Adsorption kinetics of low-density lipoproteins with Langmuir monolayer

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Abstract The present work utilizes the Langmuir monolayer technique to detect the adsorption kinetics of native low-density lipoproteins and their oxidized form with the lipid monolayer. We found that low-density lipoproteins and oxidized low-density lipoproteins are able to penetrate the LM up to pressure $\pi=9.9$ and 11.6 mN/m. Also, the adsorption constants of both particles were found to depend strongly on the monolayer initial pressure. It is found that less compressed lipid monolayers could accommodate more native low-density lipoproteins than the oxidized ones due their higher binding affinity toward monolayers. The probable α -helical regions along the apoproteinB-100 secondary structure and average hydrophobicity could explain partially their adsorption kinetics into lipid monolayers. This simplified ‘in vitro’ study of low-density lipoprotein–monolayer interaction may serve as a step further to understand the mechanism and bioactivity of the atherosclerotic process. Also, it may shed light on the oxidized low-density lipoprotein’s role in plaque formation in the innermost arterial wall in blood vessels.

Keywords Langmuir monolayer · Atherosclerosis · LDL oxidation · Adsorption kinetics

1 Introduction

Atherosclerosis is a complex chronically developing human disease initiated by and progressing as a result of excessive accumulation of lipids (e.g., mostly cholesterol in human blood) in the smooth arterial wall [1–3]. Cardiovascular disease is first distinguishable by a fatty streak, which is lipid-rich macrophages and T lymphocytes trapped by the artery wall (i.e., intima) [4]. As oxidization continues, fatty steaks decompose to polyunsaturated

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fatty acids (PUFA) [5]. Finally, PUFA decomposes further into aldehydes and other water-soluble compounds. These highly potent substances react with the protein moiety of the native low-density lipoprotein (nLDL), which ultimately results in non-recognized LDL-particles by their own receptor [6]. Then, this oxidized low-density lipoprotein (oxLDL) generates in the blood vessel wall an inflammatory accumulation which, in turn, results in the recruitment of circulating monocytes. The latter process is not well recognized by the LDL receptor pathway, leading to unregulated cholesterol accumulation and to the development of macrophage foam cells, the hallmark of the artery lesion, and finally to the progression of arteriosclerosis [5, 6]. These characteristic alterations in size, chemical composition, and structure are assuming important clinical significance [6].

Among several lipoproteins, LDL has been identified as one of the cholesterol-rich lipoproteins that transport it to the inner artery wall membrane [3–6]. LDL (515 kDa) are spherical particles composed of a hydrophobic core (mostly triglycerides and cholesteryl esters) surrounded by an amphipathic monolayer of phospholipid and cholesterol in which a single protein molecule, apolipoprotein B-100 (ApoB-100), is located. ApoB-100 is a hydrophobic protein of 4563 residues [7]. The diameter of the LDL particle is in the range of 17–60 nm [8]. In its native state, it is believed that ApoB-100 forms a ring around the hydrophobic core of the LDL particle with a known secondary structure [8]. The data deduced from several studies indicate that the amino acid sequence of ApoB-100 is composed of an alternating five domains of amphipathic α -helical and β -sheet structure (NH_2 - $\beta\alpha_1$ - β_1 - α_2 - β_2 - α_3 -COOH). A first suggestion is due to Gotto, Levy, and Fredrickson [9] who estimated using CD spectra of isolated LDL to have 15–25% β -sheet structure, while infrared spectroscopy determined the content of β -sheet structure as much as 30–40% and 25% α -helix [7]. The latter methods also suggested that the β -sheets of ApoB-100 is oriented parallel to the LDL particle phospholipid monolayer. Relevant to these results, other reports have postulated that the amphipathic α -strands contribute to the high affinity of ApoB-100 for the LDL lipid interface [10]. On the other hand, another CD study by Coronado-Gray and Antwerpen using CD spectra showed that 25% α -helix spans the secondary structure of ApoB-100 [4].

