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Food Chemistry 83 (2003) 175–183

Food Chemistry

www.elsevier.com/locate/foodchem

Chemical and structural characterisation of low-density lipoproteins purified from hen egg yolk

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Received 5 September 2002; received in revised form 22 January 2003; accepted 22 January 2003

Abstract

Low-density lipoproteins (LDL) are considered to be the main contributors to the exceptional emulsifying activity of hen egg yolk. However, the lack of understanding of the molecular basis for LDL functionality is a significant obstacle for good control of yolk emulsions. Consequently, we have attempted to link the structure and the characteristics of LDL with their emulsifying properties. After purification of LDL, we have determined their protein and lipid compositions, their ultrastructure, and then extracted their apoproteins for physicochemical characterisation. LDL are composed of about 12% of proteins and 87% of lipids and present a spherical shape with a mean diameter of about 35 nm. LDL solubility is high, whatever the medium conditions, because of their low density. LDL contain five major apoproteins out of which the apoprotein of 15 kDa is considered to be the most surface-active. After extraction, this apoprotein showed a high proportion of amphipathic α-helix chains, explaining the high capacity of this apoprotein to adsorb at the oil–water interface.

Keywords: Hen egg yolk; Low-density lipoproteins; Purification; Apoprotein structure

1. Introduction

Hen egg yolk is an essential ingredient for the preparation of a large variety of food emulsions, such as mayonnaises, salad dressings and creams. Particularly, it contributes to the formation and the stability of these emulsions by constituting an interfacial film between oil and water. However, yolk is still used empirically and physical properties of yolk emulsions are not entirely controlled because yolk is a complex mixture of several lipoproteins and proteins, the roles of which are not well understood.

Egg yolk consists mainly of 68% low-density lipoproteins (LDL), 16% high-density lipoproteins (HDL), 10% livetins and 4% phosvitins (McCully, Mok, & Common, 1962). All the constituents of yolk have high abilities to adsorb at the oil–water interface and to form films around oil droplets (Anton, 1998; Anton & Gandemer, 1999; Kiosseoglou & Sherman, 1983; Shenton, 1979). Recent works (Anton & Gandemer, 1997; Dyer-Hurdon & Nnanna, 1993; Le Denmat, Anton, & Beaumal, 2000; Martinet, Beaumal, Dalgalarrondo & Anton, 2002) have shown that LDL are likely to play primary roles in the formation and stabilisation of yolk-based emulsions. Consequently, LDL are considered to contribute mainly to yolk emulsifying properties. Furthermore, it has been demonstrated that LDL show better emulsifying properties than other proteins, such as bovine serum albumin (Mizutani & Nakamura, 1985).

LDL are large spherical particles of about 35 nm diameter with a core of triglycerides, cholesterol, and cholesteryl esters surrounded by a layer of apoproteins and phospholipids (Martin, Augustyniak, & Cook, 1964). Among LDL components, apoproteins are the main molecules that take part in the adsorption at the oil–water interface (Kiosseoglou and Sherman, 1983; Mizutani & Nakamura, 1984). Shenton (1979) and Kiosseoglou and Sherman (1983) hypothesised that, at the oil–water interface, LDL are disrupted and then, apoproteins and phospholipids adsorb whereas neutral lipids coalesce with oil droplets. The role of phospholipids in adsorption of yolk components is not completely

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doi:10.1016/S0308-8146(03)00060-8

understood because phospholipids can interact with adsorbed apoproteins or are adsorbed themselves. It is suggested that only a small amount of the phospholipids of LDL takes part in the adsorption at the oil–water interface (Kiosseoglou and Sherman, 1983; Mizutani & Nakamura, 1984, 1985). In a recent study (Martinet et al., 2001), we have confirmed the driving contribution of the proteinaceous part of yolk, especially apoprotein of LDL, in the formation and stability of emulsions made with yolk.

Proteins constituting mainly the interfacial film, have two major roles: (1) to cause a substantial decrease in the interfacial tension due to the adsorption of the proteins at the oil–water interface and (2) to form a mechanical barrier due to the viscoelastic properties of this protein film, which protects against disruption. They also control the colloidal interactions between coated oil droplets, thus regulating aggregation and flocculation.

