled discoidal complexes (designated UC-class 4; approx. 291 kDa) (12) (Table 4). UC-class 4 (with 3AI/particle) and UC-class 1 (with 2AI/particle) complexes are the almost exclusive products formed when cholate-dialysis reassembly is performed using a mixture of 88:44:1, PC:UC:AI molar ratio (Figure 3). The formation of such complexes appears to be uniquely determined by the stoichiometry of the interaction mixture and in particular by presence of UC in the reassembly mixture. In the absence of UC, the major product is a discoidal complex (designated Class 3) of molecular weight approx. 204 kDa with 2 AI per particle. In view of the above, origins of

pre-β1 and pre-β2 HDL *in vivo* may include UC-induced transformation of PC-AI surface remnants (generated during lipolysis of triglyceride-rich lipoproteins) by mechanisms comparable to those that determine formation of UC-class 1 and UC-class 4 complexes *in vitro*.

Metabolic and Compositional Factors in Subspeciation of Small HDL

Evidence from a series of studies suggests that lipolysis and/or lipolysis-derived products may play a role in formation and subspeciation, as well as in cellular secretion of small HDL particles. Thus, formation of pre-β HDL particles has been noted during lipolysis of triglyceride-rich lipoproteins (13); and, multicomponent pre-β HDL have been described in metabolic states elevated in plasma free fatty acids (FFA) (e.g., Type II diabetes) (14). Lipolysis-derived products, such as lysophosphatidylethanolamine and diacylglycerol, have also been identified in newly secreted lipoprotein particles from human AI- and AII-transfected cells (15) and apolipoprotein E-transfected cells (16) in culture . In addition, the latter studies suggest that specific phospholipids, such as phosphatidylethanolamine (PE), together with lipolysis-derived products may be important in determining structural and functional properties of nascent HDL particles. Based on these observations, we have investigated the influence of type of phospholipid (1-palmitoyl-2-oleoyl [PO]PE and POPC) and of lipolysis-derived products (specifically, sodium salt of oleic acid, Na($18:1_c$)) on formation of small AI particles.

Cholate-dialysis reassembly of AI with POPC, at increasing molar ratios of POPC:AI, results in formation of a highly reproducible series of discoidal complexes with unique particle sizes and POPC-AI stoichiometry (17) (Figure 4). Under identical conditions and comparable range of mixture molar ratios, no complex formation is observed when POPE is substituted for POPC. Such differential reactivity of POPE and POPC in forming stable complexes may reflect

differential phase preferences of these two phospholipid species when undergoing cholate-dialysis reassembly in the presence of AI. In aqueous media containing cholate, POPC exhibits discoidal bilayer structures that are capable of incorporating AI, when AI is added and the cholate is removed by dialysis. The phase status of POPE under similar conditions is undefined. It is of interest to note that while unsaturated PE (e.g., dioleoylPE) under physiological conditions are usually found in a non-bilayer organization (hexagonal phase), a bilayer organization can be stabilized by addition of detergents, including FFA (e.g., Na(18:1_c))(18). The presence of lipolysis-derived products in PE-containing HDL, secreted by transfected cells, may also contribute to stabilization of their structure. We therefore evaluated the influence of Na(18:1_c) when incorporated into cholate-dialysis reassembly mixtures containing AI and POPE (Figure 4). With addition of Na(18:1_c), discrete small AI particles, containing both POPE and Na(18:1_c), are the almost exclusive product, even at higher molar ratios (e.g., 100:50:1, POPE:Na(18:1_c):AI). When POPC is substituted for POPE in reassembly mixtures containing Na(18:1_c), monodisperse small AI particles are also formed, but only at a low mixture molar ratio (25:25:1, POPC:Na(18:1_c):AI). At mixture molar ratio of 25:1 POPC:AI, in the absence of Na(18:1_c), a paucidisperse series of discrete POPC-AI complexes is formed. Thus, lipolysis-derived products, such as FFA, can make significant contribution to the formation of monodisperse small AI particles, containing one or the other of the two major phospholipid species (POPC or POPE) identified in human plasma lipoproteins. Interestingly, unlike the results obtained with POPE, reassembly with POPC at higher POPC:Na(18:1_c):AI mixture molar ratios (e.g., 100:50:1) results in formation of larger complexes. GGE profiles of these complexes show features similar to those observed for POPC-AI discoidal complexes formed, in the absence of Na(18:1_c), in mixtures of identical POPC:AI molar ratio (i.e., 100:1).