Oxidation of LDL solutions is able to generate large sequential structural and physico-chemical alteration of nLDL, which may progressively undergo self-reorganization and modification of ApoB-100 proteins [11]. These specific studies were concerned with evolution of the secondary structure of ApoB-100 and the decomposition of the LDL particles upon oxidation [12]. Their results have been classified according to different stages of LDL oxidation: lag, propagation, and decomposition phase. Of interest is the latter phase, where all the LDL particles have oxidized and no additional structural, physical, or chemical changes can occur [8]. Detection of ApoB-100 conformation changes during oxidation has long been a subject of extensive investigations (reviewed in [8]). The early structural modifications of ApoB-100 have been studied by Krisko et al. [13]. They showed, using the ATR-FTIR method, that LDL particles experience dramatic change when entering into the decomposition phase (usually after 120 min). In this final stage of oxidation, it has been reported that both α - and β - structures are modified with an increase in β as well as unordered structures percentage of ApoB-100. Similar findings have been previously observed upon extensive oxidation of LDL particles [14]. These authors have calculated the secondary structure percentage of human LDL using infrared spectrum as 17% α -helix and about

54% β -structures. Other oxidation-structural change studies on LDL particles show almost the same ratios [15, 16].

As a biological membrane model system, the Langmuir monolayer (LM) has been routinely used by many researchers to investigate the interactions between various lipids and interfacial active molecules. LM has been long thought to function as a simple biosensor for adsorption and detecting of biological active macromolecules (e.g., DNA, LDL, etc.) [17]. The characteristic adsorption properties of lipid–protein interaction at the air–water interface are generally inferred from pressure–time (π -t) kinetic curves. These curves may be important in understanding these types of interactions at the molecular level [18]. For example, adsorption/desorption rate constants of the active molecules with the monolayer at the air–water interface are determined primarily by a single exponential least square fitting of the adsorption kinetics curves. It is also known that the trend and shape of the π -t curves depend on many experimental variations including initial lipid molecule packing, temperature, pH, and compression rate. Analyzing π -t curves led simply to some essential physical properties of the protein-monolayer system. These physical constants can be extracted from the behavior of the π -t exponential-growing curve. The effects of hydrophobic, hydrophilic, and electrostatic interactions are factors that determine the phospholipid monolayer structure and properties at the air–water interface. Moreover, the different phases which many phospholipid molecules exhibit in this pseudophysiological environment can be used as a model for “in vivo” experiments [18]. The relevance between the LM and cell membrane emerges from the interfacial pressure withstood by the monolayer at 30 mN/m. This pressure is assumed to be the physiological pressure sustained by the cell membrane [17, 18]. Owing to the interfacial pressure differences occurring between these experiments performed on monolayers and that being sustained by the cell membrane, these simplified membrane-mimicking studies still contain in their nature the essence of ‘in vivo’ process appearing at the molecular level in the arterial wall [19].

To understand how the LDL particles behave toward the cell membrane, we used the phospholipid monolayer as a biological model to study in vitro the adsorption/interaction kinetics of nLDL and oxLDL with the phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) monolayer at the air–water interface. Measuring the LDL adsorption capability as a function of lipid packing density, we show that the two LDL isomers are able to penetrate into lipid films at interfacial pressures equal to or lower than a certain maximal spreading pressure of the monolayer film. Such models may mimic the phospholipid interface exposed to lipoproteins in the blood vessel. This membrane-like environment enables the investigation and adsorption kinetics of LDL in the air–buffer interface and in the DMPC/ aqueous solution. The DMPC monolayer/LDL complex on a buffered subphase (see Materials and methods) are studied by means of LM technique at these physiological-related conditions. Thus, the information gathered from these initial and biologically relevant investigations can be projected to in vivo situations [20]. The results obtained from this simple model can reveal the role of LDL in pre-clinical observations on risk factors for atherosclerosis.

