Apolipoprotein A-I Conformation in Discoidal Particles: Evidence for Alternate Structures†

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ABSTRACT: To define the conformation of apolipoprotein A-I in discoidal particles, the immunoreactivity of a series of epitopes distributed along the apoA-I sequence has been evaluated in lipid-free apoA-I and in lipid-bound form. To this end, reconstituted discoidal lipoproteins, here called LpA-I, and defined by number of apoA-I per particle (e.g., Lp2A-I), have been prepared with palmitoyloleoylphosphatidylcholine, cholesterol, and apoA-I. Four LpA-I have been obtained and studied: two in the Lp2A-I class, 7.8 and 9.6 nm in diameter, and two in the Lp3A-I class, 10.8 and 13.4 nm. The immunoreactivity of all the epitopes tested was significantly different in LpA-I particles compared to lipid-free apoA-I, demonstrating that binding to lipids produces a drastic change in apoA-I conformation. Specific domains in the primary sequence become highly exposed while others are masked. Although the variation in immunoreactivity of the epitopes between various LpA-I was not drastic, significant differences in the calculated ED_{50} values were observed for a number of antibodies in small versus large particles within each class (Lp2A-I or Lp3A-I), indicating that particle size can modulate apoA-I conformation. In addition, when the competition between pairs of mAbs was analyzed in order to understand the relative position of epitopes, highly significant differences were observed as a function of particle size within each class. In particular, the competition between mAbs recognizing epitopes in the central region of apoA-I was greater in the large particles than in their small counterparts. This seemingly antithetic finding can be rationalized with the introduction of alternate structures, such as a hinged domain constituted by two adjacent antiparallel α-helices, probably at residues 99–143, or distinct conformations dependent upon particle size.

Apolipoprotein A-I (apoA-I),[1] a polypeptide chain of 243 amino acids, is the major protein constituent of high-density lipoproteins (HDL). In addition to stabilizing HDL structure, apoA-I is the best activator of lecithin:cholesterol acyltransferase (LCAT) *in vitro* (Jonas, 1991) and plays a role in the reverse cholesterol transport (Oram et al., 1981). The entire sequence of the apoA-I gene has been determined (Karathanasis et al., 1983). The gene is composed of 4 exons, with the fourth one containing multiple intragenic repeats coding for 22 amino acid sequences (22-mers) (Karathanasis et al., 1983; McLachlan, 1977). These 22-mers are believed to form repetitive antiparallel amphipathic α-helices, interrupted by β-turns occurring at proline and glycine residues, and are thought to be responsible for the interaction with lipids (Segrest et al., 1974). The association with lipids produces a large increase in α-helical content of apoA-I, up to 85% (Morrisett et al., 1977), indicating a remarkable degree of structural adaptability of the protein.

Although plasma apoA-I is mostly lipid-bound, a lipid-free form has been shown to be generated *in vitro* under conditions which could also exist *in vivo*, such as by action of lipase on HDL (Hara & Yokoyama, 1991; Ryan et al., 1992). It is also assumed that lipid-poor forms of apoA-I exist which, although evanescent, may be important in cellular cholesterol efflux (Hara & Yokoyama, 1992). ApoA-I has also been shown to transfer between lipoproteins, and it is generally assumed that it is mostly a lipid-poor, if not a lipid-free, apoA-I which is thus transferred. In the first part of the present studies, we have used an immunochemical approach to understand how the presence of lipid affects apoA-I structure and whether apoA-I can assume a stable and different conformation in the absence of lipid, such as those proposed for the two apolipoproteins which have been crystallized to date (Breiter et al., 1991; Wilson et al., 1991).

Plasma HDL consist of heterogeneous particles differing in size, density, and lipid and apolipoprotein content. Thus, study of the structure and function of apolipoproteins and lipids in the native lipoproteins is very difficult. Several investigators (Nichols et al., 1983; Matz & Jonas, 1982; Jonas et al., 1989, 1990; Wald et al., 1990) have developed methods for the preparation of chemically and physically defined, apoA-I-containing discoidal particles, called here LpA-I, consisting of a defined molar ratio of phosphatidylcholine, cholesterol, and apoA-I, that are analogous to the precursors of the mature plasma apoA-I-containing lipoproteins. The LpA-I particles prepared with specific phospholipids, such as palmitoyloleoylphosphatidylcholine (POPC) or dipalmitoylphosphatidylcholine, and with specific ratios between the components have very reproducible sizes and compositions. These particles can be grouped into classes according to the number of apoA-I molecules per particle, such as Lp2A-I, Lp3A-I, and Lp4A-I, and within each class into particles of different sizes and lipid

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[1] Abbreviations: apoA-I, apolipoprotein A-I; HDL, high-density lipoprotein(s); LCAT, lecithin:cholesterolacyltransferase; LDL, low-density lipoprotein(s); LpA-I, apoA-I containing discoidal particles; mAb, monoclonal antibody; POPC, palmitoyloleoylphosphatidylcholine; RIA, radioimmunoassay.

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contents. These subclasses are thought to arise from different apolipoprotein conformations (Brouillette et al., 1984). Jonas and collaborators have developed a model for apoA-I structure bound to discoidal particles (Jonas et al., 1989). In this model, apoA-I is bound to the edge of the disk with up to eight helices oriented parallel to the axis of the disk. The calculated number of α-helices that can be accommodated around the periphery of the complexes varies with the size of the particle within each class. The Lp2A-I exist as particles of 7.8, 8.6, and 9.6 nm with increasing α-helical content, and they can accommodate six, seven, and eight α-helices, respectively, for each apoA-I molecule. In the second part of the present studies, using this model as a reference, we have undertaken to study the immunoreactivity of apoA-I epitopes in order to define the changes in apoA-I conformation which allow its association with different size particles.

MATERIALS AND METHODS

Preparation of ApoA-I and LDL. The apoA-I used in these studies was obtained from pooled plasma from normolipidemic subjects according to an established procedure (Brewer et al., 1986). The purity (>95%) of the apoA-I preparations was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using Coomassie protein staining. Aliquots of apoA-I were stored at −80 °C in lyophilized form. Before use, apoA-I preparations were dissolved in 4 M guanidine hydrochloride and extensively dialyzed against 10 mM Tris buffer, pH 8.0, 150 mM NaCl, 1 mM NaN_3, and 1 mM EDTA (recombinant buffer).

Low-density lipoproteins (LDL) (d = 1.006–1.050 g/mL) were isolated and washed at their upper density by salt density ultracentrifugation (Havel et al., 1955). LDL were free of HDL and plasma proteins as judged by nondenaturing gradient gel electrophoresis.