Preliminary compositional data for the small AI particles, isolated by equilibrium density gradient ultracentrifugation after cholate-dialysis reassembly, are presented in Table 5. Apparent molecular weights of the POPE- and POPC-containing particles are 75 and 87 kDa, respectively, and approach values reported for human plasma pre-β HDL. The number of AI molecules per particle calculated for both species from their apparent molecular weights (obtained by GGE) and chemical compositions is 1.4. The reported value for human plasma pre-β1 HDL, also as calculated from apparent molecular weight and composition, is 1.2 AI molecules per particle (11). By chemical crosslinking of the protein moieties of the isolated small AI particles (Table 5), the number of AI per particle for both species is predominantly 2. Since the molecular weights and relative contents of PL and protein of the Na($18:1_c$)-containing small AI particles approximate those of pre-β1 HDL, it seems likely that both are comprised largely of species with 2 AI per particle. This would be consistent with most data for nascent small AI HDL. Interactive properties of the Na($18:1_c$)-containing small AI particles as well as their effectiveness in incorporating cell-derived cholesterol are currently under investigation in our laboratory.

Incubation of small AI particles (7.4 nm, HDL_{3c} interval; wt% 46.3 AI, 38.2 POPC, 15.5 Na($18:1_c$)), with LCAT in a medium containing low density lipoproteins (LDL) and FA-free human serum albumin, results in their transformation to core-containing particles (8.1 nm) in the HDL_{3b} size interval. Apparently, under the experimental conditions used, the level of Na($18:1_c$) incorporated into the small AI particles is not inhibitory to LCAT activity and preliminary data suggest that it may even be stimulatory. In the absence of LCAT activity, the size profile of the small AI particle is reduced with apparent redistribution of complex mass to LDL that is included in the incubation mixture as

a source of substrate cholesterol. FFA-mediated association of AI with apolipoprotein B-containing lipoproteins has been recently described (19).

Summary

Our survey of compositional properties of small HDL particles indicates considerable variation in core lipid content, reflecting in large part the origins of such particles. Whether small HDL particles of different core content and apolipoprotein composition differ in their metabolic properties and function in reverse cholesterol transport remains to be established. Our studies demonstrate that lipolysis-derived products such as FFA can facilitate formation *in vitro* of small AI particles with properties approximating those of plasma pre-β HDL. Of particular interest is our observation that small AI particles are an exclusive reassembly product in mixtures containing POPE and FFA. This observation may be relevant to the physiologic origins of PE in lipoprotein structure and its role in metabolism and secretion of nascent HDL. Lastly our observations on the reactivity of small AI particles, containing FFA, with LCAT and LDL suggest further linkages between triglyceride and HDL metabolism.

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Table 1. Properties of Small HDL Particles: Group I (Elevated Core Lipid Wt%)

	Size (nm)	Pr[a]	Composition (wt%) Surface Lipids PL[a]	UC[a]	Core Lipids CE[a]	TG[a]
Small product from incubation of HDL_3 with lipoprotein-deficient plasma (3)	7.6	60.6	22.2 (23.9)[b]	1.7	14.3 (16.2)[c]	1.9
Small product from incubation of HDL_3 + Intralipid with lipoprotein-deficient plasma (4)	7.6	64.3	20.7 (21.0)[b]	0.3	9.1 (14.7)[c]	5.6
Small (AIw/oAII) HDL from incubation of hypertriglyceridemic plasma + β-mercaptoethanol (5)	7.5, 7.3	58.0	20.4 (25.3)[b]	4.9	4.9 (17.3)[c]	12.4
Small (AIwAII) HDL from incubation of hypertriglyceridemic plasma + β-mercaptoethanol (5)	7.6	57.6	17.9 (20.5)[b]	2.6	5.4 (21.8)[c]	16.4
Small product from action of hepatic lipase on triglyceride-enriched HDL_3 (from incubation of HDL_3 + Intralipid + LTP) (6)	7.4	56.3	33.6 (33.9)[b]	0.3	7.5 (9.7)[c]	2.2

[a] Pr, protein; PL, phospholipid; UC, unesterified cholesterol; CE, cholesteryl ester; TG, triglyceride.