2 Materials and methods

2.1 Materials

Low-density lipoprotein (LDL), DMPC, and copper (II) chloride hydrate were purchased from Sigma Chemical (purity > 99%). Butylated hydroxytoluene (BHT), disodium

ethylenediamine-tetraacetic acid (EDTA), phenylmethsulphonyl fluoride (PMSF), and Tris-buffered saline (TBS) (0.14 M NaCl and 5 mM Tris-HCl, pH 7.4) were also purchased from Sigma Chemical. All the chemicals were of analytical research grade and used without further purification.

2.2 Lipid solution and monolayer preparation

Lipid was dissolved in chloroform and an appropriate amount of solution (10 μ l) was spread with a Hamilton syringe onto aqueous subphase. Monolayers at the air–water interface were left at least 40 min to allow solvent evaporation and to reach interfacial equilibrium before compression was applied to the DMPC monolayer. This was observed for an adequate time to achieve constant surface tension and removal of chloroform. Initial interfacial pressure of the monolayer (π_{lipid}) was then measured.

2.3 Lipoprotein solution preparation

After adjusting the LDL concentration to 1.5 mg/ml (expressed as total LDL concentration), lipoprotein preparations were dialyzed against 100 volumes using phosphate-buffered saline (PBS) in the absence of any antioxidants. The composition of PBS was 10 mM sodium phosphate, containing 150 mM sodium chloride, pH = 7.4. The dialyzed LDL was stored in the dark at 4 °C and used within 1 week after preparation for peroxidation experiments.

2.4 In vitro LDL oxidation

Aliquots of LDL were subject to one of the following conditions, either (a) autoxidation: 0.2 or 0.8 mg/ml of LDL in TBS (1 or 5 ml final volume), placed in loosely capped glass tubes, were exposed to air, in the dark, at 37 °C for a time period of more than 96 h to achieve full oxidation [11], or (b) copper-induced oxidation: 0.2 or 0.8 mg/ml LDL protein in 10 mM TBP (pH = 7.4), was added to solutions of 1–5 μ M CuSO₄, and the samples were treated as in (a) for a time period of more than 24 h to achieve full oxidation, as reported previously in [11, 20].

In each condition, oxidation was ceased by adding 0.01% EDTA, 0.25 μ M BHT, and 0.2 μ M PMSF to the samples, and placing the tubes in the dark, at 4 °C. For subsequent experiments, the buffers used were supplemented with the above antioxidants and proteolytic inhibitors.

The evaluation of the oxidative process leading to oxLDL formation was essentially the same during autoxidation or copper-induced oxidation. In fact, for the same incubation time, the number of oxLDL particles recognized by autoxidation was lower than upon copper-induced oxidation. Also, the observed similarity in the changes in oxLDL morphology and biochemistry by these two procedures led us to carry out most of the experiments with copper-oxidized LDL, in which the rapidity and intensity of the modifications were highest [11].

Aliquots from these experiments were examined using the Langmuir trough and Wilhelmy balance techniques to determine the adsorption rate at which the LDL or oxLDL penetrate the DMPC monolayer at the air–buffer interface.

2.5 Langmuir trough and Wilhelmy balance

A Nima Langmuir–Blodgett trough (Model 312D, England) was used to measure the adsorption kinetic of the native- and ox-LDL at the air–water interface. A Teflon trough with a working area of $30 \times 10 \text{ cm}^2$ was placed on a vibration isolation table and sealed in an environmental chamber. The interfacial pressure (π) as a function of time (t) was measured utilizing the well-known method of the Wilhelmy balance. The setup consists of a computer-interfaced film balance yielding digital data of the surface pressure at 0.1% precision. All experiments were carried out on a buffer solution subphase of 150 mM NaCl, 0.25 μM BHT, and 0.2 μM PMSF and 0.01% EDTA (pH 7.4). Water was purified with a Millipore desktop system. The subphase temperature was kept at $T = 37^\circ\text{C}$ ($\pm 0.2^\circ\text{C}$) by a circulating water using Lauda circulation bath (Model RK20). Reproducibility of the results were checked by repeating each curve at least twice.

