Recent advances concerning the functional properties of egg yolk low-density lipoproteins

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Hen egg yolk is a natural supramolecular assembly of lipids and proteins with different organisation levels. These assemblies consist mainly of interactions between proteins and phospholipids, and these interactions are essential in view to understand and to control the production of food made with yolk, and particularly emulsions.

Yolk is a complex system containing several particles in suspension in a clear yellow fluid: the plasma. The main types of particles are spheres, profiles and granules. Spheres are minor components (1 % of yolk dry matter) and have a diameter between 4 and 150 μm. Granules (20 %) consist in circular complexes ranged in diameter from 0.3 μm to 2 μm. Profiles (2/3 of yolk dry matter) are spherical particles of 12-48 nm diameter and are considered as low-density lipoproteins (LDL).

The emulsifying properties of egg yolk are principally attributed to its LDL. LDL are an assembly of neutral lipids (triglycerides and cholesterol esters) surrounded by a monofilm of proteins and phospholipids. The structure of LDL seems essential to ensure their interfacial properties because LDL serve as a vector of surfactants, like proteins and phospholipids, up to the interface. Once at the interface, the protein part of LDL plays the essential role in destabilizing all the LDL structure by their initial anchorage and denaturation. Then the LDL spread and the proteins and the phospholipids adsorb at the interface. Thus, interfacial films made with LDL are constituted by a blend of proteins and phospholipids that assure both the decrease of interfacial tension and the resistance to the rupture. This permits the formation and the stability of food emulsions made with yolk. In this review, I propose to explain the evolution which allowed us to arrive at these findings.

Keywords: hen egg yolk, low-density lipoproteins, emulsions, oil-water interfaces, assembly

Introduction

Hen egg yolk is widely used as ingredient in food notably for its exceptional emulsifying and gelling properties. It contributes to the formation and the stability of yolk emulsions like mayonnaises, salad dressings, and creams. Yolk is still used empirically and physical properties of yolk emulsions are not entirely controlled. Indeed, yolk is a very complex supramolecular assembly of lipids and proteins with different organization levels. These assemblies consist mainly of interactions between proteins and phospholipids, and these interactions are essential in view to understand and control the production of food made with yolk, and particularly emulsions.

Egg yolk can be fractionated by dilution and mild centrifugation into two fractions: plasma and granules (McBee and Cotterill, 1979). Plasma is mainly constituted by 85% low-density lipoproteins (LDL) and 15% livetin, and granules by 70% high-density lipoproteins (HDL), 16% phosvitin and 12% LDL (McCully et al., 1962). Plasma represents 75-81% of the yolk dry matter and gathers 85% of the phospholipids and 52-58% of the proteins. Granules account for the remaining 19-25% of yolk dry matter and contain 15% of the phospholipids and 42-48% of the proteins (Burley and Cook, 1961; Saari et al., 1964).
LDL have been designed as the main contributors of yolk emulsifying properties (Kiosseoglou, 1989; Martinet et al., 2002, 2003; Dauphas et al., 2006a). LDL form a film at the interface between oil and water, and this film contribute to the stabilisation of emulsions made with yolk. LDL are the main egg yolk constituent: they represent 2/3 of yolk dry matter and 22% of yolk proteins (Cook and Martin, 1969). LDL are spherical particles (17-60 nm diameter with a mean of about 35 nm) with a lipid core in a liquid state (triglycerides and cholesterol esters) surrounded by a phospholipid and protein (called apoproteins) monofilm (Evans et al. 1973). LDL are soluble in aqueous solution, whatever the pH and ionic conditions, due to their low density (0.982). LDL consist of 11 to 17% proteins and 83 to 89% lipids which are divided into 69% triglycerides, 27% phospholipids and 4% cholesterol and cholesterol esters. Phospholipids take an essential part in the stability of the LDL structure (Burley 1975). Some cholesterol is included in the outer monofilm to rigidify it. LDL particles comprise at least 6 different apoproteins: their pI varie from 6.3 to 7.5 and apoproteins, with about 40% of hydrophobic amino acid residues, present mainly a random coil structure or a β-sheet conformation (Kojima and Nakamura, 1985).