Many studies have focussed on the relationships between protein structure and composition, and emulsifying properties (Kato & Nakai, 1980; Shimizu, Takahashi, Kaminogawa, & Yamauchi, 1983). The most important factors influencing emulsifying properties of proteins appear to be molecular flexibility, solubility and hydrophobicity (Graham & Phillips, 1979; Kinsella, 1979; Nakai, 1984). These factors are derived from physicochemical characteristics, such as molecular size, amino acid composition and sequence, conformation and net charge. In addition to intrinsic molecular factors, several extrinsic factors, such as method of isolation, pH, ionic strength, interactions with other components, and technological treatments, also affect functional properties of proteins (Kinsella, 1979). Most studies on protein emulsifiers have concerned milk proteins (casein, β-lactoglobulin) and also bovine serum albumin and lysozyme as model proteins (Dickinson, 1994). Until recently, research concerning the emulsifying properties of LDL apoproteins has been hindered by the difficulties in extracting individual apoproteins from these particles (Dalgleish, 1996). We know that apoproteins of LDL consist of 5 major polypeptides between 15 to 130 kDa, the principal one of which is the apoprotein of 130 kDa (Nakamura, Hayakawa & Sato, 1977). Their average isoelectric points are situated in the range of 6.5–7.3 (Kojima & Nakamura, 1985). Amino acid compositions of LDL apoproteins consist of about 40% hydrophobic amino acids (Tsutsui & Obara, 1982) but the structure of these apoproteins has not been intensively studied. Despite the wide use of yolk as an emulsifier in food emulsions, the relationship between yolk LDL apoprotein structure and composition, and lipoprotein emulsifying properties in oil-in-water emulsions remains unclear because of a lack of specific studies.

The aim of this study was to purify LDL from egg yolk and study the composition and structure of LDL and their apoproteins from the specific perspective of emulsifying properties.

2. Materials and methods

2.1. Purification of LDL from egg yolk

Fresh hen eggs were manually broken and albumen was eliminated. 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 (1979). 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 sediment (granules). Plasma was again centrifuged under the same conditions for a complete removal of granules.

Ammonium sulphate (40%) was added to the plasma which was then stirred for 1 h at 4 °C and centrifuged at 10,000 × *g* for 30 min at 4 °C. The precipitate was discarded and the supernatant was dialysed against deionized water for at least 6 h (the bath being changed every 2 h), and then centrifuged at 10,000 × *g* for 30 min at 4 °C. The resulting floating material containing LDL was pooled.

The floating material was dissolved in 0.05 M Tris–HCl buffer (pH 7) and applied to a glass column (4.5×100 cm) of Ultrogel AcA 34 (Sepracor/IBF, Villeneuve-La-Garenne, France). Absorbancy at 220 nm was recorded and samples were eluted with 0.05 M Tris-HCl buffer (pH 7). Ribonuclease A (1.37×10^4 Da), ovalbumin (4.3×10^4 Da), bovine serum albumin (6.7×10^4 Da), aldolase (1.58×10^5 Da), catalase (2.32×10^5 Da), ferritin (4.4×10^5 Da), thyroglobulin (6.69×10^5 Da) and blue dextran (2×10^6 Da) were used for column calibration.

2.2. SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis was used to determine the composition and the relative quantity of apoproteins from LDL. Electrophoreses were run on polyacrylamide gels (stacking: 3.5% and resolving: 10%) with a migration buffer consisting of a 0.05 M Tris–HCl (pH 8.8), 0.4 M glycine and 0.1% SDS solution. The samples were diluted (1:1 v/v) in a dissociation buffer consisting of a 0.125 M Tris–HCl (pH 6.8), 20% glycerol, 10% 2-mercaptoethanol and 4% SDS solution. The proteins were stained with a Coomassie blue solution (0.05% Coomassie blue, 25% ethanol and 10% acetic acid). Destaining procedure used a 7% acetic acid

and 40% ethanol solution. Molecular weights were estimated with the low-molecular weight calibration kit for SDS electrophoresis from Amersham Pharmacia Biotech (Upsalla, Sweden). The gels were scanned on an imaging densitometer Biorad GS710 (Ivry-sur-Seine, France) and the molecular weights and relative quantities were estimated with the Quantity One 4.1 software (Biorad, Ivry-sur-Seine, France).