Formation and Isolation of Homogeneous LpA-I Particles. Discoidal LpA-I particles were prepared by the cholate dialysis method following previously published procedures (Matz & Jonas, 1982) using POPC/cholesterol/apoA-I/sodium cholate in the molar ratios of 80/8/1/80 or 120/6/1/120 (Jonas et al., 1990). All preparations and assays were done in recombinant buffer, except for cross-linking which was done in phosphate-buffered saline (PBS). After removal of the sodium cholate by extensive dialysis, the 9.6- and 13.4-nm particles were isolated from the reaction mixture containing 120/6/1 POPC/cholesterol/apoA-I by gel filtration on two serial agarose columns (95 × 2.5 cm) (BIO-GEL 5 M, Bio-Rad Laboratories). The reaction mixture containing 80/8/1 POPC/cholesterol/apoA-I, after removal of sodium cholate, was incubated with LDL for 24 h at 37 °C. This treatment leads to the transfer of POPC from LpA-I into LDL, with the formation of two limiting particles of 7.8 and 10.8 nm in diameter (Jonas et al., 1988). Gel filtration through the agarose columns separates these two particles. The sizes of isolated LpA-I particles were determined by gradient gel electrophoresis using precast Pharmacia 4/30% slab gels (Pharmacia Fine Chemicals, Uppsala, Sweden). The reference proteins used to determine particle diameters were thyroglobulin (17.0 nm), ferritin (12.2 nm), lactate dehydrogenase (8.1 nm), and albumin (7.2 nm) (Nichols et al., 1986).

Antibodies. The antibodies used for these studies have been previously described and characterized (Milthorp et al., 1986; Marcel et al., 1987, 1991; Petit et al., 1987). The control mAb 2H2 is an antibody against rat synthetic atrial natriuretic factor (Milne et al., 1987). All mAbs were murine IgG, purified on protein G–Sepharose (Pharmacia) and proven free of murine apoA-I (not illustrated).

Solid-Phase Radioimmunoassay of ApoA-I. Solid-phase radioimmunoassays were modified from those previously described (Marcel et al., 1987) by omission of detergent in the reaction buffer. Briefly, Immulon II Removawells were coated with 0.2 μg of apo-HDL in 100 μL of 15 mM Na_2CO_3/35 mM $NaHCO_3$, pH 9.6, containing 0.02% NaN_3, washed, and then saturated with 300 μL of gelatin (0.5% in PBS, pH 7.2, and 0.02% NaN_3). The antibody, at the predetermined optimal dilution, was mixed with serial dilutions of the competitive antigen in reaction buffer (0.5% gelatin/0.02% NaN_3 in PBS, pH 7.2), added to the coated and saturated wells, and incubated for 1 h at room temperature. The solution was discarded, and the wells were washed 3 times with 0.05% Tween/0.02% NaN_3 in PBS, pH 7.2 (wash buffer). Finally, to each of the wells was added 100 μL of ^{125}I-labeled rabbit anti-mouse IgG in reaction buffer, and the wells were incubated 1 h at room temperature. The wells were washed (3 times with wash buffer) and counted in a γ counter. Results were expressed as B/B_0 where B and B_0 represent the cpm bound in the presence and absence, respectively, of competitive antigen.

Competition between Antibodies. Immulon II Removawells were coated with 100 μL of mAb 4H1 or 4A12 diluted to a concentration of 5 μg/mL with 15 mM Na_2CO_3/35 mM $NaHCO_3$, pH 9.6, containing 0.02% NaN_3 and incubated overnight at 4 °C. The coating solution was discarded, and the wells were washed once with PBS containing 1 mM EDTA (PBS–EDTA). The coated wells were then saturated with 300 μL of 0.5% gelatin in PBS, pH 7.2, for 1 h. The competition reactions were carried out in 96-well microtiter plate (Linbro). All samples were diluted with 0.1% gelatin in PBS–EDTA. Serial dilutions of competitor antibodies (starting concentration 90 μg/mL) at 100 μL per well were prepared in the microtiter, followed by addition of 100 μL of LpA-I particles at 5 μg/mL and 100 μL of ^{125}I-labeled mAb (12×10^5 cpm). The reaction mixtures were incubated at room temperature for 2 h and then transferred to the precoated Removawells for an additional incubation (2 h). The Removawells were washed 5 times with PBS–EDTA and counted in a γ counter. Results were expressed as B/B_{max} (where B and B_{max} represent the cpm bound in the presence and absence of competing antibody, respectively) at the various molar ratios between competitor and labeled mAbs.

Analytical Methods. Cholesterol and phospholipid concentrations in pure isolated LpA-I particles were determined by enzymatic methods (Roschlau et al., 1974; Takayama et al., 1977). Protein was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. The number of apoA-I molecules per particle was determined by chemical cross-linking, using dimethyl suberimidate, according to Swaney and O'Brien (1978), and the products of the reaction were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Statistical analyses were carried out by Student's t test for paired samples.

RESULTS

Four discoidal LpA-I particles (two in the Lp2A-I class, 7.8 and 9.6 nm in diameter; two in the Lp3A-I class, 10.8 and 13.4 nm) have been prepared and isolated as described by others (Jonas et al., 1990) and have been analyzed in terms of size, composition, and number of apoA-I molecules per particle (Table I). The ratio between phospholipid and apoA-I

Table I: Composition of LpA-I Particles[a]

size[b] (nm)	POPC/cholesterol/apoA-I[c] (mol/mol)	apoA-I/LpA-I[d] (mol/particle)
7.8	46.6 (2.4)/6.3 (0.7)/1	2
9.6	110.3 (7.6)/2.8 (0.4)/1	2
10.8	84.0 (2.0)/17.8 (1.1)/1	3
13.4	142.0 (6.2)/5.0 (1.7)/1	3

[a] Results are means (SD) from three different experiments. [b] The diameters were determined by GGE (Nichols et al., 1986); three different preparations of these particles gave standard deviations of about 0.2 nm. [c] POPC and cholesterol were determined by enzymatic procedures (Roschlau et al., 1974; Takayama et al., 1977); apoA-I contents was determined by the Lowry et al. (1951) assay, the SD is indicated in parentheses. [d] The number of apoA-I molecules per particle was obtained by cross-linking with dimethyl suberimidate (Swaney & O'Brien, 1978).

is positively correlated with the size of the particles ($r = 0.918$, $p < 0.001$), while the ratio between cholesterol and apoA-I is inversely correlated with the lateral disk area available to each apoA-I (ratio of disk circumference to apoA-I number) in the different particles ($r = -0.8136$, $p < 0.001$).

Comparison of the Immunoreactivity of ApoA-I in Free versus Lipid-Bound Forms. The evaluation of the immunoreactivity of a panel of epitopes provides a useful approach to probe the structure of a protein under different conditions. Here, the immunoreactivity of apoA-I in lipid-free and lipid-bound forms has been studied using the standard competitive RIA previously described (Marcel et al., 1987) without any addition of detergent in order to disrupt neither the association of apoA-I with the particles nor the folding of the apolipoprotein in the free form. Furthermore, the concentration of the latter was kept below 400 μg/mL in order to avoid the formation of oligomers (Vitello & Scanu, 1976). The antigen immobilized on plastic in this assay is solubilized apoHDL. Under those conditions, the immobilized antigen assumes a modified conformation which is neither that of the soluble lipid-bound antigen nor that of the lipid-free antigen. However, the reference antigen is informative as long as it does react with the antibodies tested and remains stable under the conditions used. We have verified that these requirements were satisfied here, or we have used HDL as the immobilized antigen for assays with mAbs that do not react with apoHDL. The ED_{50} and the slopes calculated from the displacement curves obtained with different LpA-I have been averaged in order to obtain a median value for the immunoreactivity of each epitope when apoA-I is bound to lipids (Table II), and these values have been compared with the immunoreactivity of the lipid-free form of apoA-I. All the antibodies available and reacting with well-defined epitopes (Marcel et al., 1991; Figure 1) have been tested, and the results are shown in Table II. As might be expected for mAbs mainly obtained by immunization with HDL, the analyzed epitopes are best expressed on LpA-I, and their immunoreactivity decreases significantly in the lipid-free form of apoA-I. Indeed, the difference in ED_{50} between lipid-free and lipid-bound apoA-I is for most epitopes about 10-fold or greater and highly significant (Table II). This demonstrates that a major change in the conformation of the apolipoprotein occurs in the absence of lipids.