These last years, controversies have persisted about the adsorption mechanism of LDL at the oil-water interface. It is commonly supposed that LDL particles break down when they come into contact with the interface. The lipid core coalesces with the oil phase and apoproteins and phospholipids spread at the interface (Shenton, 1979). However, this assertion has never been clearly demonstrated. Furthermore, other authors (Mizutani and Nakamura, 1985) estimated that LDL conserve their structure at the interface and that it is this preserved supramolecular assembly that ensure the remarkable interfacial properties of LDL.

This review explains the progress which led us these last years to confirm the dominance of the LDL in the emulsifying properties of hen egg yolk, and which allowed us to clarify the mechanism of adsorption and the structure of the films of LDL.

**Yolk constituents and emulsifying properties**

Emulsifying activity is related to the capacity of surface active molecules to cover the oil-water interface created by mechanical homogenisation, reducing thus the interfacial tension. Consequently, the better the emulsifying agent is active, the better the interfacial tension is lowered. Emulsion stability indicates the capacity to avoid flocculation, creaming, and/or coalescence of oil droplets. Creaming and flocculation are reversible phenomena which can be avoided by a simple agitation of the emulsion. Coalescence is the irreversible fusion of oil droplets due to the rupture of the interfacial film created by emulsifying agents. This phenomenon leads to a complete destruction of the emulsion.

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**Figure 1** Surface-volume diameter ($d_{3.2}$) of oil droplets of emulsions (H:E 30:70) made with yolk, plasma or granules, 25 mg proteins/ml
Searching the principal contributor to yolk emulsifying properties, numerous authors have separated yolk into its main fractions: plasma and granules. Large similarities have been observed between emulsifying properties of yolk and plasma, whereas emulsions made with granules behaved very distinctly (Dyer-Hurdon and Nnanna, 1993; Anton and Gandemer, 1997; Le Denmat et al., 2000). Particularly, emulsions made with granules are more coarse (more important oil droplet size) than emulsions made with yolk and plasma, and notably at acidic pH where granules are not soluble (Le Denmat et al., 2000) (Figure 1).

Concerning the parameters of emulsion stability (creaming), we evidenced (Le Denmat et al., 2000) that emulsions made with yolk and with plasma had the same creaming rate, in function of the medium conditions, whereas emulsions made with granules behaved very differently (Figure 2). Consequently, these studies demonstrated that yolk emulsifying power was situated in plasma.

Among plasma constituents, some authors demonstrated that LDL are better emulsifiers than BSA (Mizutani and Nakamura, 1984) and casein (Shenton, 1979). Even if some authors suggested that, in determined conditions, HDL were more efficient than LDL to form and stabilize O/W emulsions (Hatta et al., 1997; Mine 1998), a large amount of studies confirm the prevalent role of LDL in yolk emulsions. These findings have been confirmed recently (Aluko et al., 1998; Mine and Keeratiurai, 2000, Anton et al., 2003; Martinet et al., 2003). Particularly, we have established that LDL made emulsions finer than HDL, along different conditions of pH and ionic strength (Martinet et al., 2003) (Figure 3). Now, how to explain the exceptional efficiency of LDL at the interfaces?

![Figure 2](image2.png)  
**Figure 2** Creasing index (Icr) of emulsions (H:E 30:70) made with yolk, plasma or granules, 25 mg proteins/ml

![Figure 3](image3.png)  
**Figure 3** Surface-volume diameter ($d_{3,2}$) of emulsions (H:E 30:70) made with yolk, LDL or HDL, 25 mg proteins/ml
Role of LDL structure

Given that any destructurating treatment affects the emulsifying properties of LDL, it appears that the integrity of the structure of LDL seems essential to ensure their interfacial properties (Tsutsui, 1988). **But, is the LDL structure essential during all the process?** It is generally supposed that LDL particles break down when they come into contact with the interface. The lipid core coalesces with the oil phase and apoproteins and phospholipids spread at the interface (Garland, 1973; Shenton, 1979; Kiosseoglou, 1989). The disruption of LDL particles is attributed to a weakening of protein-protein interactions.