[b] Numbers in parentheses are total surface lipid wt%.

[c] Numbers in parentheses are total core lipid wt%.

Table 2. Properties of Small HDL Particles: Group II (Low Core Lipid Wt%)

	Size (nm)	MW[a] (kDa)	Composition (wt%)				
				Surface Lipids		Core Lipids	
			Pr	PL	UC	CE	TG
Small spherical HDL from LCAT-deficient plasma (7)		85	57.9	35.5 (39.8)[b]	4.3	0.7 (2.2)[c]	1.5
Small spherical HDL from Hep G2 conditioned media (8)	7.8	92	63.6	30.7 (31.5)[b]	0.8	4.9 (4.9)[c]	—

[a] MW, molecular weight.
[b] Numbers in parentheses are total surface lipid wt%.
[c] Numbers in parentheses are total core lipid wt%.

Table 3. Properties of Small Preβ-Particles: Group III (Essentially No Core Lipid)

	Size (nm)	MW (kDa)	Composition (wt%) Pr	Surface Lipids PL	Surface Lipids UC
Pre-β1 HDL (11)	___	71	47.5 (±8.0)	44.6 (±4.6) (52.2)[a]	7.6 (±2.3)
UC-Class 1 Complex (discoidal) (12)	8.4 x 4.1	123	45.8 (±0.5)	50.7 (±0.5) (54.2)[a]	3.5 (±0.3)

[a] Numbers in parentheses are total surface lipid wt%.

Table 4. Properties of Large Pre-β Particles (Essentially No Core Lipid)

	Size (nm)	MW (kDa)	Composition (wt%) Pr	Surface Lipids PL	Surface Lipids UC
Pre-β2 HDL (11)	___	325	21.5 (±4.3)	73.8 (±2.6) (79.5)[a]	5.7 (±1.0)
UC-Class 4 Complex (discoidal) (12)	13.5 x 4.4	291	29.0 (±0.8)	62.3 (±1.0) (71.0)[a]	8.7 (±0.6)

[a] Numbers in parentheses are total surface lipid wt%.

Table 5. Properties of Small AI Particles Containing Sodium Oleate

	Size (nm)	MW (kDA)	Composition (wt%) Pr	PL	$Na(18:1_c)$	% Helical Content
PE-$Na(18:1_c)$-AI	7.2	75	51.1	34.1	14.8	67
PC-$Na(18:1_c)$-AI	7.4	87	45.4	38.6	16.0	65

Figure 1 Particle size distribution (GGE) of (AIw/oAII) HDL and (AIwAII) HDL isolated from incubated hypertriglyceridemic plasma by immunoaffinity chromatography: Noninc (stored plasma, 4°C, 12 h); Inc (β-mercaptoethanol) (37°C, 12 h, 14mM β-mercaptoethanol); Inc (iodoacetate) (37°C, 12 h, 20mM sodium iodoacetate).

Figure 2 Size distribution (GGE) of particles in d 1.063-1.20g/ml fraction from: (upper) plasma of patient with LCAT-deficiency; (lower) HepG2 conditioned medium. Migration region of discoidal particles is indicated on the profiles.

Figure 3 Particle size distribution (GGE) of discoidal complexes formed following cholate-dialysis reassembly of: (upper) egg yolk (EY)PC:UC:AI, molar ratio 88:44:1; (lower) EYPC:AI, molar ratio 88:1. Major complexes in the EYPC:UC:AI profile are designated UC-class 4 and UC-class 1; major complex in the EYPC:AI profile is designated Class 3.

Figure 4 Particle size distribution (GGE) of products formed following cholate-dialysis reassembly of: selected mixtures of POPC-AI without (left) and with (right) Na(18:1_c), and selected mixtures of POPE-AI without (left) and with (right) Na(18:1_c). Mixture molar ratios are indicated on each profile. GGE profile of isolated AI run in absence of lipids is identical to profiles observed for mixtures containing POPE and AI, but without Na(18:1_c) (e.g., 50:0:1 and 100:0:1, POPE:Na(18:1_c):AI).