In the N-terminal region, the epitope for mAb 2F1 is completely masked in the lipid-free form of apoA-I, and the overlapping epitopes for mAbs A51 and A16 are also significantly less reactive. The epitope for 2F1 was shown earlier to be expressed only in intact apoA-I and in CNBr fragment 1 (residues 1–86), but not in any shorter fragments or fusion proteins, and was thus defined as a conformational

Table II: Immunoreactivity of ApoA-I in Free versus Lipid-Bound Forms

	apoA-I, lipid-free form		apoA-I, lipid-bound form	
mAb[g]	ED_{50} (μg/mL)	slope	ED_{50} (μg/mL)	slope[a]
4H1	56.1 (25.2)	−0.55 (0.01)	83.0 (33.3)	−0.93 (0.05)[c]
2F1	>400		2.1 (2.3)[b]	−0.95 (0.05)
A05	2.3 (1.4)	−0.25 (0.01)	2.4 (0.7)	−0.42 (0.02)[d]
A51	29.5 (2.3)	−0.58 (0.02)	1.1 (0.5)[b]	−0.33 (0.009)[b]
A16	14.1 (1.2)	−0.32 (0.01)	1.1 (0.4)[b]	−0.31 (0.01)
2G11	15.4 (3.8)	−0.37 (0.01)	56.6 (19.4)	−0.64 (0.06)[e]
A11	69.1 (17.2)	−0.51 (0.06)	34.3 (9.6)	−0.46 (0.02)
A17	0.7 (1.1)	−0.30 (0.40)	1.3 (0.3)	−0.47 (0.006)[b]
3G10	49.2 (25.6)	−0.37 (0.03)	0.9 (0.4)[b]	−0.52 (0.04)
5F6	*f*		7.5 (2.7)[b]	−0.58 (0.02)
4F7	>400		2.3 (0.2)[b]	−0.95 (0.02)
A03	11.5 (0.5)	−0.30 (0.06)	1.1 (0.1)[b]	−0.48 (0.02)[c]
A07	16.7 (0.5)	−0.81 (0.09)	13.2 (3.3)	−0.62 (0.03)
A44	12.2 (1.3)	−0.49 (0.03)	1.4 (0.3)	−0.40 (0.01)[d]
4A12	>400		11.0 (2.3)[b]	−0.64 (0.01)

[a] The ED_{50} (SE) and the slope (SE) for the lipid-free form are the average of four experiments, and those for the lipid-bound form are the mean of the ED_{50} and the slope obtained with the four species of LpA-I in multiple experiments. [b] $p < 0.0001$. [c] $p < 0.005$. [d] $p < 0.002$. [e] $p < 0.02$, lipid-bound versus lipid-free forms. [f] In the competitive RIA, the lipid-free form of apoA-I does not generate a displacement curve with antibody 5F6, and the results are not reproducible. [g] mAbs are separated in three groups representing the N-terminal region (4H1 to 2G11), the central region (A11 to A03), and the C-terminal region (A07 to 4A12).

epitope contained within residues 8–86 (the sequence 1–7 was excluded because it had been shown to react with other mAbs, such as 4H1, but not 2F1). Given their similar variation in immunoreactivity, with and without lipid, the epitopes for 2F1, A51, and A16 overlap significantly. On the other hand, the immunoreactivity of the epitopes for mAbs 4H1 and A05 is relatively unaffected by the presence of lipids, and the epitope for mAb 2G11 is more reactive in the lipid-free form than in the lipid-bound one, a finding not unexpected, since 2G11 had been obtained by immunization with lipid-free apoA-I (Petit et al., 1987).

In the middle region of the protein, only the epitopes for mAbs A11 and A17, which overlap on residues 99–105, are not differently immunoreactive in the two apoA-I forms. In contrast, the epitopes for mAbs 3G10, 5F6, 4F7, and A03, which overlap to various degrees over residues 118–148, are significantly more reactive in all the LpA-I particles than on the lipid-free apoA-I. Interestingly, the epitope for mAb 5F6 shows unusual behavior in lipid-free apoA-I, in that it does not generate a normal displacement curve, but only scattered points. In contrast, antibody 5F6 displays a normal immunoreactivity with LpA-I (Table II) and is, in fact, a very efficient mAb in immunoprecipitation of all LpA-I particles or HDL (Meng et al., 1993). The unstable expression of the epitope for 5F6 in the absence of lipids suggests that it is located in a region unstable or mobile in the lipid-free form.

In the C-terminal region of apoA-I, the epitopes for A44 and 4A12 are more immunoreactive in the lipid-bound form, while the epitopes for A07 are unaffected by the presence of lipids. Therefore, although the epitopes for A44 and A07 have been found to overlap completely (Marcel et al., 1991), their specificity is clearly different as shown here and in our study of apoA-I activation of the LCAT reaction (Meng et al., 1993).

The slopes obtained with lipid-free apoA-I are different, in some cases significantly, from the mean of the slopes obtained with the four species of LpA-I (Table II). This suggests a change in the relative binding affinity, which prevents the accurate comparison of apoA-I concentration in lipid-free

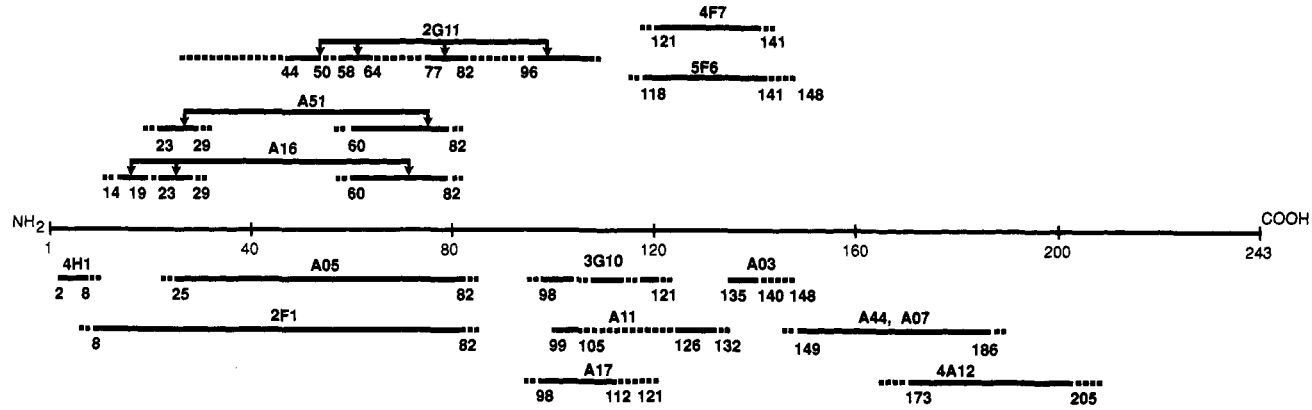

FIGURE 1: Epitope map of apolipoprotein A-I. The positions of epitopes recognized by these mAbs were previously defined and described (Milthorp et al., 1986; Marcel et al., 1987, 1991; Petit et al., 1987). The names of mAbs are placed above the solid line bars which represent the sequences of apoA-I recognized by these mAbs. The dashed lines on the bars mean that the antigenic recognition at this site may extend somewhat further.

