A Comparison of Structure and Thermal Behavior in Human Plasma Lipoprotein(a) and Low-Density Lipoprotein. Calorimetry and Small-Angle X-ray Scattering†

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ABSTRACT: Differential scanning calorimetry (DSC) and small-angle X-ray scattering (SAXS) studies have been performed to investigate the structural properties of lipoprotein(a) [Lp(a)] and low-density lipoprotein (LDL) obtained from the same donor. In addition, a comparison was made between autologous LDL and the remnant particle Lp(a−) obtained by removal of apo(a) through chemical reduction. With Lp(a), three distinct thermal transitions have been observed: the first one around 20 °C, arising from the core-located apolar lipids, similar to LDL but with a significantly lower melting temperature as compared to LDL of the same donor. The second one, at 55.7 ± 0.25 °C, can be attributed to apo(a), since it was found to be absent in Lp(a−) and LDL, whereas isolated apo(a) in aqueous solution exhibited a similar transition. The third transition, at 80.4 ± 0.9 °C, corresponds to apo-B100 protein unfolding. The low melting temperature of the core lipids in Lp(a) is preserved in Lp(a−); this suggests that the apolar lipid interactions are unaffected by apo(a) binding, and that the difference in the core melting behavior between Lp(a) and LDL is due to a different stabilization through interaction between the apolar core and the surface monolayer lipids. SAXS curves exhibited qualitatively the same characteristic features for LDL, Lp(a), and Lp(a−). Thus, the SAXS results showed that no major deviations from spherical particle shape occur with Lp(a), indicating that apo(a) wraps around the particle surface without major globular protrusions into the aqueous surrounding. Overall, the calorimetric and X-ray results show that LDL and Lp(a−) are similar but not identical in structure and thermal stability, which may be of metabolical interest.

Low-density lipoprotein (LDL)[1] and lipoprotein(a) [Lp(a)] have been found to be two independent risk factors for coronary heart disease (Goldstein et al., 1977; Dahlen et al., 1988); therefore, special attention has been paid to these lipoprotein classes (Steinberg et al., 1981; Scanu et al., 1990). Structurally, LDL is a species of quasi-spherical particles consisting of a central core of apolar lipids, mainly cholesteryl esters and triglycerides, stabilized by an external shell of polar lipids, i.e., phospholipids and cholesterol and one single polypeptide chain, apolipoprotein-B100 [Laggner et al., 1976; Deckelbaum et al., 1977; Müller et al., 1978; for a review, see Kostner and Laggner (1989)].

The composition of Lp(a) is essentially that of a LDL particle with a second polypeptide, apo(a), which is covalently linked by a disulfide bond to apo-B100 (Utermann et al., 1983; Gaubatz et al., 1983; Sommer et al., 1991). This additional protein conveys to Lp(a) distinct immunochemical and physicochemical properties (Armstrong et al., 1989). Apo(a) is a high molecular glycoprotein with about 30% carbohydrate content and a remarkable heterogeneity in size. Several molecular weight isoforms have been described with masses ranging from 200 to 700 kDa depending on the donor (Utermann, 1989). Apo(a) can be easily removed by chemical reduction (Armstrong et al., 1985; Fless et al., 1985). The resulting lipoprotein remnant, designated as Lp(a−), contains apo-B100 as its sole protein and is electrophoretically and immunologically similar but not identical to autologous LDL (Fless et al., 1986). For instance, Fless et al. (1986) report that Lp(a−) particles are distinctly larger than their respective autologous LDL. In comparison to what is known about the structure of LDL and the underlying molecular interactions, the knowledge of Lp(a) is still poorly developed and, therefore, any structure-function relationships defining the differences in atherogenicity are at this stage speculative.

The aim of the present study is to better define structural similarities and differences between LDL, Lp(a), and Lp(a−) regarding the size and shape of the particles, their temperature-induced alterations in the core lipid organization, and the conformational stability of the protein moiety at the particle surface. In this context, in native LDL a reversible thermotropic order−disorder transition of the core components is observed at temperatures between 15 and 40 °C (Deckelbaum et al., 1975, 1977; Laggner et al., 1977). However, apart from a broad knowledge on the biochemical and genetic properties of Lp(a) and apo(a), little is known

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[1] Abbreviations: LDL, low-density lipoprotein; Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); apo-B100, apolipoprotein-B100; Lp(a−), remnant lipoprotein particle obtained upon reduction and subsequent removal of apo(a) from Lp(a); LDL(−), low-density lipoprotein treated with dithiothreitol for control experiments; DTT, dithiothreitol; SAXS, small-angle X-ray scattering; DSC, differential scanning calorimetry; T_m, midpoint temperature of calorimetric transitions; CE, cholesteryl ester; TG, triglyceride.