2.6 Monolayer penetration

Either native LDL or oxLDL solutions were slowly infused into the subphase by direct injection at different places underneath the DMPC monolayer at the air–water interface. This injection was done gently in order to not disturb the monolayer interfacial pressure. Prior to each injection, an equivalent volume of the subphase was slowly withdrawn separately. The samples were equilibrated to constant interfacial pressure prior to making a final measurement of the interfacial pressure ($\pi_{\text{lipid-LDL}}$). Then, detecting the changes in the interfacial pressure vs. adsorption time was done immediately after the injection of the protein under constant area conditions of the LM. This method allows direct detecting of monolayer compression isotherms during the adsorption process at the air–buffer interface as well as a good temporal resolution ($< 1 \text{ s}$) of the interfacial pressure.

The interfacial measurements were carried out using two different procedures. The first procedure took the well-defined initial pressures (π_i) of the monolayer at fixed values by keeping the trough barrier positions at a defined place. Then, a fixed concentration of LDL (or ox-LDL) aqueous solution underneath the monolayer was added. The prepared monolayer was selected to be at an initial pressure of $2 < \pi_i < 8 \text{ mN/m}$. In the second procedure, another monolayer of DMPC with fixed pressure was spread at the air–water interface, while different aliquots solutions of LDL (or oxLDL) were injected into the subphase to a final concentration between $5 \times 10^{-7} \text{ mol/l}$ and $9 \times 10^{-6} \text{ mol/l}$.

The results reported in the subsequent sections were obtained from experiments in which copper was used to oxidize lipoproteins for a period of more than 24 h [11].

3 Results and discussion

The ability of LDL and oxLDL particles to interact with the phospholipid monolayer is investigated by measuring the increase in interfacial pressure at constant monolayer film area when the protein is injected underneath it.

Figure 1 shows the interfacial pressure increase $\Delta\pi$ as a function of the initial monolayer's pressure π_i . The data is fitted by a linear regression and extrapolated to $\Delta\pi = 0$. This extrapolated value gives the pressure above which the LDL particles are no longer able to penetrate into the phospholipid monolayer film at the air–water interface (i.e., exclusion

Fig. 1 Influence of the initial interfacial pressure (π_i) on the maximal pressure variation ($\Delta\pi$) induced by the protein adsorption onto the monolayer at the air–water interface with different initial pressure. Each point was calculated by the difference from the final interfacial pressure plateau and the initial monolayer pressure. Final protein concentration was 0.2 nM and the subphase is a buffer solution of 150 mM NaCl and 0.01% EDTA. Each pressure variation point is a representative average of two independent measurements

pressure). The exclusion pressures induced by the LDL adsorption into lipid monolayer at various initial interfacial pressures were obtained from the linear fitting (see Fig. 1) after measuring the monolayer's pressure increase due to its adsorption at lipid–water interface. The exclusion pressure is the minimal monolayer interfacial pressure at which the adsorption/penetration of any external interface-active agents to the monolayer will not induce any interfacial pressure increase [19]. The exclusion pressure of oxLDL and LDL for the DMPC monolayer is estimated to be 9.9 and 11.6 mN/m, respectively. Interestingly, the exclusion pressure of the oxLDL is lower than that of the native LDL protein but for both of them it is lower than the pressure which is supposed to exist in the real biological cell membrane [19]. Both native and oxidized LDL particles have similar exclusion pressures for DMPC monolayer, which suggests that their interactions with the phospholipid monolayer has been affected by their secondary structures, as discussed below.

Earlier data concerning LDL particles suggested that the Apo-B100 sequence might be implicated in LDL–membrane interactions [21]. To verify the contribution of the Apo-B100 protein in these interactions, we chose to utilize the phospholipid monolayer as a simple biomimetic membrane model to follow the adsorption of LDL particles into the monolayer film with an increase in the interfacial pressure at constant interfacial molecular area. We investigated the adsorption of these particles into neutral zwitterionic phospholipid monolayer (DMPC) at a buffered subphase. This was done to gain insight into the interaction tendency of LDL particles toward the monolayer and also into the ApoB-100 secondary structure contribution to the exclusion pressure induced in the lipid monolayers. The observed pressure increase can be explained by a two-step process, initially, the LDL particles are recognized and bound by the lipid monolayer and consequently insert themselves between the lipid molecules, presumably within the lipid headgroups, at the air–water interface by electrostatic interactions. This part of the adsorption process is possible since the lipid monolayer was not fully compressed and could accommodate LDL particles at the phase boundaries or between the monolayer domains [22]. During this step, the LDL particles are causing the phospholipid tails to re-arrange into a liquid or condensed phase almost instantaneously [22]. In the second step of adsorption, which occurs with a negligible delay time, the ApoB-100 proteins