Table III: Immunoreactivity of ApoA-I in Lp2A-I and Lp3A-I Particles[a]

	Lp2A-I		Lp3A-I	
mAb[g]	7.8 nm	9.6 nm	10.8 nm	13.4 nm
4H1	19.1 (3.6)	131.6 (24.2)[b]	32.4 (7.6)	149.0 (32.4)[e]
2F1	2.3 (0.8)	1.5 (0.1)	3.1 (0.5)	1.8 (0.3)[c]
A05	2.9 (0.5)	1.2 (0.3)[b]	4.1 (1.1)	1.4 (0.3)[c]
A51	2.3 (0.3)	1.3 (0.2)[c]	0.2 (0.05)	0.4 (0.07)
A16	2.4 (0.3)	0.4 (0.1)[c]	0.6 (0.2)	0.6 (0.1)
2G11	107.4 (42.1)	63.7 (27.1)[c]	39.0 (5.9)	16.4 (2.9)[c]
A11	28.4 (7.9)	62.8 (11.0)[c]	25.5 (3.4)	20.5 (6.7)
A17	1.1 (0.2)	0.7 (0.2)	2.0 (0.4)	1.3 (0.1)
3G10	0.1 (0.03)	0.4 (0.1)[c]	1.5 (0.3)	1.7 (0.2)
5F6	2.8 (0.5)	15.3 (1.9)[d]	4.6 (0.4)	7.2 (1.5)
4F7	1.9 (0.3)	2.2 (0.5)	3.4 (0.7)	2.2 (0.5)
A03	0.7 (0.2)	0.5 (0.1)	1.8 (0.3)	1.2 (0.2)
A07	7.4 (1.0)	22.8 (2.8)[b]	10.7 (0.8)	12.0 (2.6)
A44	2.3 (0.4)	0.9 (0.2)[b]	1.3 (0.2)	1.1 (0.2)
4A12	17.1 (4.4)	6.8 (1.6)[e]	12.0 (2.2)	8.4 (1.3)[e]

[a] Results are means (SE) from six experiments. [b] $p < 0.005$. [c] $p < 0.05$. [d] $p < 0.001$. [e] $p < 0.02$ large versus small particles. [g] mAbs are separated in three groups representing the N-terminal region (4H1 to 2G11), the central region (A11 to A03), and the C-terminal region (A07 to 4A12).

versus lipid-bound forms with most of these antibodies. The results obtained with the competitive RIA demonstrate that the association with lipids induces a drastic change in the conformation of the entire apoA-I molecule as specific domains distributed along most the sequence become highly immunoreactive while others are masked.

Comparison of the Immunoreactivity of ApoA-I in Different LpA-I Particles. Within each class of LpA-I, we have compared the Lp2A-I particles, 7.8 versus 9.6 nm in diameter, and the Lp3A-I particles, 10.8 versus 13.4 nm, to study the apoA-I immunoreactivity and understand the effect of particle size on apoA-I structure. None of the RIAs carried out with the available antibodies have displayed any significant difference in the calculated slopes of the displacement curves obtained with the different LpA-I particles (data not shown), reflecting very similar binding affinities of each mAb with the various particles. Table III summarizes the ED_{50} values calculated for small and large particles in the Lp2A-I and Lp3A-I classes.

The epitope for mAb 4H1, located at residues 2–8 in the apoA-I sequence, is 5-fold more reactive with small than with large particles, demonstrating a significantly different conformation of the extreme N-terminal region of the molecule. In contrast, five antibodies—2F1, A05, A51, A16, and 2G11—reacting further in the N-terminal region between residues 8 and 100 (Figure 1), are less immunoreactive with small than with large Lp2A-I (the difference is significant for all but 2F1). This difference is maintained with the small versus large Lp3A-I, except for mAbs A51 and A16, which show no significant difference (Table III).

In the middle of apoA-I, out of six epitopes tested (A11, A17, 3G10, 5F6, 4F7, and A03) which overlap between residues 98 and 141, only three are more reactive in the small Lp2A-I (7.8 nm) compared to the large Lp2A-I (9.6 nm). These are the epitopes for mAbs A11, 3G10, and 5F6 which overlap between residues 118 and 121. There is no significant difference in the expression of any of the six epitopes in the large and small Lp3A-I (Table III).

In the C-terminal half of apoA-I, we have studied three antibodies: mAbs A07 and A44, overlapping and reacting with residues 149–186, and mAb 4A12, which recognizes the sequence 173–205 and is the most C-terminal epitope identified from our group (Marcel et al., 1991). Despite the significant overlap of these epitopes, they are differentially expressed in the different LpA-I. A07 and A44 must clearly react with different contact residues within their common immunoreactive sequence as the first mAb is significantly more reactive with small Lp2A-I and the second with large Lp2A-I. The epitope for mAb 4A12 is significantly more immunoreactive in large particles in both Lp2A-I and Lp3A-I (Table III).

The analysis of the correlations between the immunoreactivity of different antibodies and the lipid composition of the particles demonstrates that the phospholipid to apoA-I ratio has specific effects on apoA-I structure, as only specific epitopes are affected by the phospholipid concentration. Antibody 4H1 immunoreactivity is inversely related to phospholipid concentration ($r = -0.926$), while mAbs A51 ($r = 0.7011$), A16 ($r = 0.8027$), and 2G11 ($r = 0.8570$) in the N-terminal third and mAbs A44 ($r = 0.850$) and 4A12 ($r = 0.8897$) in the C-terminal third of the molecule define two domains where the expression of the epitopes is positively related to phospholipid concentration. All the correlations are significant ($p < 0.005$).

