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Structures and rheological properties of hen egg yolk low density lipoprotein layers spread at the air–water interface at pH 3 and 7

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Abstract

Low density lipoproteins (LDL) from egg yolk have a classical structure of lipoprotein with a core of neutral lipids surrounded by a monolayer of apoproteins and phospholipids. This structure collapses during adsorption and all constituents spread at the interface. To understand better the nature of the interactions between apoproteins and lipids at the interface, we have deposited LDL at an air–water interface and analysed the isotherms during their compression on a Langmuir trough. Then, these LDL films were studied by atomic force microscopy (AFM) imaging. To identify the protein and lipid structures, we imaged films before and after lipid solubilisation by butanol. To study the interactions in the LDL films, we have varied the pH, ionic strength and used simplified model systems. We also studied the correlation between observed structures and interfacial rheology of the film. The isotherms of interfacial LDL films were similar for pH 3 and 7, but their structures observed in AFM were different. At surface pressures below the transition corresponding to the demixion of apoprotein–neutral lipid complexes, the LDL film structure was not governed by electrostatic interactions. However, above this surface pressure transition (45 mN/m), there was an effect of charge on this structure. Around the transition zone, the rheological properties of LDL films at pH 3 were different as a function of pH (viscous at pH 3 and visco-elastic at pH 7). So, the rheological properties of LDL films could be linked to the structures formed by apoproteins and observed in AFM.

Keywords: LDL; Interface; Structure; pH; Electrostatic repulsion; Charge; Interfacial rheology

1. Introduction

The low density lipoproteins (LDL) from egg yolk are spherical particles comprised of an outer film of different hydrophobic proteins (Apo) and of different phospholipids (PL) (mainly phosphatidylcholine and phosphatidylethanolamine) surrounding a core of different neutral lipids (NL) (mainly triglycerides and cholesterol) [1]. Their diameter is in the range of 17–60 nm [2]. They contain 11–17% (w/w) of proteins and 83–89% (w/w) of lipids which are composed of 74% (w/w) neutral lipids and 26% (w/w) phospholipids [3]. As LDL is the main emulsifying component in egg yolk, it was appropriate to study the organisation of these different constituents at the interface and to investigate the structures and the rheological properties of LDL interfacial films.

When proteins and lipids form mixed interfacial films, different structures can be observed, depending on the charge and hydrophobic character of proteins or peptides, and charge of lipids. The majority of the studies have been performed with phospholipids, which can be zwitterionic or un-charged in which case, the hydrophobic interactions may dominate; or with charged phospholipids where interactions are mostly electrostatic.

In the hydrophobic interaction domain, Vié [4] studied a synthetic peptide, mainly composed of β-sheet, mixed with DOPC (dioleoylphosphatidylcholine) and DPPC (dipalmitoylphosphatidylcholine) at the air–water interface. The interactions between DOPC and this peptide resulted in an increase of the β-sheet structure and in an expansion of the molecular area. During this study, atomic force microscopy (AFM) showed an

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evolution of the observed structures depending on molar fraction of peptide. At low molar fraction (1%), small circular particles of peptide appear in the lipid film. For molar fractions from 5% to 25%, a phase separation occurs due to the presence of the peptide. Moreover, filamentous structures were observed, which become more numerous and stretched out when the molar fraction increased from 1% to 25%. At 25%, the hydrophobic interactions seem highest between DOPC and the peptide.

Concerning the electrostatic interactions, Vié [4] also observed using AFM, after transfer from an air–water interface, the structures of the same peptide in the presence of charged phospholipids, DOPG (dioleoylphosphatidylglycerol) and DPPG (dipalmitoylphosphatidylglycerol). For the same β-sheet structure of peptide, there were different organisations depending on molar fraction of this peptide. In the DOPG-peptide films, many filaments were observed, but at a peptide molar fraction of 75% compared to 25% for the DOPC-peptide films. The peptide can reorganize at the air–water interface and optimize hydrophobic interactions that could induce an increase of the molecular area. Aliphatic chains being more flexible for DOPG, can improve lipid–peptide hydrophobic interactions that increases the expansion of molecular area, contrary to the DPPG where it is more restricted.