2.3. Chemical analysis

Dry matter of LDL was determined after desiccation for 48 h at 104 °C and expressed as g of dry matter per 100 g of fresh sample.

Protein content was determined by the procedure of Markwell, Haas, Bieber, and Tolbert (1978). Protein content (between 10 and 100 μg/ml) was calculated using a linear regression equation giving the absorbency of bovine serum albumin solutions as a function of their concentrations. Results were expressed as g of protein per 100 g of dried matter.

Lipid content of LDL was determined after extraction by hexane/isopropanol. Eight millilitres of a 0.73% NaCl solution was added to 1 g of sample, and then 10 ml of hexane/isopropanol (3:2 v/v) were added and mixed before centrifugation at 1000 × *g* during 10 min. The upper organic phase was collected and solvents were evaporated with a rotavapor. Lipid content was estimated by weighing lipid extract after solvent evaporation. Results were expressed as g of lipid per 100 g of dried matter.

Total lipids were fractionated into neutral lipids and phospholipids on Sep-pack silica cartridges (Waters, Milford, USA) by the procedure of Juaneda and Rocquelin (1985).

Phospholipid content (PL) was determined by the measure of phosphorus content (P) in lipid extract (PL = P × 25) (Leseigneur-Meynier & Gandemer, 1991). Results were expressed as g of phospholipid per 100 g of dried matter. Phospholipid composition was determined by high performance liquid chromatography, using a light scattering detector as described by Leseigneur-Meynier and Gandemer (1991). Extracted phospholipid (100 μg) were injected in a silica column (SI 60; 5 μm; 25 cm length × 4.4 mm internal diameter). The column was equilibrated in chloroform. Phospholipids were separated by a gradient of methanol/water/conc. ammonia/chloroform (92:5:2:1) at a flow rate of 1.5 ml/min. Only two phospholipid classes were present in a significant amount: phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The results were expressed as g of PC or PE per 100 g of dried matter.

Cholesterol of LDL was quantified by gas liquid chromatography, as described by Beyer, Milani, Dutelle, and Bradley (1989). Trimethylsilyl derivatives of cholesterol were prepared from 0.5 g of LDL and 5α-cholestan was added as internal standard. The gas chromatograph (HP 5890) was equipped with a flame ionization detector, a split injector and a 7.5 m × 0.32 mm diameter DB5 capillary column (J&W Scientific, Millstadt, USA). The bound stationary phase was a 5% diphenyl–95% dimethyl-polysilicone and the film thickness was 0.1 μm. The operating conditions were: hydrogen carrier gas flow 2 ml/min, split ratio 1/10, injector and detector temperature 300 °C, oven temperature raised from 170 to 230 °C in 10 min. Cholesterol content was calculated using 1.12 as the relative response factor of cholesterol to standard. Results were expressed as g of cholesterol per 100 g of dried matter.

Triglyceride content was calculated as the difference between total lipid, phospholipid and cholesterol contents. Results were expressed as g of triglyceride per 100 g of dried matter.

Fatty acid composition of total, neutral and polar lipids was determined by gas chromatography of fatty acid methyl esters (fame), prepared as described by Morrison and Smith (1964). The gas chromatograph (HP 5890) was equipped with a flame ionization detector, a split injector and a DB225 capillary column (J&W Scientific, Millstadt, USA): 30 m × 0.32 mm diameter, 100% cyanopropylphenyl, 1 μm film thickness. The operating conditions were: hydrogen carrier gas flow 1.5 ml/min, split ratio 1/10, injector and detector temperature 250 °C, oven temperature raised from 150 to 210 °C at a rate of 5 °C/min. Fatty acid peaks were identified by comparing their retention times with those of known standards and expressed as percent of fame.