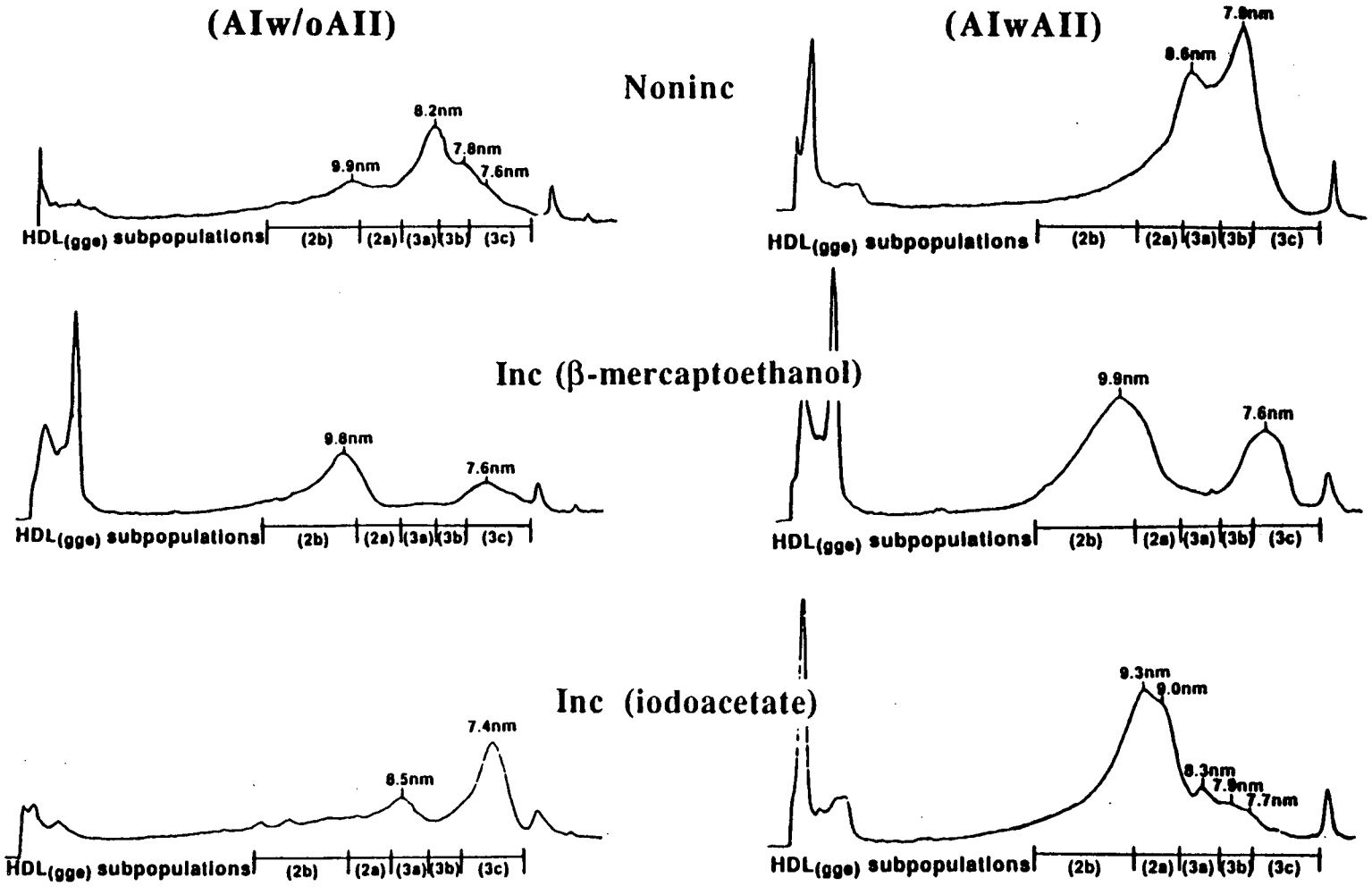
(AIw/oAII)
(AIwAII)
Noninc
8.2nm
9.9nm
7.8nm
7.6nm
8.6nm
7.8nm
HDL(gge) subpopulations
(2b)
(2a)
(3a)
(3b)
(3c)
Inc (β-mercaptoethanol)
9.8nm
7.6nm
9.9nm
7.6nm
Inc (iodoacetate)
7.4nm
8.5nm
9.3nm
9.0nm
8.3nm
7.9nm
7.7nm