have to re-insert or re-locate their favorable-interaction side (i.e., their hydrophobicity) properly when facing the lipid monolayer in proximity to the lipid headgroups [23]. This explanation is also assumed for the interface-active apolipoproteins A-I and A-II at the lipid–water interface. The average residue hydrophobicity and the probable helical structure of these types of proteins may be the key factor for this binding affinity with the phospholipid monolayer at the air–water interface [23, 24]. Also, the same interaction procedure has been discussed by Donald and Patterson [25] when studying the adsorption and insertion of β -lactoglobulin from solution into the lipid monolayer at the air–water interface. Another study of Bordi et al. [18], who analyzed the insertion of two different anthracyclines (e.g., adriamycin and daunorubicin) into DMPC monolayer at the air water–interface, pointed out that these effects strongly depend on the molecular packing of the lipid film. They found that the insertion of the drug molecules was favored at the gas–liquid coexistence phase [18].

Previous data concerning the LDL adsorption at a bare air–water interface suggested that an alteration in π was detected, indicating that a Langmuir or Gibbs monolayer was already formed [26]. To verify the involvement of nLDL or oxLDL particles in the membrane interactions, we used interfacial lipid monolayers as bio-mimetic membranes to follow the adsorption of LDL particles into the DMPC monolayer as an increase in the interfacial pressure at constant molecular packing. We chose the initial interfacial pressure of 8 mN/m and aqueous solutions of LDL protein at different concentrations. Then it was injected into the subphase underneath the monolayer to follow the interfacial pressure increase. The final concentrations of LDL or oxLDL ranged between 0.28 and 5.8 μM /l. No lag-time was observed for the adsorption of the proteins into the lipid monolayer at this initial pressure. From Fig. 2, one can observe that interfacial pressure variations depend on the LDL concentration injected in the subphase. These variations are due to the protein adsorption within the lipid monolayer and can be attributed to their interaction with the polar headgroups or even penetration within the lipid film at the air–water interface. This typical adsorption isotherm interfacial pressure increase can be characterized by the well-known mono-exponential relaxation (see also Fig. 3) [27]. Notably, if the surface was already covered with DMPC monolayer at a given pressure, the resulting adsorption kinetics were different from what has been observed on bare air–water interface [26].

Fig. 2 Adsorption kinetics of LDL into the DMPC monolayer at the air–water interface. The subphase is the same as mentioned in Fig. 1. The LDL concentration is given in the legend. Each curve is well described by mono-exponential behavior

Fig. 3 The adsorption kinetics of oxLDL into the DMPC monolayer at the air–water interface. The subphase is the same as mentioned in Fig. 1. The LDL concentration is displayed in the figure. Each data set is well described by mono-exponential behavior

The adsorption of proteins or peptides at bare air–water or lipid film interface is a well-studied interfacial phenomenon [17]. The general accepted behavior is that the proteins lose their native biological activities when exposed to the air–water interface or when interacting with the lipid monolayer by changing their secondary structures [28]. It is also a well-accepted phenomenon that the protein's adsorption kinetics is a nonlinear process and the interfacial pressure reaches a plateau within a certain time after the injection into the subphase. The latter behavior depends on different factors like lipid monolayer composition, molecular packing, interfacial-active macromolecules, and the buffer solution in subphase. In our case, the LDL interfacial pressure reaches the plateau within 30–40 min after the injection into the subphase, indicating that both LDL and oxLDL were adsorbed well into the DMPC monolayer.