Direct adsorption of apoproteins and phospholipids from LDL is not easy because of the non-solubility of these species in water or in aqueous buffer. So the interactions between apoproteins and lipids to assemble the LDL particles are essential to transport the surfactants in a soluble form at the neighbouring of the interface and then to release them at the interface.

Using Langmuir film balance (air-water interface), we have detected three phase transitions in compression isotherms and we have attributed these three transitions (19, 41 and 54 mN/m) respectively to neutral lipids, apoproteins and phospholipids by comparison with films of neutral lipids, phospholipids and total lipids extracted from LDL (Figure 4) (Martinet et al., 2003). 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. These different transitions evidenced that LDL actually break down when they come into contact with the interface to release neutral lipids, phospholipids and apoproteins from the lipoprotein core and to allow their spreading.

![Compression isotherms of LDL and extracted LDL constituents spread at the air-water interface](image)

**Figure 4** Compression isotherms of LDL and extracted LDL constituents spread at the air-water interface, LDL (160 µg), total lipids (287 µg), phospholipids (198 µg), neutral lipids (85 µg), compression rate: 100 cm²/min

So we have deducted that LDL serve as vectors of surfactant constituents (apoproteins and phospholipids) that could not be soluble in water, until the interface. At this step the conservation of the LDL structure is essential. Once LDL are near the interface, the structure is then broken up to release surfactant constituents at the interface (Figure 5). At this moment, we had the certitude that LDL were destructured at the interface but we had no idea about the respective importance of protein and phospholipid parts, and we did not know the mechanism of LDL disruption.
Mizutani and Nakamura (1985) demonstrated that increasing treatments of LDL by proteases (trypsin and papaïn) produced a decrease of their properties of formation and stabilization of emulsions. It was then suggested that only a limited amount of the phospholipids of LDL takes part in the adsorption at the oil-water interface and that the protein part of LDL played the essential role. We have confirmed these findings (Le Denmat et al., 2000) by measuring the interfacial concentration in proteins and in phospholipids in emulsions made with yolk, plasma and granules. We have observed that if interfacial protein concentration was correlated with the granulometry and the stability of emulsions, interfacial phospholipid concentration behaved very differently. This suggests a major role of the protein part.

In another way, Bringe et al. (1996) noticed that emulsifying properties of yolk was not affected by the elimination of triglycerides and cholesterol from LDL. Furthermore, Aluko and Mine (1997) observed that cholesterol was not adsorbed at the oil-water interface. In a recent study (Martinet et al., 2002), we have confirmed the driving contribution of the proteinaceous part of yolk, specially apoprotein of LDL, in the formation and stability of emulsions made with yolk.

Other studies in our laboratory (Sirvente et al., 2004) intended to explain, using extracted phospholipids or apoproteins, or recombined apoproteins-phospholipids vesicles, the respective role of phospholipids and apoproteins in LDL interfacial properties. Compression isotherms of phospholipids organised in small unilamellar vesicule (SUV or liposome) showed a very low value of the surface pressure all along the isotherm. This signifies that liposomes are not able to spread at the air-water interface. In contrast, when recombination of phospholipids/apoproteins were used, values near those obtained with native LDL were observed (2-5 mN/m). This confirms that the protein part of LDL is essential to understand interfacial properties of yolk LDL. Now, it remained to clarify by which mechanism proteins induce the disruption of the LDL.