Figure 1

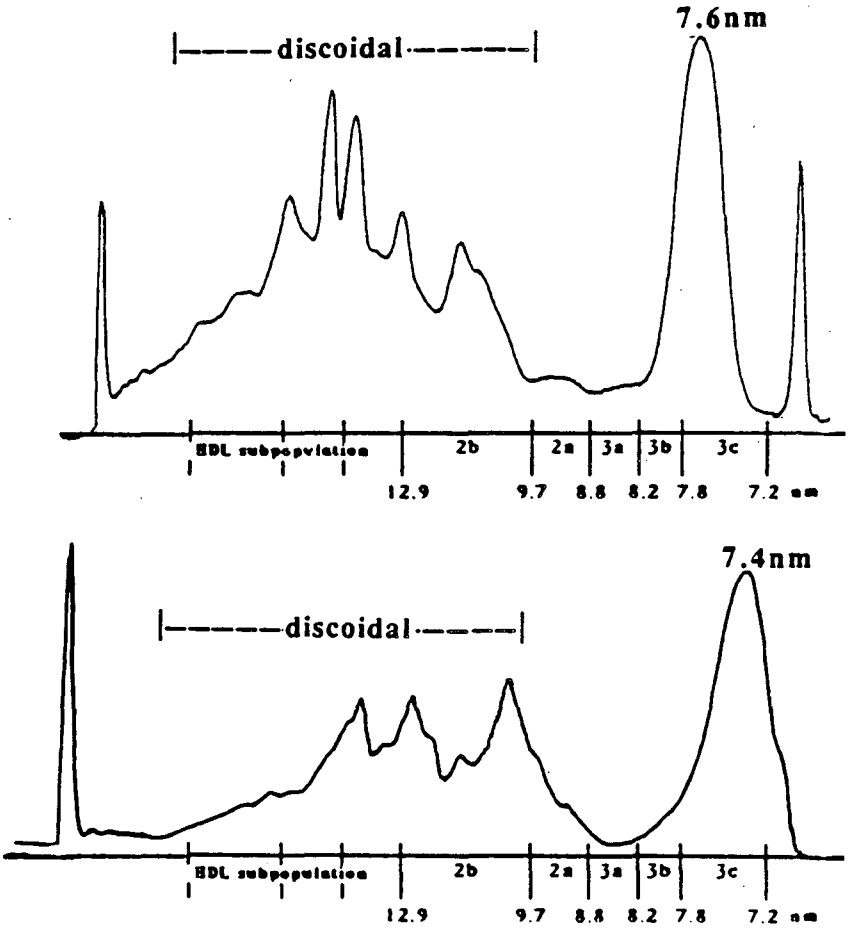
7.6nm
|-----discoidal------|
HDL subpopulation
2b
2a
3a
3b
3c
12.9
9.7
8.8
8.2
7.8
7.2 nm
7.4nm
|------discoidal-----|
HDL subpopulation
2b
2a
3a
3b
3c
12.9
9.7
8.8
8.2
7.8
7.2 nm

Figure 2

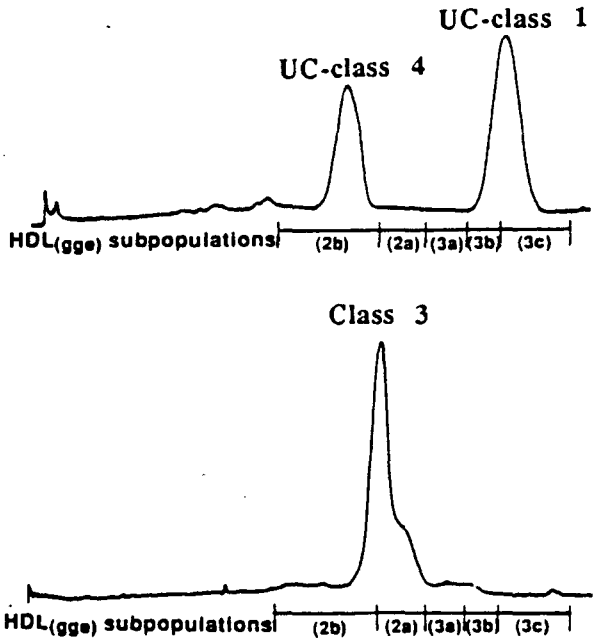
UC-class 1
UC-class 4
HDL(gge) subpopulations
(2b)
(2a)
(3a)
(3b)
(3c)
Class 3
HDL(gge) subpopulations
(2b)
(2a)
(3a)
(3b)
(3c)