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about the temperature-dependent phase behavior of lipids in Lp(a).

In this work, we show that Lp(a) exhibits a similar order−disorder transition of cholesteryl esters in the core, as reported for LDL, but with a strikingly lower melting temperature (T_m) and a significantly higher enthalpy as compared to autologous LDL. The difference in T_m is still conserved in Lp(a−) after reductive removal of apo(a).

Additionally, we report on the thermotropic behavior of the polypeptide apo(a) in aqueous solution and within native Lp(a), and on the thermal unfolding and denaturation of apo-B100. The current study is a combination of two temperature-sensitive methods, i.e., differential scanning calorimetry and small-angle X-ray scattering, to obtain information both on the thermal properties and on the structural characteristics.

MATERIALS AND METHODS

Isolation of Lipoproteins. LDL and Lp(a) were isolated from fasting normolipidemic male donors aged <35 years as described earlier (Steyrer & Kostner, 1990; Kostner & Grillhofer, 1994). In brief, freshly drawn blood was allowed to clot at room temperature for 30 min, centrifuged for 15 min at 4000*g* to separate serum from blood clots, and immediately fractionated by ultracentrifugation. LDL was prepared at a density of 1.025−1.055 g/mL and washed twice by recentrifugation. Lp(a) was isolated at a density of 1.063−1.125 g/mL followed by column chromatography using Biogel A-5m. Both fractions were electrophoretically homogeneous and >97% pure as judged by SDS−polyacrylamide gel electrophoresis and immunochemistry. Finally, all samples were dialyzed against degassed working buffer containing 10 mM phosphate, pH 7.2, 0.9% (w/v) NaCl, 0.01% (w/v) EDTA, and 50 μg/mL gentamicin base and stored under argon (Argon 5.0; $\leq 2 \times 10^{-4}$ vol % O_2; Alpenländische Sauerstoffwerke, Graz, Austria) at 4 °C in the dark.

Dissociation of Apo(a) from Lp(a). In order to prepare Lp(a−), apo(a) was dissociated from Lp(a) by incubation with dithiothreitol (DTT) followed by heparin−Sepharose column chromatography as described by Armstrong et al. (1985). For control, LDL was treated in an identical manner as Lp(a) in order to account for possible alterations in conformation of apo-B100 caused by chemical reduction of existing disulfide bonds.

Chemical Analysis. The chemical composition of the lipoprotein fractions was estimated using commercially available enzymatic kits. Free and total cholesterol were determined using the Boehringer (Mannheim, Germany) assay kits based on the methods of Röschlau et al. (1974) and Siedel et al. (1983), respectively. The cholesteryl ester content was calculated from the difference between the total and free cholesterol content using an average molecular weight of 650 for cholesteryl esters. Phospholipids and triglycerides were estimated according to the procedures of Takayama et al. (1977) and Fossati and Prencipe (1982), respectively, using the assay kits of BioMerieux (Marcy L'Etoile, France). Protein was measured by the bicinchoninic acid assay (Pierce, Netherlands) as described by Smith et al. (1985), using bovine serum albumin as standard.

Differential Scanning Calorimetry (DSC). Calorimetry experiments were performed in a high-sensitivity, adiabatic DASM-4 calorimeter (Biobribor, Pushtchino, Russia) designed by Privalov et al. (1975) at a heating rate of 1 °C/min. To avoid air bubble formation or vaporization of solvent during high-temperature heating, the cells were kept under about 2 bar nitrogen pressure. The calorimeter was calibrated by the internal electric power signal and connected via an electronic interface to a personal computer for automatic data collection. Heat capacity functions were obtained after subtraction of the buffer base line and mass normalization of experimental data. Prior to use, the samples were diluted with working buffer to a final protein concentration of about 1 mg/mL, and the working buffer was used to fill the reference cell.

Table 1: Chemical Composition[a] of LDL, Lp(a), LDL(−), and Lp(a−)

	samples					
component	LDL#1[b]	Lp(a)#1	LDL#2	Lp(a)#2	LDL(−)#2	Lp(a−)#2
cholesteryl ester	41.6	36.6	40.9	35.3	39.4[c]	34.7[c]
free cholesterol	9.9	8.5	8.9	8.1	9.0	9.3
triglyceride	5.5	6.1	5.3	8.1	5.3	9.5
phospholipid	23.2	20.2	21.3	20.6	21.8	21.8
protein	19.8	28.6	23.6	27.9	24.5	24.7

[a] Relative percent (w/w). [b] The numbers 1 and 2 refer to different donors. [c] For LDL(−) and Lp(a−), the compositional data were determined after treatment with dithiothreitol.