2.4. Lipoprotein and apoprotein solubility

LDL and apoLDL were diluted with different buffers corresponding to different pH and ionic strength combinations to a final protein concentration of 1 mg/ml. The diluted samples (24 ml) were equilibrated for 1 h at ambient temperature. Four milllitres were taken aside (initial dilution) and 20 ml were centrifuged at 10,000 × *g* for 20 min at 10 °C. Protein content was determined on the initial dilution and the supernatant after centrifugation according to the procedure of Markwell et al. (1979). A calibration curve was established with bovine serum albumin and an index of protein solubility was calculated as mg protein in supernatant/mg protein in initial dilution × 100.

To measure the combined effects of pH and ionic strength, we have used a central composite model design. It is constituted of 10 tries distributed on a circle. The central try is replicated twice to estimate the repeatability. A response surface is then fitted to the experimental points following a quadratic equation. The validity of the surface response is rendered by the R^2 coefficient, expressing the proportion of experimental variance reproduced by the model. The range used for pH was 3–10 and 0.05–0.55 M

for NaCl. The pH–NaCl associations were: pH 6.5–0.05 M NaCl, 6.5–0.3, 6.5–0.55, 3–0.3, 10–0.3, 4.03–0.12, 8.97–0.12, 4.03–0.48, and 8.97–0.48. The buffers used were: glycine-HCl for pH 3.00, sodium acetate for pH 4.03, imidazole-HCl for pH 6.5, Tris–HCl for pH 8.97, and sodium carbonate for pH 10.

2.5. Transmission electron microscopy of LDL

The LDL solutions (1 mg protein/ml in 0.05 M Tris–HCl pH 7, 0.1 M NaCl buffer) were observed using transmission electron microscopy with a JEOL JEM 1010 (Tokyo, Japan) at 80 kV. The LDL solutions were diluted with an equal volume of 2% sodium phosphotungstate (pH 7.4); then a small droplet of this mixture was placed on a Formvar carbon-coated grid and observed with the electron microscope. The following technological treatments were performed on LDL solutions before microscopy preparation: heating at 75 °C/10 min, freezing at −80 °C, hydrostatic high-pressure (5000 bars), and high-pressure homogenisation (165 bars).

2.6. Apoprotein extraction and separation

The LDL solutions were extracted with ether-ethanol (1:3 v/v) for 12 h at −20 °C and centrifuged at 1500 *g* for 10 min at −20 °C. The solvent was removed and the precipitate was extracted again with several volumes of ether-ethanol for 10 min, then centrifuged under the same conditions. Three extractions were carried out and the resulting protein sediment was dried under nitrogen flow.

The protein mixture (2 mg/ml) was dissolved in a 0.05 M Tris–HCl buffer (pH 8.2) containing 0.5% SDS and separated by gel filtration. Samples were applied to a glass column (1.5 cm × 50 cm) of Ultrogel AcA 34 (Sepracor/IBF, Villeneuve-La-Garenne, France) and eluted with the same buffer as above. Optical density was recorded at 220 nm to monitor the column effluent. SDS-polyacrylamide gel electrophoresis was used to identify fractions from the gel filtration.

2.7. N-terminal sequence determination

The exact identity of purified apoprotein C was determined by sequencing the N-terminal extremity following the method of Edman with an Applied Biosystem 477A (Perkin-Elmer, Foster City, USA) sequencer. The hydropathy profile was deducted from the N-terminal sequence of apoprotein C according to the method of Kyte and Doolitle (1982).

2.8. Secondary structure determination

Circular dichroïsm spectra of apoprotein C solution were recorded on a Jobin-Yvon CD6 spectrometer (Longjumeau, France). Measurements were carried out at 25 °C in 0.01 cm path length quartz plates with protein concentration of 1.2 mg/ml in a 0.05 M Tris–HCl buffer (pH 8.2) containing 0.5% SDS. Spectra were recorded over the 190–250 nm wavelength range with 0.2 nm increments and an integration time of 2 s. The baseline-corrected spectra were smoothed by using a third-order least-squares polynomial fit.

2.9. Statistical analysis

Except for the experimental design, three replicates were made for all the measurements. The results of chemical analysis and solubility were subjected to a 1-way analysis of variance using STATGRAPHICS software (Statistical Graphics Corporation, Rockville, USA). Confidence intervals were set at 95% ($P < 0.05$).