Then, we apply the well-known Langmuir adsorption isotherm to study the dependence of the observed pressure increase as a function of LDL concentration as follows [18]:

$$C + N_f \xrightleftharpoons[k_d]{k_a} N_0 \quad (1)$$

where C represents the free LDL concentration, N_f and N_0 are the free and occupied adsorption sites on the lipid film, and k_a and k_d are the rate constants for the adsorption and desorption process, respectively. The pressure increase ($\Delta\pi$) as a function of LDL particle adsorption into the lipid monolayer at time t can be modeled as [20, 27]

$$\Delta\pi \approx 1 - \exp(-t/\tau) \quad (2)$$

where the time constant τ is given by

$$\tau = \frac{1}{k_d + k_a[C]}. \quad (3)$$

It is evident from Figs. 2 and 3 that the LDL particle adsorption into the lipid film follows very well the mono-exponential relaxation in the investigated concentration range. The statistical analysis of the fitted data are presented in Tables 1 and 2 in the supplementary

materials. Figure 4 shows the dependence of the time constant-reciprocal on the LDL concentration $[C]$. The constants k_a and k_d can be inferred from the linear fit of data presented in Fig. 4. Our fit results are, for nLDL $k_a = 1.15 \pm 0.012 \cdot 10^3 \text{ M}^{-1} \text{ s}^{-1}$ and $k_d = 3.21 \pm 0.044 \cdot 10^{-3}$ and for oxLDL $k_a = 2.62 \pm 0.013 \cdot 10^3 \text{ M}^{-1} \text{ s}^{-1}$ and $k_d = 1.06 \pm 0.032 \cdot 10^{-3}$, and the equilibrium association constant, $K = k_a/k_d$ is $3.6 \cdot 10^5 \text{ M}^{-1}$ and $2.5 \cdot 10^6 \text{ M}^{-1}$, respectively. Finally, the pressure increase at constant molecular packing as a function of the concentration is depicted in Fig. 5. The values obtained here clearly indicate the successful binding of LDL onto the lipid monolayer at the air–water interface. Also, it shows that the nLDL has a greater ability to alter the lipid monolayer interfacial pressure, suggesting qualitative agreement with the rate constants obtained from Fig. 4. Similar values have been found by Matharu et al. [29] based on surface plasmon resonance and quartz crystal microbalance fabricated by immobilizing anti-apolipoprotein B into a self-assembled monolayer of 4-aminothiophenol. These interesting results showed that the adsorption of oxLDL was slightly different than that of nLDL on the lipid monolayer as discussed below.

To explain the differences in interfacial behavior of the native nLDL and oxidized oxLDL particles toward the lipid monolayer, we look for the influence of the modified structures of the amino acids along the sequence of ApoB-100 upon oxidation. At pressures lower than the exclusion pressure, where the phospholipid monolayer is mostly found in the gas–liquid expanded coexistence region, the lipid acyl chains have considerable degrees of freedom allowing for an excellent interactions with the whole LDL particles at the air–water interface. The primary and secondary structure of apolipoproteins nLDL and oxLDL have been known since the seminal work of several research groups [6–]. The nLDL has a higher molecular weight than oxLDL for obvious reasons. It is unlikely that the difference in molecular weights or their weight ratios with respect to the total lipid content of the whole LDL particles of these proteins influences their interfacial activities and their affinities for phospholipids because ApoB-100 is flexible and contains several β -sheets and α -helical segments along its backbone, which may behave as independent sub-units. Apolipoprotein hydrophobicity is a property that has a significant effect on the binding to model membranes; more hydrophobic molecules bind better. To compare nLDL and oxLDL, it is necessary to know the average residual hydrophobicity of the amino acid sequence of both proteins. As this piece of information is lacking in

Fig. 4 The dependence of the time constant inverse ($1/\tau$) as a function of protein concentrations. The data points presented here are extracted from Figs. 2 and 3 for LDL and oxLDL respectively