Competition between Antibodies. In contrast to the N-terminal domain for which no structural model has been proposed, the periodic pattern of the 11- and 22-mer repeats that stretch from residue 66 to the C-terminal forms the basis of the amphipathic α-helices which have been identified as the specific structural motif of apoA-I and other apolipoproteins (Segrest et al., 1974, 1992; Andrews et al., 1976; Boguski et al., 1986; Brasseur et al., 1990). Using this model

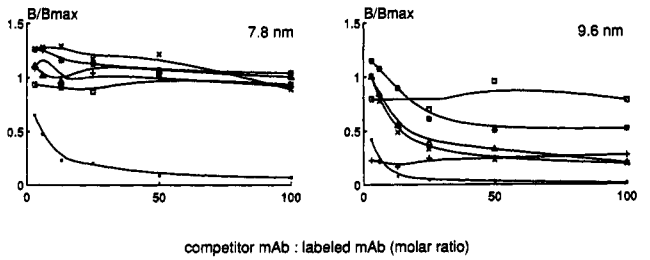

FIGURE 2: Competition of antibodies with mAb A44 for binding to Lp2A-I. The ability of mAbs A44 (■), 4A12 (+), 5F6 (*), A03 (×), 3G10 (▲), and 2H2 (□) to compete with A44 for binding to Lp2A-I 7.8 nm (*left*) and 9.6 nm (*right*) was tested in the solid phase at room temperature for 2 h. Each reaction includes 50 ng of LpA-I bound by 4H1-coated solid phase, 10^5 cpm of ^{125}I-A44, and serial dilutions of competitor mAb (competitor mAb to labeled mAb molar ratios between 0 and 100).

as a reference, we have studied the spatial relationships between epitopes identified on this portion of the molecule as they are expressed on the different discoidal LpA-I. We have especially concentrated our efforts to understand the relative positions of the well-defined epitopes downstream of residue 98 (Figure 1), using competition binding assays between pairs of mAbs reacting with each of these epitopes. We have selected four mAbs reacting with high affinity in this region of apoA-I (3G10, 5F6, A03, and A44) and another reacting in the N-terminal (A16), which is used as a negative control. Another criterion for the selection of these mAbs was their ability to react after iodination. This problem limited the assay for A05 which could be tested only as a cold competitor. For these competitive binding assays with the mAbs indicated above, the different LpA-I were captured using a noninterfering mAb immobilized on plastic, in this case and as described under Materials and Methods, antibody 4H1 which reacts at the N-terminal. Figure 2 shows typical competition curves obtained with labeled mAb A44. The results obtained with the Lp2A-I particles are summarized in Table IV. Experiments carried out with the Lp3A-I give similar results (data not shown).

Highly significant differences in the competition of mAbs are observed as a function of particle size but independently of the number of apoA-I molecules per particle. The most significant differences are observed with mAbs recognizing epitopes in the central region of apoA-I where the competition is greater when the mAbs react with the large particles than with the smaller counterparts. MAb 3G10 (residues 98–121) binding to Lp2A-I particles is significantly displaced by mAbs A03 (residues 135–148) and A44 (residues 149–186) in the 9.6-nm particle (55% and 80%, respectively) while no competition was observed in the 7.8-nm particle. When mAb A03 is labeled, and tested in competition with the different mAbs, similar results are obtained. MAbs 3G10, 5F6 (residues 118–148), and A44 compete for the binding of mAb A03 in the large particle but significantly less in the small Lp2A-I (Table IV). Furthermore, when the competition of these antibodies with mAb A44 binding is analyzed, the difference between the two particles (7.8 nm and 9.6 nm) is even more pronounced. MAbs 3G10, 5F6, and A03 are able to strongly compete for binding with mAb A44 (65%, 40%, and 50%, respectively), but only in the large Lp2A-I. The decrease in competition in the small particles does not result from decreased affinity of mAbs for small versus large particles. In fact, as shown in Table III, all the mAbs tested in the competition assays are either equally reactive with the two different particles or even more immunoreactive with the smaller one. Furthermore, the relative binding affinity is very similar in the various particles with all the tested mAbs, as shown by the mean (and SE) of the slopes calculated from the displacement curves for the four LpA-I. These results can only be interpreted as evidence for different conformations of apoA-I in large and small LpA-I, which impose different orientations to the respective mAbs when bound to these particles.

DISCUSSION

The majority of circulating apoA-I is found in HDL which consist of very heterogeneous particles, differing in physical properties and lipid and apolipoprotein composition. According to the apolipoprotein content, HDL can be separated into two major subclasses, particles containing only apoA-I (LpA-I) and particles containing apoA-I and apoA-II (LpA-I,A-II particles) (Cheung et al., 1984). The heterogeneity of HDL apolipoproteins is the major determinant of the heterogeneity of the particles present in this density class as has been shown in the early studies on apoA-I immunoreactivity (Schonfeld et al., 1976, 1977; Curtiss et al., 1985). In addition, conformational studies have indicated that apoA-I possesses a remarkable degree of flexibility manifested by a low free energy of denaturation (Tall et al., 1976) and a large increase in α-helical content (up to 85%) upon association with lipids (Morrisett et al., 1977).

The first aim of this study was to evaluate the changes in apoA-I conformation that occur upon binding to lipids as expressed by the different immunoreactivity of apoA-I in the lipid-free and lipid-bound forms. Our results are consistent with a drastic change in the apolipoprotein conformation upon association with lipids. The differences in immunoreactivity of antibodies reacting with epitopes covering most of the apoA-I sequence demonstrate that multiple regions are differently exposed in the two apoA-I forms. Most epitopes are less reactive in the lipid-bound form, and some of them are totally masked in the absence of lipids such as 2F1, in the N-terminal region, 4F7, in the middle region, and 4A12, in the C-terminal end. Three short epitopes are equally immunoreactive in the two forms: the epitope for mAb 4H1, located in the extreme N-terminal region, and the epitopes for mAbs A11 and A17, in the middle region, at residues 99–112 and 126–132. Only the epitope for mAb 2G11, which was obtained by immunization with apoA-I (Petit et al., 1987), is more immunoreactive in the lipid-free form. From our immunoreactivity studies and by analogy with the molecular structures described for other apolipoproteins in the lipid-free form and their proposed structures in lipid-bound forms (Breiter et al., 1991; Wilson et al., 1991), we can hypothesize that the existence of a condensed structure for the soluble lipid-free apoA-I (i.e., by formation of up and down bundles of amphipathic α-helices stabilized by hydrophobic interactions) must be responsible for the lack of immunoreactivity of most of the epitopes along the sequence, while in the lipid-bound form the apolipoprotein probably spreads on the lipid surface, exposing most of the epitopes. These types of structure have been suggested by Breiter and co-workers (Breiter et al., 1991), who reported the first X-ray determination of the structure of an insect apolipoprotein of 18–20 kDa referred to as apolipophorin III (apoLp-III) (Shapiro et al., 1988). The structure determined for apoLp-III is that of five long amphipathic α-helices connected by short loops to form a five-helix bundle globular protein. The authors proposed that hinged sequences linking two pairs of adjacent helices allow the change in conformation from the soluble globular structure to the lipid-bound conformation on the surface of lipophorin. More recently,

Table IV: Competition between MAbs for Binding to Lp2A-I[a]

	B/B_{max} (%)[b]									
	Lp2A-I, 7.8 nm					Lp2A-I, 9.6 nm				
mAbs	^{125}I-A16	^{125}I-3G10	^{125}I-5F6	^{125}I-A03	^{125}I-A44	^{125}I-A16	^{125}I-3G10	^{125}I-5F6	^{125}I-A03	^{125}I-A44
A05	93 (5)	nd	62 (4)	nd	85 (12)	100 (5)	nd	64 (6)	nd	68 (4)
3G10	106 (7)	0 (2)	87 (3)	50 (7)	91 (6)	101 (4)	0 (2)	97 (3)	30 (2)	36 (3)[e]
5F6	102 (4)	92 (5)	37 (5)	94 (8)	102 (4)	95 (7)	88 (2)	36 (5)	77 (5)	62 (12)[c]
A03	nd	84 (3)	103 (1)	29 (4)	98 (4)	nd	44 (9)[c]	101 (4)	37 (4)	49 (11)[c]
A44	87 (2)	96 (4)	85 (3)	43 (7)	12 (2)	91 (4)	22 (3)[d]	59 (6)[c]	23 (1)[c]	19 (10)
4A12	nd	nd	97 (4)	nd	96 (1)	nd	nd	106 (2)	nd	47 (15)[c]
2H2	98 (3)	99 (5)	99 (2)	97 (5)	102 (1)	101 (9)	109 (9)	101 (3)	93 (4)	103 (1)