Small-Angle X-ray Scattering (SAXS). Small-angle X-ray scattering was measured by an integrated MBG-SAX Camera system (MBraunGraz-Optical Systems, Graz, Austria), based upon the Kratky line collimator, equipped with a position-sensitive detector, a semitransparent beam-stop and monitor for on-line measurement of the primary beam, and an automatic temperature/time programmer and data collection unit. The camera was operated at a rotating-anode generator (RU-200B; Rigaku Denki, Japan) with Ni-filtered Cu-radiation (wavelength 0.154 nm). The lipoprotein concentration was routinely 10−20 mg/mL, and the exposure time was 8 h. Even with prolonged irradiation, no signs of a drift in the scattering patterns have been observed, indicating that eventually occurring radiation damage has no influence on the morphology. The measurements were performed at 10 and 40 °C, respectively. All scattering curves were normalized to integral primary beam intensity, buffer background-corrected, and normalized to total lipoprotein concentration. Desmearing of the data obtained with slit-collimation was performed by using the ITP program (Glatter, 1977) as described earlier (Müller et al., 1978).

RESULTS

Chemical Composition. The compositional data of the lipoproteins used in this study are listed in Table 1. There are no significant differences in the lipid composition between LDL and Lp(a) except for an increase in triglyceride relative to cholesteryl ester content in Lp(a) and Lp(a−), respectively, which is known to be important with respect to the transition temperature of the lipid core (Deckelbaum et al., 1977). This difference is expressed in a molar ratio of CE to TG of 10.0 ± 0.1 and 6.8 ± 1.1 for LDL and Lp(a), respectively, and is in accordance with findings reported by Fless et al. (1986). The compositional results are in good agreement with published data (Fless et al., 1984; Sattler et al., 1991).

Calorimetry. For differential scanning calorimetry, lipoprotein preparations from two different single donors have

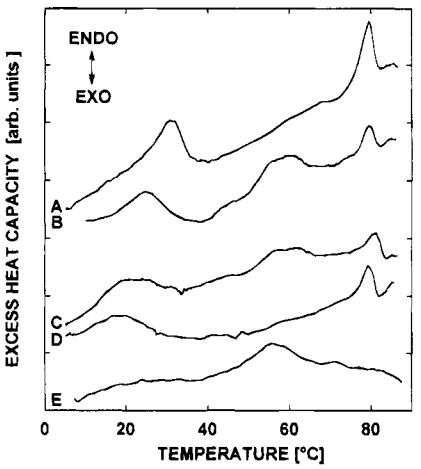

FIGURE 1: Excess heat capacity versus temperature functions for LDL#1 (A), Lp(a)#1 (B), Lp(a)#2 (C), Lp(a−) (D), and apo(a) (E). The numbers 1 and 2 refer to different donors. The heating rate was 1 °C/min. For all samples, the protein concentration was about 1 mg/mL. Buffer base lines were subtracted. The data are not normalized to the total lipoprotein concentration; for quantitative analyses, see Table 2.

