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Conference Paper

Conformation and Physical Structure of Tropoelastin from Human Vascular Cells: Influence of Cells Lipid Loading

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Aggregated low density lipoproteins (agLDL) contribute to massive intracellular cholesteryl ester (CE) accumulation in human vascular smooth muscle cells (VSMC). Our aim was to determine the conformational and physical structure of agLDL and elastic material produced either by control human VSMC or by agLDL-loaded human VSMC (agLDL-VSMC). At the conformational level scanned by FTIR spectroscopy, a new undefined, probably non-H-bonded, structure for tropoelastin produced by agLDL-VSMC is revealed. By differential scanning calorimetry, a decrease of water affinity and a drop of the glass transition associated with aggregated tropoelastin (from 200°C to 159°C) in the supernatant from agLDL VSMC are evidenced. This second phenomenon is due to an interaction between agLDL and tropoelastin as detected by the weak specific FTIR absorption band of agLDL in supernatant from agLDL-loaded VSMC.

1. Introduction

VSMC in atherosclerotic lesion are unable to produce normal elastic fibers due to atherosclerotic risk factors such as diabetes and associated hyperglycemia, endothelial dysfunction, and inflammation [1, 2]. If experimental hypercholesterolemia decreases the wall elastin content *in vivo* [3] and *in vitro* systems [4], the role of hypercholesterolemia in the altered VSMC elastogenic capacity and the possible mechanisms involved are not yet elucidated. VSMC become foam cells through the uptake of diversely modified LDLs [5, 6], whereas the aggregation of LDLs (agLDL) seems to be a key condition for lipid accumulation in VSMCs [7, 8]. Intracellular cholesterol accumulation alters proteoglycan composition [9] and collagen assembly [10] in VSMC, but it is unknown whether intracellular lipid may change the physical characteristics of the tropoelastin synthesized by human VSMC.

The aim of this work was to characterize agLDL as well as tropoelastin produced by agLDL-lipid-loaded human VSMC versus that produced by control VSMC using polymer characterization techniques that were previously shown to be efficient in checking the molecular architecture and chain dynamics of proteins [11, 12].

2. Materials and Methods

2.1. Human VSMC. Primary cultures of human VSMC were obtained from nonatherosclerotic areas of human coronary arteries from hearts explanted during heart transplantation at Hospital of Santa Creu i Sant Pau as previously described

[5, 6]. Donors of the explanted hearts were men between 40 and 60 years old.

Explants were incubated at 37°C in a humidified atmosphere of 5% CO_2. Cells grown out of explants were suspended in a solution of trypsin/EDTA and subcultured. They grew in monolayers in medium 199 supplemented with 20% fetal calf serum and 2% human serum, 2 mmol/L L-glutamine, 100 U/mL penicillin G, and 100 μg/mL streptomycin. The study was approved by the institutional ethics committee at Hospital of Santa Creu i Sant Pau and conducted in accordance with the Declaration of Helsinki.

2.2. LDL Isolation and Modification. Human LDL ($d_{1.019}$ – $-d_{1.063}$ g/mL) were obtained from pooled sera of normocholesterolemic volunteers by sequential ultracentrifugation and were <48 hours old. AgLDLs were prepared by vortexing LDLs in PBS at room temperature. The formation of LDL aggregates by vortexing was monitored by measuring the turbidimetry (absorbance at 680 nm).

2.3. AgLDL-Loaded Human VSMC. VSMC were incubated with agLDL (50 μg/mL) for 15 days. Following the lipoprotein incubation period, cell supernatants were centrifuged to remove lipids not taken up by the cells and cellular debris. The same procedure was applied to extract the supernatants from control VSMC. Samples were then freeze-dried before the physical experiments were performed.

2.4. Fourier Transform Infrared Analysis (FTIR). Fourier transform infrared spectroscopy/attenuated total reflectance (FTIR/ATR) spectra were collected using a Nicolet 5700 FTIR equipped in ATR device. Spectra were recorded over the region of 4000–450 cm^{-1} with a spectral resolution of 4 cm^{-1} and 64 accumulations. Fourier self-deconvolution (FSD) of the infrared spectra that allows resolution of several overlapping bands was performed in the amide I/II region. The decomposition of the amide I/II bands on the FSD trace was performed with a Gaussian curve fitting procedure.

2.5. Differential Scanning Calorimetry (DSC). Analyses were performed using a DSC Pyris calorimeter. The calorimeter was calibrated at low temperatures using Hg and In as standards, resulting in a temperature accuracy of ±0.1°C and an enthalpy accuracy of ±0.2 J/g. Samples, 10 mg in weight, were set into aluminium pans and equilibrated at the initial temperature for 5 minutes before the heating ramp at 20°C/min.