[a] Results are the means (SE) from three experiments. The competition reaction was carried out at room temperature for 2 h. Each reaction mixture includes 50 ng of LpA-I precipitated by 4H1-coated solid phase, 10^5 cpm of ^{125}I-labeled mAb, and serial dilutions of competitor mAb (competitor mAb to labeled mAb molar ratio between 3 and 100). The data in this table were chosen at saturation level (competitor mAb to labeled mAb molar ratio = 50). [b] B/B_{max} (%) = (*competitor cpm* − *background cpm*)/(maximum cpm − background cpm)]^{100}. [c] $p < 0.05$. [d] $p < 0.005$. [e] $p < 0.002$, Lp2A-I 9.6 nm *versus* Lp2A-I 7.8 nm.

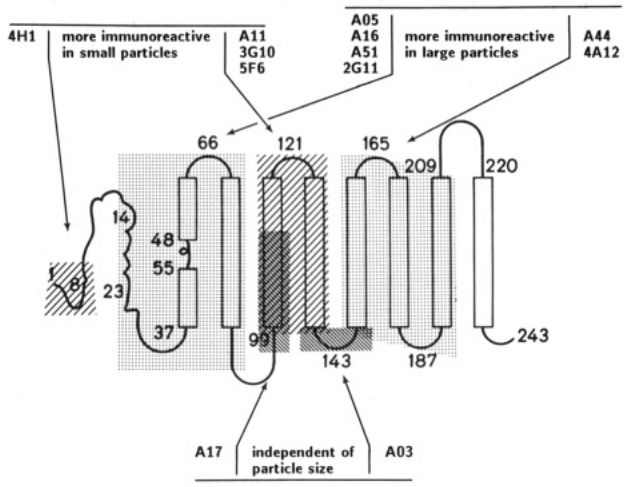

FIGURE 3: Location of domains that are differently immunoreactive in small *versus* large LpA-I particles in relation to a planar representation of apoA-I supersecondary structure. The predicted elements of secondary structure are drawn on a line proportional to their position in the primary structure. Amphipathic α-helices, β-turns, and random coils and β-sheets are respectively indicated with *boxes*, *curvilinear sections*, and *herring bones*; the numbers along the *line* and the *boxes* refer to residues and are intended as approximate reference points for the elements of secondary structure. Well-defined epitopes (Marcel et al., 1991) are regrouped according to the immunoreactivity in the two different sized LpA-I. Different domains are indicated with particular shading of the area.

the three-dimensional structure of a large portion of human apoE (residues 1–191) has been described by Wilson and coworkers (Wilson et al., 1991). The protein was determined to form a four-helix bundle, where the helices are oriented up and down, forming a globular structure. A mechanism similar to the hinged sequences of apoLp-III has been proposed for the change in conformation that is known to occur when apoE binds to lipids, that is, a mobile sequence linking helices 2 and 3 which would allow helices 1 and 2 to separate from helices 3 and 4 and spread onto the lipid surface (K. Weisgraber, personal communication).

The second aim of our studies was to understand the different conformations that apoA-I may assume when it is bound to lipids especially in the well-defined discoidal lipoproteins which are characterized by a precise stoichiometry of two, three, and four molecules of apoA-I per particle and which, within each class, are discretely sized (Nichols et al., 1983; Jonas et al., 1989, 1990; Wald et al., 1990). A summary of the immunoreactivity of the various epitopes as a function of particle size in Lp2A-I and Lp3A-I is presented in Figure 3. Using the planar model of apoA-I supersecondary structure recently described by our group (Marcel et al., 1991), we pooled neighboring epitopes, based on the similarity of their immunoreactivity as a function of particle size or phospholipid concentration (two interrelated parameters) within each LpA-I class, to form what appears to be structurally homogeneous domains. The first domain is defined by antibody 4H1 which reacts with the extreme N-terminal region (residues 2–8) and seems to be masked in the large particles and inversely related to phospholipid concentration. Two large domains of similar immunoreactivities are defined by antibodies A05, A16, 2F1, and 2G11, which react with discontinuous sequences between residues 14 and 96 on the N-terminal third of the molecule, and by mAbs A44 and 4A12 on the C-terminal. The epitopes in these two domains (with the exception of A51 in large Lp3A-I) are significantly more immunoreactive in the large particles within each class, and their immunoreactivities are positively related to phospholipid concentration. Between these two domains, an intermediary region is defined by mAbs A11, 3G10, and 5F6 (residues 99–143, Figure 3) which is more immunoreactive in small particles. As should be expected for such a region located between two domains more immunoreactive in large particles, its borders defined by mAbs A17 and A03 (residues 98–110 and 135–148, Figure 3) have an immunoreactivity independent of particle size. We believe that these findings are consistent with the existence of a mobile domain constituted by the central epitopes (residues 99–143) in which the sequences around residues 99 and 143 constitute the hinges, and we propose that the formation of particles of discrete size within each class of LpA-I, containing two, three, or four apoA-I, could be due to such a central mobile domain, able to assume different conformations (Figure 4A,B). This region has been shown to have a particularly high antigenicity (Marcel et al., 1991) that could be explained by the conformational flexibility of a hinged pair of helices. The existence in apoA-I of such a hinged domain constituted by a pair of amphipathic α-helices which could exist in two configurations, either bound to the lipoprotein surface or free in the aqueous milieu, has been proposed to be a possible mechanism to explain the discontinuous sizes observed in HDL particles (Brouillette et al., 1984; Cheung et al., 1987).

The examination of the correlations observed between epitope immunoreactivity and particle size or phospholipid to apoA-I ratios supports the concept of distinct structural domains on apoA-I as defined above. Several of the epitopes analyzed here have also been studied earlier in native HDL where phospholipid and cholesterol levels varied (Collet et al., 1991). It is noteworthy that the correlations between immunoreactivity and lipid concentration can markedly differ in HDL and in discoidal particles for specific epitopes. This is especially evident for mAb 2G11, reacting with a discon-