been used and referred to as samples #1 and #2, respectively. The isoforms of apo(a) according to Utermann (Seed *et al.*, 1990) were determined to be S-1 for Lp(a)#1 and S-1/B in a ratio of 1:1 for Lp(a)#2. From Lp(a)#2, apo(a) was removed by chemical reduction, and the apo(a)-free Lp(a) remnant, designated as Lp(a−), was compared to autologous LDL and parent Lp(a). As a control, LDL was treated in the same manner and referred to as LDL(−). Finally, we investigated the thermal-induced changes of the reduced protein apo(a) in aqueous solution. Representative calorimetric curves for the preparations are shown in Figure 1. The differential scanning thermograms of LDL and Lp(a) show that the particles undergo a series of thermal transitions. For LDL, two endothermic transitions are observed, while for Lp(a) three peaks can be distinguished. Upon heating from 0 to 40 °C, both LDL and Lp(a) exhibit one endothermic transition, which is reversible on second heating to 40 °C. For LDL, this transition corresponds to the well-characterized order−disorder transition of the core-located apolar lipids (CE and TG) from a liquid-crystalline to a liquid-like phase (Deckelbaum et al., 1977). Considering the compositional similarities between LDL and Lp(a), the transition observed within Lp(a) can be also attributed to the melting of the core lipids. In both series, it has been found that the thermotropic core transition temperature (T_m), which is defined by the point of maximum excess heat capacity, is lower in Lp(a) than in LDL of the same donor (Table 2). This decrease was found to be $\Delta T_m = 6$ and 10.6 °C, for Lp(a)#1 and Lp(a)#2, respectively. The thermodynamic parameters calculated from calorimetric experiments are summarized in Table 2. The corresponding calorimetric enthalpies (ΔH_{cal}) were calculated by integrating the peak areas. There are some inaccuracies concerning the absolute values caused by the uncertainty in the base line setting in the DSC thermograms, in particular at the low-temperature side. Therefore, the values represent the average of several heating scans with an experimental error within ±10%. The ΔH_{cal} of 2.65 ± 0.07 kJ/kg of CE for LDL is in good agreement with the value of 2.89 ± 0.25 kJ/kg of CE as reported by Deckelbaum et al. (1977), while for Lp(a) the calorimetric enthalpy for the core melting is significantly higher (5.35 ± 0.9 kJ/kg of CE). After chemical reduction, i.e., splitting off of apo(a), the transition temperature for the core melting was found to remain constant for both Lp(a−) and control LDL(−), leading to the conclusion that the chemical treatment does not alter the phase behavior of the core lipids. Moreover, it is clearly shown that the additional protein apo(a) is not responsible for the decrease in the core melting temperature. On the other hand, the calorimetric enthalpy seems to be affected by chemical reduction; i.e., a significant decrease for Lp(a−) as compared to parent Lp(a) is observed; ΔH_{cal} is now comparable to native LDL.

Upon heating to about 65 °C, no additonal transition is observed in native LDL, while for Lp(a) a broad, endothermic transition is detected with a T_m of about 56 °C. The fact that this transition is not present in Lp(a−) leads to the notion that this transition can be undoubtely attributed to apo(a) bound to apo-B100. Taking into account the molecular weights of the apo(a) isoforms [according to Utermann (1989), the molecular masses are about 520 kDa for isoform S-1 and about 460 kDa for isoform B, respectively], a calorimetric enthalpy of 8.1 ± 1.5 kJ/kg could be obtained for apo(a) bound to apo-B100. The results are in good agreement with a value of ΔH_{cal} = 8.8 kJ/kg which we observed for isolated apo(a) in aqueous solution.

It is known that apo(a) shows a high homology to plasminogen with respect to the kringle-4 domain (Eaton et al., 1987; Kratzin et al., 1987; McLean et al., 1987). Each kringle contains six Cys residues forming three internal disulfide bonds, thus stabilizing the characteristic triple-loop structure. It should be mentioned, therefore, that treatment with reducing agents may result in the loss of kringle structure by cleavage of disulfide bonds. Nevertheless, upon heating from 0 to 90 °C, only one single endothermic transition could be observed in isolated apo(a) with a T_m of about 54 °C and the above-mentioned enthalpy of about 8.8 kJ/kg. Repeated heating of the scanned sample showed that the transition peak almost disappears (data not shown), suggesting that the unfolding process of apo(a) is irreversible.

In both lipoproteins, an irreversible transition at a temperature of about 80 °C is observed, which can be associated with irreversible protein unfolding and denaturation (Deckelbaum et al., 1977; Walsh et al., 1990). This coincides with particle aggregation, as clearly visible in cloudy precipitation. While exhibiting almost the same transition temperature, there are differences in the calorimetric enthalpies, namely, H_{cal} = 2.55 ± 0.07 kJ/kg of apo-B100 and ΔH_{cal} = 1.38 ± 0.04 kJ/kg of apo-B100 + apo(a) in LDL and Lp(a), respectively. Comparing the widths and shapes of these high-temperature transitions, they seem to be identical within experimental limits. This leads us to the assumption that in Lp(a) as well as in LDL only apo-B100 is involved in this transition with similar denaturation enthalpies for LDL and Lp(a), respectively (see Table 2). This confirms that solely apo-B100 contributes to the high-temperature transition. In support of this conclusion, the thermograms of isolated apo(a) showed no transition at this temperature range.