3. Results and Discussion

Figure 1 shows the FTIR/ATR spectra of ag-LDL, supernatant from control VSMC, and supernatant from agLDL-VSMC.

The agLDL FTIR spectrum is characterized by the H-bonded (O–H) and (N–H) stretching vibrations between 3000 and 3500 cm^{-1}, the quartet of asymmetric and symmetric (CH_3) and (CH_2) stretching vibrations from lipids chains between 2875 and 2825 cm^{-1}, the very specific absorption

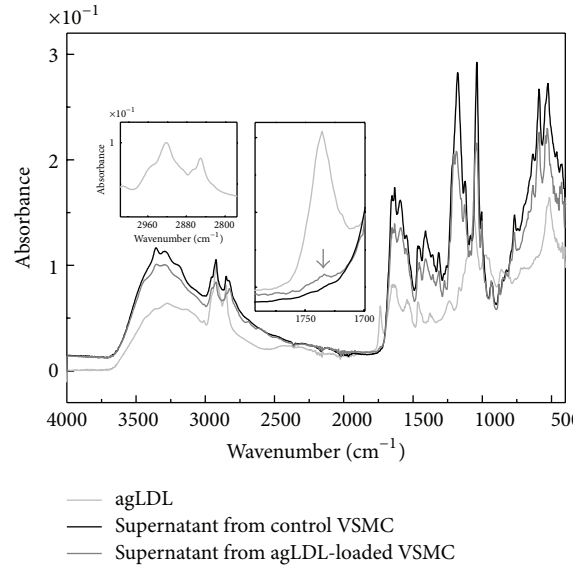

Figure 1: FTIR/ATR spectra of agLDL and purified supernatants.

of the (C=O) stretching of ester groups at 1734 cm^{-1}, the proteins vibration between 1660 and 1500 cm^{-1}, and the lipids fingerprint between 1300 and 1000 cm^{-1}. The ratio of absorbances $A_{2927/I2866} = 1.99$ can be considered as the measure of environment polarity [13] and it is found lower than in LDL (3.78, not shown). The ratio of absorbances $I_{2855}/I_{2866} = 1.48$ correlates with the looseness of chain packing [14] and it is found lower than in LDL (2.27, not shown) confirming the increase of chain packing with aggregation.

The classical absorption bands of proteins (amide A, amides I, II, III) are found on the spectra of both supernatants and their positions are coherent with the literature data on tropoelastin [14–17]. The two fractions of supernatants exhibit quite similar profiles, suggesting a very similar chemistry. A slight shoulder at 1734 cm^{-1} in the spectrum of the supernatant from agLDL-VSMC is indicative of a weak fraction of agLDL in the sample that could remain after the purification, suggesting an interaction between tropoelastin and agLDL, as previously reported for tropoelastin and LDL [18].

The decomposition of the amide I/II region of the supernatants from control VSMC and from agLDL-VSMC are shown in Figures 2 and 3, respectively, with the probable assignments of the different components according to the literature data [14–20].

The band located at 1591 cm^{-1}, observable in both cases, was not associated with a peculiar structure of tropoelastin and it certainly arises from water absorption. The appearance of the vibration at 1667 cm^{-1} in the supernatant from agLDL-VSMC could be attributed to irregular structures [14–17], to another undefined component [20], or to non-H-bonded groups [19]. This assumption is confirmed by the intensification of the vibration band at 1555 cm^{-1} in the amide II region, attributed to non-H-bonded groups.

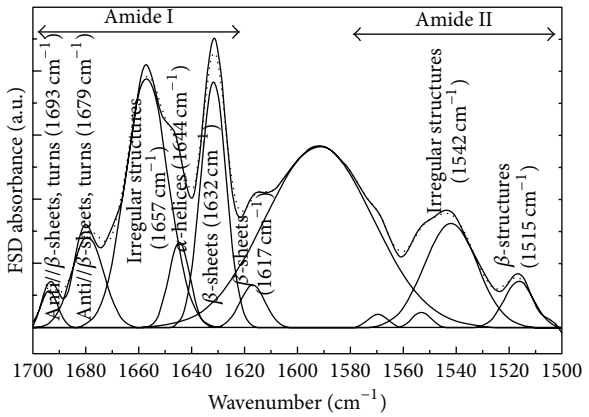

Figure 2: Decomposition of the amide I/II region of supernatant from control VSMC.