Fig. 5 The interfacial pressure increase ($\Delta\pi$) as a function of protein concentrations. The data points presented in the figure are extracted from Figs. 2 and 3 for LDL and oxLDL, respectively. The solid line corresponds to Langmuir adsorption isotherm fits

the literature, we speculate that nLDL is more hydrophobic than oxLDL, as is clear from the adsorption kinetic isotherms shown in Figs. 2 and 3. Thus, we shall discuss their different affinities toward the lipid monolayer in an analogous approach adopted for apolipoproteins A-I and A-II at the lipid–water interface [23]. This may partially explain why nLDL has a higher affinity for the lipid monolayer than oxLDL. Several reports from different laboratories have pinpointed that hydrophobic forces are essential for the formation of apolipoprotein–lipid complexes at the air–water interface [23, 30]. On the other hand, secondary structure of nLDL and oxLDL has been known for some time due to sophisticated methods like CD and IR-spectra [11–16]. This allows us to propose that the apolipoproteins containing more amphipathic α -helical segments interact better with the lipid monolayer. The ability of these proteins to form α -helical structures is reduced when oxidized as indicated in the introduction. Although it is probable that the α -helical percentage of nLDL (i.e., 25%) and oxLDL (i.e., 17%) per sequence cause a difference in their affinities toward the monolayer, the role of their α -helix domains along the protein backbone can't ruled out in this situation. Chakarborty et al. [16] used oxidative foot-printing to map the ApoB-100 surface using a concentration gradient technique with oxidant agents. They have explained for their result with the fact that loss of sequence coverage of the apolipoproteins limits peptide oxidation. Their surface mapping approach of ApoB-100 elucidated the oxidative accessible residues for prediction of the secondary structure of oxLDL [16]. They found apolipoprotein mapping patterns with specific residue oxidation with a possibility to alter the properties of the helical segments in the oxLDL. This led to a change in the average hydrophobicity and may affect the percentage and location of α and β structures in the apolipoproteins. This suggests that the interplay between the hydrophobicity and structural content of the apolipoproteins may play a critical role in their binding affinity for lipid monolayers. Also, it is difficult to speculate on other effects of helical or sheet amphiphilicity on interfacial-active apolipoproteins because of the lack of certainty regarding their exact position in apoproteins. It is also possible that other helical properties such as length and number of α -helices per protein are important when adsorbing at the interface. However, helix-helix interactions may be of merit in determining the interfacial activities of apolipoproteins [31].

3.1 Future study

Phospholipids are well known for their different headgroups, chain length, and degree of unsaturation and their molar ratios in the native cell membrane [17]. Also, conformational and structural changes of human plasma of native and oxidized ApoB-100 proteins are different in their intrinsic physico-chemical properties. Moreover, an information lack about the average hydrophobicity and the exact percentage of α -helices during the oxidation process in ApoB-100 and partially within the native ApoB-100 would advance an understanding of their absorption kinetics toward the inner artery wall in blood vessels. From a medical viewpoint, it would be of great interest to investigate the different role of the lipid properties on the adsorption effects of ApoB-100 (e.g., native and oxidized) upon adsorption into the monolayer at the air–water interface. A more relevant issue would be studying the effects of the cholesterol component on the molecular recognition and kinetics between the various lipid–cholesterol mixtures and ApoB-100 ratio. These future investigations will have significant impacts on biological and medical applications.

4 Conclusions

In conclusion, the adsorption kinetics of native LDL and oxLDL in a cell-mimicking environment was investigated using the well-known Langmuir monolayer technique. The investigated LDL particles showed different adsorption affinity towards the lipid monolayer at the air–buffer interface. The adsorption was significantly higher for the LDL when compared with the oxLDL as the later changes its structural and conformational nature upon oxidation by the copper ions. LDL particles are well-known risk factors for atherosclerosis. Despite the fact that this study has been performed using a model interface mimicking the endothelial vessel wall, it still serves as a step toward understanding in vivo biological activities in the human body. These results may be of great significance for at least some aspects of atherosclerosis.

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