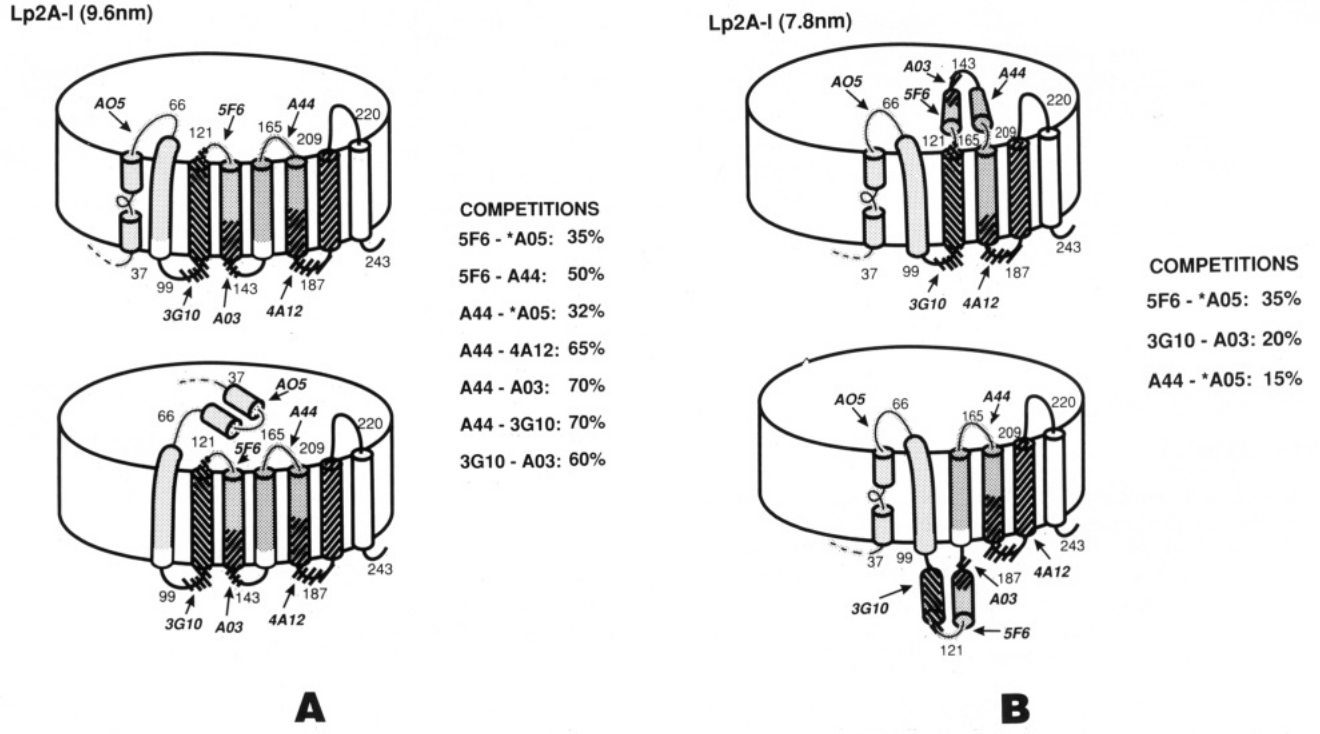

FIGURE 4: Theoretical structure of apoA-I bound to discoidal particles including the proposed hinged domains. The different amphipathic α-helices (represented by cylinders) run parallel to the axis of the disk and antiparallel to each other and are linked by coiled regions and β-turns (represented by curvilinear tracing). Panel A (top) represents apoA-I bound to lipids with eight helices as in Lp2A-I (9.6 nm). Panel A (bottom) represents an intermediate structure of apoA-I in Lp2A-I (9.6 nm) with seven helices bound to the edge of the disk and residues 37–66 located on the top of the disk. Panel B represents two versions of apoA-I in Lp2A-I (7.8 nm) bound to the edge of the disk with six helices and with an alternative hinged domain located either between residues 121 and 165 or between residues 99 and 143. In each case, the hinged domain is pictured as extended far away from the disk's edge for clarity reasons only. In each panel, the location of each epitope is identified by a shaded or hatched area, and the overlap between these areas represents the overlap between certain epitopes. On the side of each panel, a summary of the competitions between pairs of mAbs reacting either with the 9.6-nm or with the 7.8-nm Lp2A-I particles is presented. These summary values represent the average of the competitions observed with either mAb labeled (with the exception of A05). As such, the values may differ from those in Table IV.

tinuous epitope in the N-terminal region, which is negatively correlated to phospholipid concentration in HDL (Collet et al., 1991) but positively in discoidal LpA-I. This observation suggests that the significantly different spatial arrangement of apoA-I in relation to lipids which must exist in discoidal versus spherical particles also introduces different conformations in the protein. Other studies which demonstrated that the charge of apoA-I differs in discoidal versus spherical particles (Sparks & Philips, 1992) also support the concept of a different structure for apoA-I in these two types of lipid association.

The competition studies, conducted in order to better understand the spatial relationship between epitopes in the central region of apoA-I, also support the concept of a mobile domain which enables apoA-I to assume different conformations. The competition between the analyzed mAbs is greater in the large Lp2A-I and Lp3A-I particles (9.6 and 13.4 nm, respectively) than in the smaller counterparts (7.8 and 10.8 nm). If the conformation of apoA-I remained the same in the two particles, a greater competition (i.e., greater steric hindrance) would be expected in the small particles as a result of a tighter packing of the chain. Since it is not the case, a different folding of the protein has to exist in the two different size particles which results in a different orientation of the epitopes and in turn of the mAbs bound to these epitopes. This provides direct evidence for alternative conformations of the central domain.

On the basis of competitions between pairs of antibodies binding to the large LpA-I species, several models of apoA-I conformation can be considered. The classic model (Jonas et al., 1989; Brasseur et al., 1990a,b) in which the amphipathic α-helices of apoA-I are bound to the edge of the disk with their axes parallel to the main axis of the disk is represented in Figure 4A (top). This model is compatible with most of the competitions observed here, with the exception of that between mAbs A05 and A44, which react with epitopes separated by two helices. A better fit of the model with the competition data can be achieved if we consider a model where part of the sequence constituting the epitope for mAbs A05 is present on the top side of the disk (Figure 4A, bottom).

We have illustrated in Figure 4B (top and bottom) two models representing the hypothesis of a hinged region in the center of apoA-I allowing a pair of adjacent α-helices, either between residues 99 and 143 (Figure 4B, bottom) or between residues 121 and 165 (Figure 4B, top), to be free of the lipid surface and exposed to the aqueous milieu. The model in Figure 4B (top) would explain satisfactorily the remaining competitions between A05 and either 5F6 or A44, but to account for the competition between 3G10 and A03, the pair of mobile helices cannot be extended very far away from the edge of the disk. The model in Figure 4D (bottom) explains satisfactorily the competition between 3G10 and A03 and between A05 and A44, and can be compatible with the competition between 5F6 and A05.

In conclusion, the competition studies are informative for the structure of apoA-I on the large LpA-I, but not on their small counterparts. However, they do show that the apoA-I conformation cannot be the same on both types of particles and that alternate structures exist for apoA-I on discoidal lipoproteins. This is compatible with earlier evidence that apoA-I denaturation curves are indicative of a loosely bound and flexible structure and that there may be a reversible

unfolding of lipid-bound apoA-I (Reijngoud & Phillips, 1984). The existence of these alternate structures for apoA-I may be important for its role in cholesterol transport, whether in cellular efflux (Castro & Fielding, 1988) or in cholesterol esterification (Meng et al., 1993). If the classic model of apoA-I bound to discoidal particles where the amphipathic α-helices are parallel to the disk's axis is correct, then the competition data presented here support the concept of hinged domains constituted by pairs of α-helices.