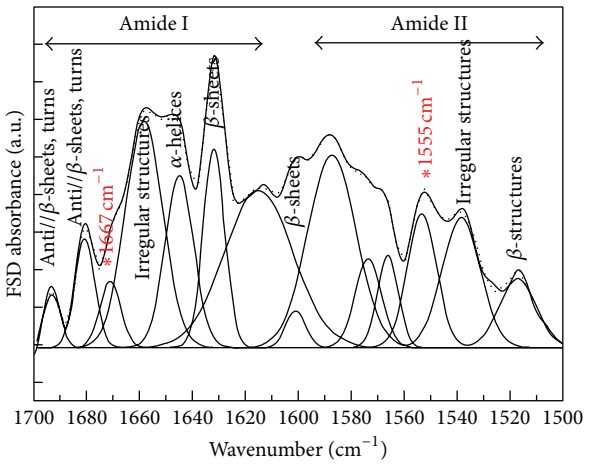

Figure 3: Decomposition of the amide I/II region of supernatant from agLDL-VSMC.

The thermogram of agLDL recorded between −100 and 100°C is shown in Figure 4. It is characterized by a glass transition at $T_g = -33$°C, ascribed to the amorphous phase of agLDL, and an endothermic transition at $T_m = 38$°C, associated with the apolar lipids constituting the central core [21].

The two thermograms corresponding to the first and second scans of purified supernatants are superimposed in Figure 4. The first scans were performed between 30 and 180°C (a departure from the ambient temperature was chosen to avoid a possible crystallisation of water in the samples). They are characteristic of water-proteins interactions and present the general feature of a broad endothermic peak associated with bound water departure. The enthalpy associated with this transition is connected to the water content of the supernatant; the largest value of the enthalpy is found for supernatants from control VSMC (160 J/g versus 120 J/g), meaning that this fraction corresponds to the more hydrated sample. The second scans were performed on a larger temperature scale (−60 to 250°C) to explore the possible transitions at low and high temperature (up to

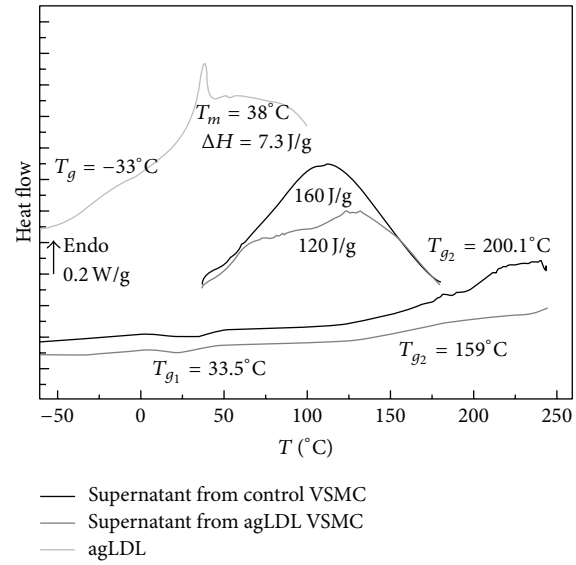

Figure 4: DSC thermograms of agLDL and purified supernatants.

the degradation temperature). On these thermograms two glass transitions phenomena (T_{g_1}, T_{g_2}) are observed for both supernatants.

The presence of two glass transitions is indicative of two kinds of amorphous phases in the purified supernatants. By comparison with previous studies [12, 16], the second glass transition (at $T_{g_2} = 200.1$°C) of supernatant from control VSMC could be addressed to the thermal answer of aggregated tropoelastin. The lower glass transition (at $T_{g_1} = 41.4$°C) could be attributed to nonaggregated tropoelastin. In the case of supernatant from agLDL-VSMC, both tropoelastin and aggregated tropoelastin undergo a glass transition at lower temperature, with a spectacular decrease of the glass transition associated with aggregated tropoelastin ($T_{g_2} = 159$°C). This phenomenon could be due to the interaction between agLDL and tropoelastin as evidenced by the weak specific FTIR absorption band of agLDL in supernatant from agLDL-VSMC. It is also noteworthy that the physical structure of agLDL must be modified in the supernatant, since the specific thermal answer of ag-LDL is not observed in the supernatant from agLDL-VSMC that was shown by FTIR to contain a lipid fraction.

4. Conclusion

Aggregated LDL is one of the main mechanisms by which VSMC become foam cells during atherosclerosis. We showed physical modifications on secreted tropoelastin when VSMC are agLDL loaded. The affinity with water is decreased, suggesting that a loss of elasticity since water is essential in the elasticity of elastin. At the conformational level a new undefined, probably non-H-bonded, structure is revealed for tropoelastin that could be due to an interaction of the protein with remaining agLDL, inducing the decrease of the glass transition of tropoelastin and aggregated tropoelastin. These alterations in the physical structure of tropoelastin will

unfailingly induce alterations of the mechanical properties of elastic fibers.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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