Regarding the heat capacity functions of LDL(−) and Lp(a−), it should be noted, that treatment with DTT may cause some cleavage of disulfide bonds within apo-B100 and therefore alter the protein conformation. However, this effect on the denaturation temperature of apo-B100 is relatively small; i.e., a decrease in T_m by about 1−2 °C was observed in both fractions, while the calorimetric enthalpy is only half

Table 2: Calorimetric Parameters of Lipoprotein Samples

sample	T_m(CE) (°C)	ΔH_{cal}[b] (kJ/mol of CE)	ΔH_{cal} (kJ/kg of CE)	ΔH_{vH} (kJ/mol of CE)	$\Delta H_{vH}/\Delta H_{cal}$	T_m (a)[c] (°C)	ΔH_{cal} [kJ/kg of apo(a)]	T_m (b)[c] (°C)	ΔH_{cal} (kJ/kg of apo-B100)
LDL#1[a]	30.7	1.7	2.6	339	199			79.6	2.6
Lp(a)#1	24.7	3.0	4.6	268	89	55.5	9.2	79.5	2.7
LDL#2	29.3	1.8	2.7	414	230			80.3	2.5
Lp(a)#2	18.7	4.0	6.1	184	46	56.0	7.1	81.3	2.7
LDL(−)#2	29.3	2.4	3.7	280	117			79.3	1.5
Lp(a−)#2	18.6	1.8	2.8	218	121			79.3	1.6
apo(a)						54.4	8.8		

[a] The numbers 1 and 2 refer to different donors. [b] The values are the average of several heatings; the experimental error is within ±10%. A mean molecular weight of 650 was used for CE. [c] T_m(a) refers to the first protein peak, observed in Lp(a), while T_m(b) corresponds to the denaturation peak at high temperatures.

the value found for native LDL and Lp(a), indicating that as a consequence of chemical reduction at least parts of the protein are already unfolded and changes of intramolecular interactions have occurred. As judged by immunoblotting of DTT-treated LDL with anti-apo-B100, Zawadzki et al. (1988) showed that DTT treatment has no effect on the immunoreactivity of LDL apo-B100. The same group reported that Lp(a) is a poorer competitor for the LDL receptor than labeled LDL independent of the apo(a) isoform and that after removal of apo(a) the uptake by the LDL receptor is improved which makes Lp(a−) an equally efficient ligand as LDL.

Small-Angle X-ray Scattering. Several X-ray studies have been reported providing information about the overall particle dimensions and the internal structural organization of LDL, indicating quasi-spherical particles with diameters between 22 and 25 nm (Laggner et al., 1976, 1977; Laggner & Müller, 1978; Müller et al., 1978; Luzzati et al., 1979; Baumstark et al., 1990). It was shown that the core melting around 30 °C is reflected in changes of the small-angle X-ray scattering pattern (Deckelbaum et al., 1975, 1977; Atkinson et al., 1977; Laggner et al., 1977; Laggner & Müller, 1978): mainly, the characteristic maximum around $h = 1.7$ nm^{-1} observed below the core transition temperature disappears above the transition temperature. In the present paper, we give the first report on a comparative structural X-ray study of Lp(a) and LDL, respectively. Since the details of SAXS data evaluation and interpretation from LDL have been given elsewhere (Laggner & Müller, 1978; Müller et al., 1978), the presentation will be focused only on a comparison of the results.

Figure 2A and Figure 2B show the scattering curves of LDL and Lp(a) at 10 and 40°C, respectively. The scattering curves were measured in the angular range 0.1 nm^{-1} < h < 2 nm^{-1} [h = $(4\pi \sin \theta)/\lambda$, 2θ = scattering angle, $\lambda = 0.154$ nm]. Both curves exhibit qualitatively very similar characteristics with regard to the existence of well-resolved maxima and minima in the scattering function. The angular positions of the maxima of Lp(a) are shifted to slightly lower angles as compared to LDL, indicating a larger particle size for Lp(a). The pronounced maximum around $h = 1.7$ nm^{-1} (arrow in Figure 2A) at 10 °C was found at the same angular position in both samples and disappeared above the transition temperature. The determination of the innermost part of the scattering curve, especially at 40 °C, becomes difficult, because the central scattering maximum could not be detected owing to the low contrast. In Figure 2C and Figure 2D, the desmeared scattering curves are shown for the reduced samples LDL(−) and Lp(a−) at 10 and 40 °C, respectively, indicating that the basic scattering features are largely