Dubreil et al. [5] reported that puroindoline, a cationic lipid binding protein, interacts strongly with phospholipids and specially with DPPG which is anionic. Yan et al. [6] studied the effect of NaCl concentration and pH on DPPE (dipalmitoylphosphatidylethanolamine)-β-lactoglobulin mixed films at a chloroform–water interface. In the presence of NaCl, the DPPE increased the surface pressure of β-lactoglobulin because NaCl decreased the electrostatic repulsion between β-lactoglobulin and DPPE. However, at protein concentrations higher than 3.8 mg/L, the adsorption behaviour is independent of the NaCl concentration. These authors have observed an adsorption rate of β-lactoglobulin was lower at pH 5 than pH 8.

In our case, lipids are a mix of neutral and polar lipids. The neutral lipids are, in the majority, composed of triglycerides, which stay neutral, whatever the pH. Whereas phospholipids and proteins, being zwitterionic and polyionic compounds, have their charge influenced by the pH.

A recent study [7] compared films of LDL with films of neutral lipids, phospholipids and total lipids extracted from LDL at the air–water interface, using Langmuir balance. During the compression of the LDL film, three transitions were observed out of which two were identified by comparison with the compression of pure lipid films. The transition observed at 19 mN/m corresponds to the collapse of neutral lipids, and the transition at 54 mN/m corresponds to phospholipid collapse. LDL being composed of three constituents, the transition observed at 41 mN/m has been attributed, by deduction, to the apoproteins. These apoproteins being insoluble, it was not possible to study them separately. The three components were spread to form an interfacial film and the compression induced their collapse sequentially. Another study [8], suggested that, for the isotherms of LDL at the air–water interface, the second transition was not due to apoproteins alone, but to apoprotein–lipid complexes. As LDL is composed of a mixture of proteins and lipids, there are likely to be protein–phospholipid and protein–neutral lipid interactions at the interface. At the air–water interface, LDL form interfacial films composed of neutral lipids, apoproteins and phospholipids, whereas at the oil–water interface LDL forms interfacial films composed primarily of apoproteins and phospholipids [8]. So at the oil–water interface, the interfacial properties of LDL should be due to apoproteins and phospholipids present at the interface, and neutral lipids should partition into the oil droplets [9]. However, at the air–water interface, the film properties will be due to the three individual LDL constituents that stay at the air–water interface alone or in association [7]. Furthermore, during compression of LDL films, whatever the pH (3 or 7), the compression isotherms have shown a similar evolution with the same three transitions [8].

In a previous study [10], we observed LDL films at pH 7, spread on the air–water interface, using AFM after Langmuir–Blodgett transfer onto a mica sheet. During the compression of this film, we observed a homogeneous structure below a surface pressure of 30 mN/m but at higher pressure, this film becomes heterogeneous with circular domains composed of small separated grains surrounded by smooth, flat domains with irregular edges. Above the apoprotein–neutral lipid transition ($\pi = 45$ mN/m), the interfacial structure is more heterogeneous containing smooth domains together with others of different roughness, and circles whose interior is composed of compact grains. The structures observed in the LDL films are a function of surface pressure, the structures formed between the apoprotein–neutral lipid transition and the phospholipid collapse were attributed to the demixing between apoproteins and neutral lipids.

Our aim is to understand better the interfacial organisation of the different constituents of LDL, and the nature of the interactions in the complex structures, over a wide range of surface pressures. Therefore we have followed this study using different strategies: addition of butanol which solubilises lipids to help to identify the protein and lipid structures; modification of pH or ionic strength to study the role of electrostatic interactions on film structure; utilization of simplified model systems composed of the purified constituents. We have also studied the correlation between observed structures and interfacial rheology of the film.

2. Materials and methods

2.1. Low density lipoproteins

LDL were extracted from hen egg yolk and purified according to the method of Anton et al. [11]. Fresh hen eggs were manually broken and albumen was removed. Yolks were carefully rolled on a filter paper (Whatman, Springfield Mill, England) to remove albumen and chalazes adhering to the vitellin membrane. This membrane was then perforated to collect unspoiled egg yolk in a beaker cooled in iced water. Yolk was fractionated into plasma and granules according to the method of McBee and Cotterill [12]. Yolk was diluted with an equal volume of a 0.17 M NaCl solution and stirred with a magnetic stirrer for 1 h at 4 °C. This solution was then centrifuged at 10,000 × g for 45 min at 4 °C and the supernatant (plasma) was separated from the sedi-