3. Results

3.1. LDL composition

Complete purification of LDL was achieved by submitting the floating material extracted with ammonium sulphate to gel filtration chromatography. This step allowed the separation of contaminants corresponding to α, β, and γ livetins (results not shown). We obtained 37.5 g of pure LDL from 100 g of dry yolk.

The electrophoretic pattern showed that LDL consists of five major apoproteins with molecular weights of about 130, 80, 65, 60 and 15 kDa (Fig. 1). Densitometric

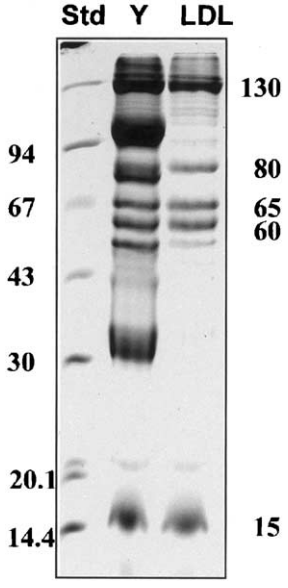

Fig. 1. SDS-polyacrylamide gel electrophoresis of yolk (Y) and LDL solution. Low-molecular weight calibration Kit (Amersham Pharmacia Biotech) was used as protein standard (Std). Stacking and running gels: 3.5% and 10% acrylamide, respectively.

Table 1
Composition of LDL (g/100 g dry matter)

	LDL
Proteins	12.0
Lipids	86.7
Triglycerides (TG)	62.0
Phospholipids (PE)	21.5
Phosphatidylcholine (PC)	18.4
Phosphatidylethanolamine (PE)	3.0
Cholesterol	3.2
Lipids/proteins	7.2
PL/TG	0.35
PC/PE	6.1
Fatty acid composition of LDL (% of fatty acids of total lipids)	
Palmitic acid (C16:0)	24.7
Oleic acid (C18:1)	41.1
Linoleic acid (C18:2)	16.0
PUFA/SFA	0.60

Polyunsaturated fatty acids = PUFA. Saturated fatty acids = SFA

analysis revealed that the relative proportions of the apoproteins were: 34, 16, 16, 19, and 15%.

One hundred grams of dried LDL contain about 12 g of proteins and 87 g of lipids (Table 1). The lipids are composed of about 62 g of triglycerides, and 22 g of phospholipids [out of which 18.4 g was phosphatidylcholine (PC), 3.0 g phosphatidylethanolamine (PE)] and 3.2 g of cholesterol. This corresponds to 71% of triglycerides, 25% of phospholipids and 4% of cholesterol on the basis of total lipids. Consequently, the ratios PL/TG and PC/PE were, respectively, 0.35 and 6.1.

LDL total lipids were composed of about 34% of saturated fatty acids (SFA), 45% of monounsaturated fatty acids and 21% of polyunsaturated fatty acids (PUFA). The main fatty acids were 41% oleic acid (C18:1), 25% palmitic acid (C16:0) and 16% linoleic acid (C18:2). The PUFA to SFA ratio was 0.60.

3.2. LDL solubility

LDL solubility was high (more than 90%), whatever the pH and salt concentration (Fig. 2). With a density close to that of water (0.98; Martin et al., 1964), LDL do not sediment on centrifugation. This point is relevant as it means that LDL particles do not precipitate and hence are available, whatever the medium conditions, to participate at the formation of the interfacial film between oil and water.