**Adsorption mechanism of LDL**

Liposomes have a spherical structure surrounded by phospholipids. Furthermore, the hydrodynamic diameter we obtained (50 nm) was close to that of LDL (30-70 nm). The main difference between LDL and liposomes is the presence of surface apoproteins whereas liposomes are constituted of a pure double layer of phospholipids surrounding an aqueous phase. So, we can suggest that the apoproteins situated on the LDL surface start the LDL disruption mechanism by their initial anchorage. This anchorage provokes an unfolding of the protein leading to the destabilization of the external layer of the LDL. Then this phenomenon could be followed by a deformation of the particle due to the creation of a neutral lipid lens conducing to the spreading of the LDL constituents. In the case of liposomes,
without external proteins, the structure remains steady at the interface and then this structure is not able to adsorb efficiently and to decrease interfacial tension (Figure 6).

At this time, it was not clear about the behaviour of phospholipids of LDL during the adsorption, and precisely it was not obvious if phospholipids cohabit at the interface with apoproteins of LDL, forming mixed layers, and/or interact with adsorbed apoproteins.

**Figure 6 Comparison of the adsorptions of LDL and liposomes at the air-water interface**

**Interactions between surfactants at the interface: a mixed film**

We have seen that the compression isotherms of LDL showed three transitions and that we had attributed all these transitions respectively to neutral lipids, apoproteins and phospholipids (Martinet et al., 2003). However, a in a recent study made with atomic force microscopy (AFM) after a Langmuir-Blodgett transfer of the layers from the air-water interface to a silica plate, we have evidenced that the second transition (previously attributed to apoproteins alone) should not be due to apoproteins alone, but to apoproteins-lipids complexes (Dauphas et al, 2006b). Our results have shown that the structures observed into the LDL film are different depending on the surface pressure. The first transition \( (P=19 \text{ mN/m}) \) should be attributed to the free triglycerides collapse, the second transition \( (P=41 \text{ mN/m}) \) should be attributed to the demixion of apoproteins-triglycerides complexes, and the last transition \( (P=54 \text{ mN/m}) \) should be attributed to phospholipid collapse or to demixion of apoproteins-phospholipids complexes.

More precisely, during the compression of the LDL film, we have observed an 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 of domains with irregular edges appearing smooth. After apoprotein-neutral lipid transition \( (P=45 \text{ mN/m}) \), this structure is more heterogeneous and domains looking smooth and others with different roughness, and circles whose the interior is composed of compact grains, appear. So, the apoproteins and the triglycerides miscibility seems to occur until the apoproteins-lipids transition (second transition) before a demixion. The structures observed after the apoproteins-lipids transition should be due to the demixion between apoproteins and neutral lipids. On the other hand, apoproteins and phospholipids seem miscible whatever the surface pressure.

We can remark that these interfacial studies have been realised for practical reasons at the air-water interfaces, so in this case the interfacial film formed by LDL results from proteins-phospholipids and proteins-triglycerides interactions. Whereas, at the oil-water interface (interface actually existing in
emulsions), triglycerides certainly merge with the oil phase and in this case the interfacial film formed by LDL is the result of proteins- phospholipids interactions.

Conclusion

LDL are the main contributors to yolk interfacial and emulsifying properties. These capacities are clearly due to the LDL structure through interactions between amphiphilic apoproteins and phospholipids. Indeed, proteins and phospholipids are the main surface-active compounds coming from hen egg yolk. LDL structure allows the transport through the aqueous phase and until the interface of these amphiphilic species that would be insoluble in an other form. The lipoprotein disruption at the oil-water interface already presumed in several studies concerning LDL emulsifying properties is now confirmed. Proteins are largely implicated in the LDL interfacial disruption through their own anchorage and unfolding. Then LDL spread and constitutive apoproteins and phospholipids adsorb at the interface, and the interfacial films made with LDL are constituted by a mix of apoproteins and phospholipids that assure both the decrease on interfacial tension and the resistance to rupture. This permits the formation and the stability of food emulsions made with yolk.

References