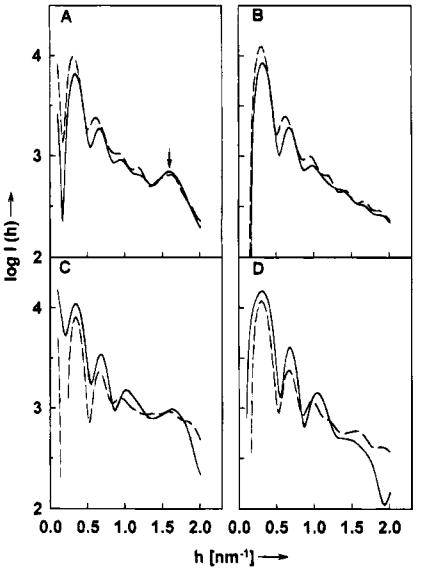

FIGURE 2: Desmeared small-angle X-ray scattering curves for LDL (—) and Lp(a) (— —). (A, B) At 10 and 40 °C, respectively. In (C) and (D), LDL(−) (—) and Lp(a−) (— —) at 10 and 40 °C are shown, respectively. The arrow in (A) indicates the pronounced maximum around $h = 1.7$ nm^{-1}.

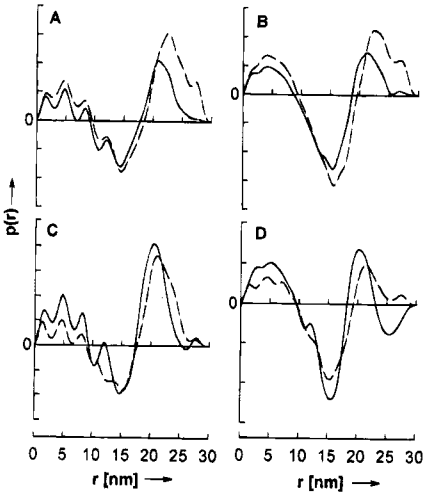

FIGURE 3: Electron-pair distance distribution functions $p(r)$ are shown, corresponding to the scattering curves displayed in Figure 2. In the present calculations a maximum fitting dimension D_{max} of 30 nm and 25 spline functions were used.

unaffected by chemical treatment. The corresponding electron-pair distance distribution functions, $p(r)$, obtained by indirect Fourier transformation (Glatter, 1977) are plotted in Figure 3. The $p(r)$ function contains information about the internal electron density distribution, and the maximum particle

diameter is defined as the distance r_{max} where $p(r)$ approaches zero. The suitability of the $p(r)$ function to obtain structural information for LDL was discussed in detail by Müller et al. (1978). The determination of the exact value of r_{max} was difficult due to the poorly defined intercept with the abscissa toward large distances r, as a consequence of the low accuracy in $I(h)$ at very low angles ($h < 0.2$ nm^{-1}). It is evident, nevertheless, that Lp(a) exhibits a larger size with a distinct shoulder at $r > 25$ nm. When the peak maximum located around $r = 20$ nm, which corresponds to the electron density autocorrelation of the phospholipid headgroups and the protein moiety, is investigated a difference of about 2 nm can be estimated by comparing LDL to Lp(a). After chemical reduction, Lp(a−) was found to be smaller in size due to the loss of protein mass at the surface, but it should be mentioned that Lp(a−) is still about 0.5−1 nm larger than control LDL(−) treated in the same manner.

All curves measured below the transition temperature showed a number of well-pronounced submaxima with a periodicity of 3.7 nm corresponding to the autocorrelation of the concentric shell architecture of the lipid core. The submaxima are transformed into broad shoulders above the melting temperature; only some remaining traces of this regularity are still present in Lp(a−) at 40 °C. The closely similar curves for LDL and Lp(a) for r values up to 15 nm lead to the conclusion that apo(a) present in Lp(a) has no influence on the structural regularity of the core.

DISCUSSION

A central task of the present study was to identify the structural relationships between LDL and Lp(a) isolated from the same donor with special attention being paid to the thermal behavior of the particle. We applied the method of differential scanning calorimetry to gain insight into both the melting behavior of the core lipids as well as the thermal stability of the protein moieties present at the particle surface. In parallel, we used SAXS to obtain information about the supramolecular arrangement. In general, the results of our investigations confirmed the LDL-like structure and thermal behavior of Lp(a) but with distinct differences, which were partially still present after reductive removal of apo(a).