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REFERENCES

Andrews, A. L., Atkinson, D., Barratt, M., Finer, E. G., Hauser, H., Henry, R., Leslie, R. B., Owens, N. L., Phillips, M. C., & Robertson, R. N. (1976) *Eur. J. Biochem. 64*, 549–563.

Boguski, M. S., Freeman, M., Elshourbagy, N. A., Taylor, J. M., & Gordon, J. I. (1986) *J. Lipid Res. 27*, 1011–1034.

Brasseur, R., De Meutteur, J., Vanloo, B., Goormaghtigh, E., Ruysschaert, J. M., & Rosseneu, M. (1990a) *Biochim. Biophys. Acta 1043*, 245–252.

Brasseur, R., De Meutteur, J., Vanloo, B., Goormaghtigh, E., Ruysschaert, J.-M., & Rosseneu, M. (1990b) *J. Biol. Chem. 265*, 22123–22129.

Breiter, D. R., Kanost, M. R., Benning, M. M., Wesenberg, G., Kaw, J. H., Wells, M. A., Rayment, I., & Holden, H. M. (1991) *Biochemistry 30*, 603–608.

Brewer, H. B., Jr., Roonan, R., Meng, M., & Bishop, C. (1986) *Methods in Enzymol. 128*, 223–235.

Brouillette, C. G., Jones, J. L., Ng, T. C., Kercret, H., Chung, B. H., & Segrest, J. P. (1984) *Biochemistry 23*, 359–367.

Castro, G. R., & Fielding, C. J. (1988) *Biochemistry 27*, 25–29.

Cheung, M. C., & Albers, J. J. (1984) *J. Biol. Chem. 259*, 12201–12209.

Cheung, M. C., Segrest, J. P., Albers, J. J., Cone, J. T., Brouillette, C. G., Chung, B. H., Kashyap, M., Glasscock, M. A., & Anantharamaiah, G. M. (1987) *J. Lipid Res. 28*, 913–929.

Collet, X., Perret, B., Simard, G., Raffai, E., & Marcel, Y. L. (1991) *J. Biol. Chem. 266*, 9145–9152.

Curtiss, L. K., & Edgington, T. S. (1985) *J. Biol. Chem. 260*, 2982–2993.

Hara, H., & Yokoyama, S. (1991) *J. Biol. Chem. 266*, 3080–3086.

Hara, H., & Yokoyama, S. (1992) *Biochemistry 31*, 2040–2046.

Havel, R. J., Eder, H. A., & Bragdon, J. H. (1955) *J. Clin. Invest. 34*, 1345–1354.

Jonas, A. (1991) *Biochim. Biophys. Acta 1084*, 205–220.

Jonas, A., Kezdy, K. E., Williams, M. I., & Rye, K.-A. (1988) *J. Lipid Res. 29*, 1349–1357.

Jonas, A., Kezdy, K. E., & Wald, J. H. (1989) *J. Biol. Chem. 264*, 4818–4824.

Jonas, A., Wald, J. H., Toohill, K. L. H., Krul, E. S., & Kezdy, K. E. (1990) *J. Biol. Chem. 265*, 22123–22129.

Karathanasis, S. K., Zannis, V. I., & Breslow, J. L. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 6147–6151.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Marcel, Y. L., Jewer, C., Vezina, C., Milthrop, P., & Weech, P. K. (1987) *J. Lipid Res. 28*, 768–777.

Marcel, Y. L., Provost, P. R., Koa, H., Raffai, E., Dac, N. V., Fruchart, J. C., & Rassart, E. (1991) *J. Biol. Chem. 266*, 3644–3653.

Matz, C. E., & Jonas, A. (1982) *J. Biol. Chem. 257*, 4535–4540.

McLachlan, A. D. (1977) *Nature 267*, 465–466.

Meng, Q. H., Calabresi, L., Fruchart, J. C., & Marcel, Y. L. (1993) *J. Biol. Chem.* (in press).

Milne, R., Gutkowska, J., Thibault, G., Schiller, P., Charbonneau, C., Genest, J., & Cantin, M. (1987) *Mol. Immunol. 24*, 127–132.

Milthrop, P., Weech, P. K., Milne, R. W., & Marcel, Y. L. (1986) in *Human Apolipoprotein Mutants* (Sirtori, C. R., Nichols, A. V., & Franceschini, G., Eds.) pp 103–117, Plenum Publishing Corporation, New York.

Morrisett, J. D., Jackson, R. L., & Gotto, A. M., Jr. (1977) *Biochim. Biophys. Acta 472*, 93–133.

Nichols, A. V., Gong, E. L., Blanche, P. J., & Forte, T. M. (1983) *Biochim. Biophys. Acta 750*, 353–364.

Nichols, A. V., Krauss, R. M., & Musliner, T. A. (1986) *Methods Enzymol. 128*, 417–431.

Oram, J. F., Albers, J. J., Cheung, M. C., & Bierman, E. L. (1981) *J. Biol. Chem. 256*, 8348–8353.

Petit, E., Ayrault-Jarrier, M., Pastier, D., Robin, H., Polonovki, J., Aragon, I., Hervaud, E., & Pau, B. (1987) *Biochim. Biophys. Acta 919*, 287–296.

Reijngoud, D.-J., & Phillips, M. C. (1984) *Biochemistry 23*, 726–734.

Roschlau, P., Bernt, E., & Gruber, W. (1974) *Z. Klin. Biochem. 12*, 403–407.

Ryan, R. O., Yokoyama, S., Liu, H., Czarnecka, H., Oikawa, K., & Kay, C. M. (1992) *Biochemistry 31*, 4509–4514.

Schonfeld, G., Bredshaw, R. A., & Chen, J. S. (1976) *J. Biol. Chem. 251*, 3921–3926.

Schonfeld, G., Chen, J. S., & Roy, R. G. (1977) *J. Biol. Chem. 252*, 6655–6659.

Segrest, J. P., Jackson, R. L., Morrisett, J. D., & Gotto, A. M., Jr. (1974) *FEBS Lett. 38*, 247–258.

Segrest, J. P., Jones, M. K., De Loof, H., Brouillette, C. G., Venkatachaiapathi, Y. V., & Anantharamaiah, G. M. (1992) *J. Lipid Res. 33*, 141–166.

Shapiro, J. P., Law, J. H., & Wells, M. A. (1988) *Annu. Rev. Entomol. 33*, 297–318.

Sparks, D. L., & Philips, M. C. (1992) *J. Lipid Res. 33*, 123–130.

Swaney, J. B., & O'Brien, K. (1978) *J. Biol. Chem. 253*, 7069–7077.

Takayama, M., Itoh, S., Nagasaki, T., & Tanimizu, I. (1977) *Clin. Chim. Acta 79*, 93–96.

Tall, A. R., Shipley, G. G., & Small, D. M. (1976) *J. Biol. Chem. 252*, 2200–2205.

Vitello, L. B., & Scanu, A. M. (1976) *J. Biol. Chem. 251*, 1131–1136.

Wald, J. H., Krul, E. S., & Jonas, A. (1990) *J. Biol. Chem. 265*, 20037–20043.

Wilson, C., Wardell, M. R., Weisgraber, K. H., Mahley, R. W., & Agard, D. A. (1991) *Science 252*, 1817–1822.