Figure 3

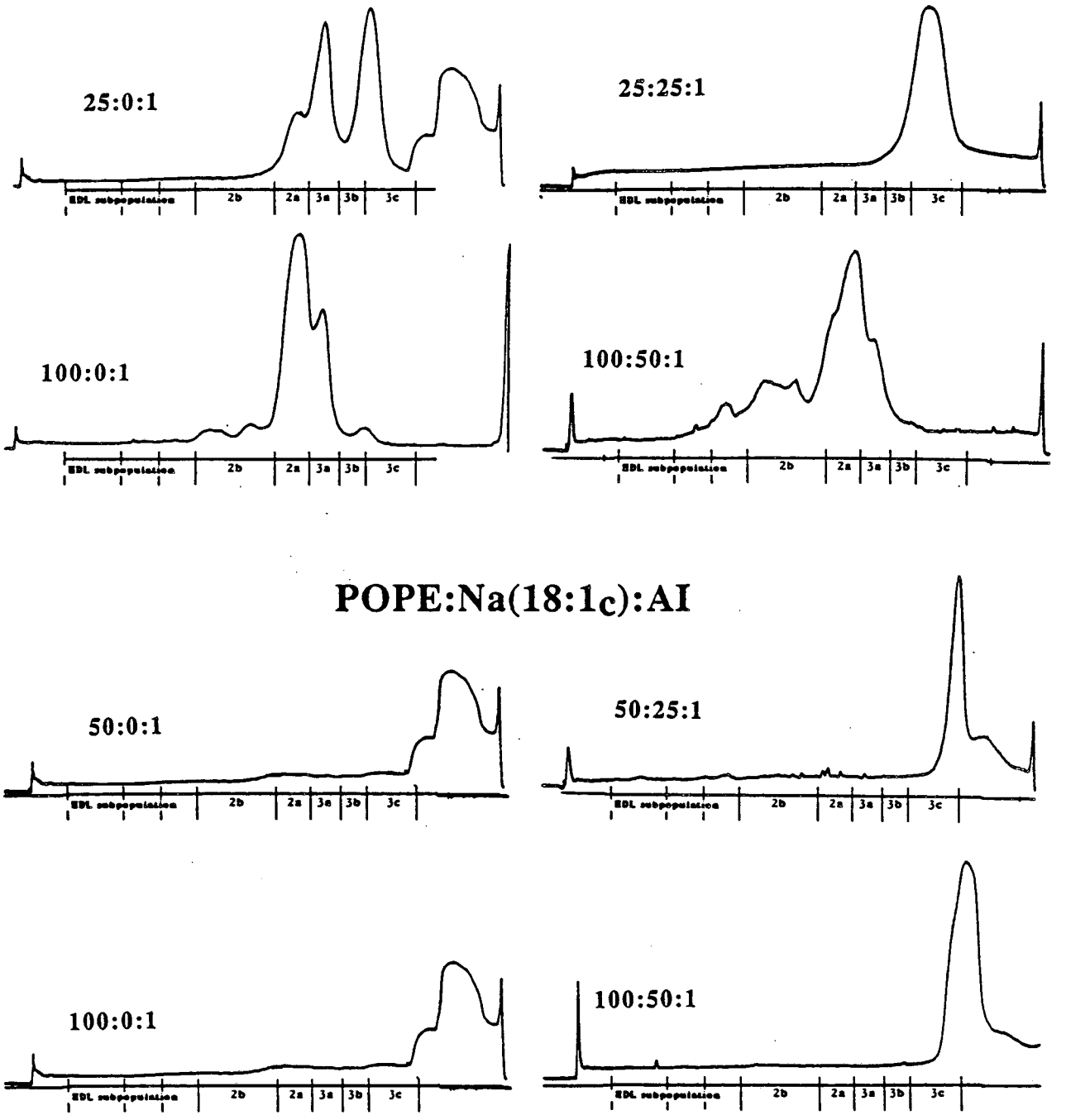
POPC:Na(18:1c):AI
25:0:1
25:25:1
100:0:1
100:50:1
POPE:Na(18:1c):AI
50:0:1
50:25:1
100:0:1
100:50:1
EDL subpopulation
2b
2a
3a
3b
3c

Figure 4

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