When LDL and Lp(a) from the same donor were compared, a significantly lower transition temperature of the apolar lipids in the core of Lp(a) was observed, indicating that the ordered lipid arrangement within Lp(a) is thermally less stable than in LDL. Furthermore, a broadening of the transition, characterized by a significant increase of the transition half-width, indicates a loss of cooperativity. This decrease in cooperativity can be estimated from the ratio of the van't Hoff enthalpy (ΔH_{vH}) to the calorimetric enthalpy (Sturtevant et al., 1974) using the standard equation for calculating ΔH_{vH} from the calorimetric curves:

$$H_{vH} = 4RT_m^2 \Delta C_{p,max}/\Delta H_{cal}$$

where $\Delta C_{p,max}$ is the maximum excess heat capacity and R is the gas constant. A ratio greater than 1 indicates that clusters of molecules are undergoing a cooperative transition. For native LDL, we found an average size for the cooperative unit of about 200 molecules, whereas for Lp(a) the number of molecules building up the cooperative unit is significantly lower (about 65 molecules). In the literature (Deckelbaum et al., 1975), a minimum domain size of about 50−100 molecules has been reported for intact LDL, possibly reflecting the heterogeneity in size and chemical composition of LDL. However, it should be emphasized that there are distinct differences between LDL and Lp(a) from the same donor.

For a reversible equilibrium process where the Gibbs free energy change at the transition temperature is zero, ΔS is equal to $\Delta H/T$, where ΔS is the change in entropy during the transition. Our results indicate a much higher entropy change for native Lp(a) than for all other samples, suggesting that within Lp(a) the cholesteryl esters are less motionally constrained above the transition or that they are more constrained below the transition temperature. This may be a consequence of more pronounced interaction forces between the inner core and the outer phospholipid monolayer within Lp(a) which are released above T_m. In support of such an interpretation, it is recalled that influences of the core transition on the surface phospholipid monolayer are known to exist within LDL (Laggner & Kostner, 1978; Fenske et al., 1990; Vauhkonen & Somerharju, 1990). Furthermore, a reduced phospholipid side chain mobility was found for Lp(a), which was attributed to the presence of apo(a) (Sommer et al., 1992). This phospholipid rigidization may in turn promote the core−surface interaction. This interpretation is supported by the fact that after removal of apo(a), at the same chemical composition, namely, CE:TG ratio, the enthalpy is drastically reduced, while the transition temperature is not influenced.

The decrease in the core melting temperature of 6.0 and 10.6 °C for Lp(a)#1 and Lp(a)#2, respectively, as compared to autologous LDL may be caused by the higher TG content in Lp(a). Triglycerides are known to have a significant effect on the thermal behavior of cholesteryl esters: increasing amounts of triglycerides lower the transition temperature by disordering the quasi-crystalline packing of cholesteryl esters (Deckelbaum et al., 1977). Several studies have shown that the triglyceride content is of physiological interest by modulating the binding of LDL to the LDL receptor. For example, LDL which has been enriched in triglycerides is less efficiently taken up by cultured cells (Aviram et al., 1988). Differences in the fatty acid composition of the core lipids would influence the transition temperature (Small, 1986), but it has been shown by Sattler et al. (1991) that the distribution of the fatty acid residues is very similar in LDL and Lp(a). Regarding the role of free cholesterol, evidence has been obtained that part of the unesterified cholesterol in LDL exchanges between the core and the outer layer (Yeagle et al., 1982; Lund-Katz et al., 1986; Smutzer et al., 1988), but the precise location and molecular environment of free cholesterol are not yet well characterized. Therefore, a different partition of unesterified cholesterol between the core and the surface domains within the particles may play a decisive role in terms of influencing the order of the surface phospholipids (Kroon, 1994). To summarize, it is clear that differences in lipid composition cause different lipid organization within the core, leading to a decrease in transition temperature.

Following our results for Lp(a−), it is apparent that the additional polypeptide apo(a) located on the particle surface has no influence on the stability of the core with respect to the transition temperature, which is mainly determined by the lipid composition. On the other hand, a striking

difference in the enthalpy of the core melting was found between Lp(a), Lp(a−), and LDL. The lower enthalpies in Lp(a−) and LDL reflect a different lipid organization of the core being less affected in terms of packing constraints. That means that distinct differences in protein−lipid interaction between Lp(a) and LDL lead to a stabilization of the ordered core lipid arrangement in Lp(a). Our interpretation, which is inferring a different lipid−protein association strength in Lp(a) and LDL, respectively, is supported by the findings of Sommer et al. (1992), who reported that the interaction between outershell lipids and the hydrophobic protein segments is much stronger in the presence of apo(a).

The protein denaturation of apo-B100 exhibits a similar characteristic for LDL and Lp(a). The calorimetric enthalpies are in good agreement with values reported for native LDL (Walsh et al., 1990). This calorimetric result indicates that apo-B100 exhibits the same overall structure in both particles, which is in accordance with circular dichroism experiments. Fless et al. (1986) showed that apo-B100 has the same secondary structure in LDL and Lp(a). Furthermore, identical secondary structure was also reported for apo(a) bound to apo-B100 and in aqueous solution from the same group. This is consistent with our results that apo(a) shows similar thermodynamic properties in intact Lp(a) and in isolated form. Despite the facts that free apo(a) exhibits more than 70% random conformation as shown by circular dichroism and behaves as a flexible, extended coil, as indicated by its extremely high intrinsic viscosity (Fless et al., 1986), it shows a cooperative melting behavior with a transition temperature similar to that observed for apo(a) covalentely linked to apo-B100. A similar transition temperature of 58 ± 1 °C has been reported (Castellino et al., 1981) for the isolated kringle-4 domain in human plasminogen, which is known to be homologous to apo(a) (McLean et al., 1987; Guevara et al., 1993) and responsible for its size polymorphism (Utermann, 1989).

Most of the current knowledge on the molecular structure of LDL has been deduced from SAXS and neutron scattering studies [for reviews, see Laggner (1982) and Kostner and Laggner (1989)]. By electron microscopy, it was shown that LDL particles are spherical with diameters of about 20−30 nm (Shen et al., 1981; Granot et al., 1985; Phillips et al., 1989; Chatterton et al., 1991). Some of the uncertainties in the observed particle sizes can be explained by differences in the methods of preparation and distortions caused by drying procedures in electron microscopy studies (Chatterton et al., 1991). On the other hand, from hydrodynamic studies on distinct LDL subfractions, diameters of about 21−28 nm were found for dense to light subspecies, respectively (Chapman et al., 1988; Nigon et al., 1991; Tribble et al., 1992). All these results also reflect the heterogeneity of LDL particles. Therefore, it should be pointed out that it was not the purpose of this paper to address the absolute size determination of LDL and Lp(a) in particular, but to compare LDL, Lp(a), and Lp(a−) obtained from the same donor. The present results show that the overall quasi-spherical symmetry is maintained for Lp(a) exhibiting larger particle dimensions as compared to autologous LDL. Regarding the question of how the additional protein apo(a) is arranged, our results (Figure 2 and 3) indicate the following. Obviously, from the larger values to which the *p(r)* functions extend, the location of the apo(a) must be above the surface of the Lp(a−). Also, the spherical symmetry, as reflected by the relatively sharp maxima and minima in the *I(h)* curves, seems not dramatically perturbed, suggesting that apo(a) wraps around the particle surface without major globular protrusions into the aqueous surrounding. We suggest, therefore, that in addition to the known disulfide linkage, noncovalent interactions between apo-B100 and apo(a) have to exist to keep apo(a) in the vicinity of apo-B100. On the other hand, Lp(a−) is smaller than Lp(a) but still slightly larger than LDL(−). Similar observations were obtained by Fless et al. (1986), who reported a greater particle mass and a greater number of lipid components in Lp(a−) as compared to LDL. In this context, Galeano et al. (1994) reported that the lipid composition, namely, the TG content, has no effect either on the apo-B100 conformation or the binding to the LDL receptor in the absence of changes in particle size. The size of LDL, however, can play an important role in determining the conformation of the apo-B100 molecule near its receptor recognition site, as demonstrated for the small and dense LDL from hypertriglyceridemic individuals (Galeano et al., 1994). In the case of Lp(a), we found both an enrichment of triglycerides accompanied by an increase of particle size, which may in this way be associated with alterations in apo-B100 conformation at specific sites, thus facilitating the binding of apo(a).

With respect to the internal structure of the core lipids as determined in the present study by small-angle X-ray scattering, LDL and Lp(a) exhibit a similar arrangement of cholesteryl esters and triglycerides at low temperatures. It can be concluded that the additional protein apo(a) bound to the surface has no influence on the size of the core as deduced from the same characteristic periodicity of the maxima and minima in the *p(r)* function.

In conclusion, it can be stated that Lp(a) and LDL are structurally related to each other concerning the molecular organization of the core but with distinct differences in the thermal stability, which is preserved in Lp(a−). These findings can only partially be interpreted in terms of variations in the chemical composition and lead to the hypothesis that different interactions exist between the core lipids and the surrounding phospholipid−protein monolayer in these two lipoprotein particle species.

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