3.3. LDL ultrastructure

An electron micrograph (TEM) of LDL, negatively stained with 2% sodium phosphotungstate, is shown in Fig. 3. Particles of non treated LDL (Fig. 3A) appeared spherical, with heterogeneous sizes between 20 and 60

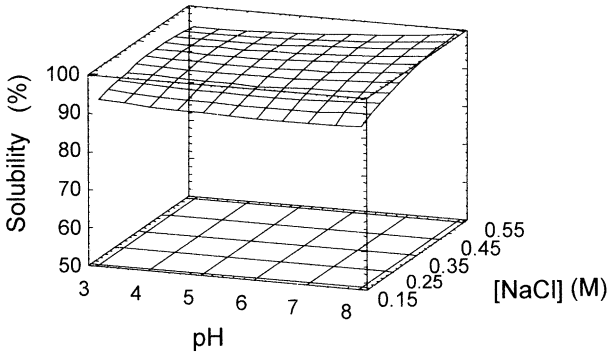

Fig. 2. Effect of pH and ionic strength on the solubility of LDL. Protein concentration: 1 mg/ml.

nm diameter and the average diameter was situated between 35 and 40 nm. Contiguous particles have flattened edges, and these polygonal shapes could be the result of particle aggregation due to dehydration on grids or to vacuum in the electron microscope during analysis. Furthermore, the same sample of LDL was analysed by photon correlating spectroscopy (results not shown) and gave an average particle diameter of 35 nm, which was in close agreement with the average value obtained from the present electron micrograph of LDL solution. Our results agree with typical values found in the literature. Martin et al. (1964) gave diameters ranging from 17 to 60 nm. Furthermore, we observed some structures of about 200 nm diameter surrounded by aggregated LDL. It is possible that these structures are very low density lipoproteins, as observed by Martin et al. (1964) or merged LDL, as noticed for human plasma LDL (Ala-Korpela, Pentikäinen, Korhonen, Hevonoja, Lounila, & Kovanen, 1998).

Considering the technological treatments, when LDL are heated for 10 min at 75 °C, we observed (Fig. 3B)

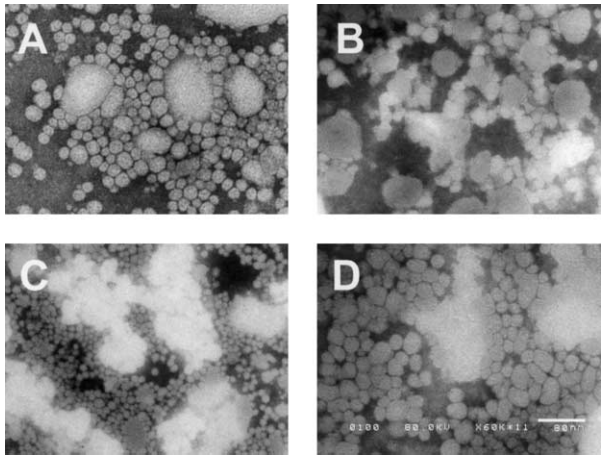

Fig. 3. Electron micrographs (TEM) of different LDL solutions after physical treatments. LDL solutions: 1 mg protein/ml in 0.05 M tris–HCl pH 7, 0.1 M NaCl buffer, coloration with 2% sodium phosphotungstate (pH 7.4). A: LDL control (×50,000). B: 75 °C/10 min (× 50,000). C: 5000 bars/10 min (× 30,000). D: −80 °C (× 60,000).

the result of a disruption and a rearrangement of the fragments into big clusters (average size of about 300 nm). The treatment by high hydrostatic pressure (5000 bars) also brought about a disruption, followed by a rearrangement of the fragments into clusters of about 90 nm diameter (Fig. 3C).

Conversely, the electron micrograph of LDL, frozen for 12 h at −80 °C and subsequently thawed at room temperature, showed (Fig. 3D) that this treatment did not change the structure of LDL.

Furthermore, (results not shown) the structure of LDL was not altered by passage through a high pressure homogeniser at up to 250 bars or through a rotor/stator homogeniser. This meant that, during emulsion formation, the homogenisation step did not destroy LDL.

3.4. Apoprotein solubility

Apoproteins extracted from LDL with ether-ethanol were re-hydrated with different buffers of varying pH and ionic strength. Protein solubility of these dispersions was analysed (Fig. 4). It was observed that acidic and neutral pH were not favourable to an efficient solubilisation of LDL apoproteins, whatever the ionic strength. Conversely, at basic pH (12) and at low ionic strength (0.1 M NaCl), there was a maximum protein solubility of about 30%.

3.5. Apoprotein separation

Apoproteins extracted from LDL by delipidation with ether-ethanol were completely solubilised in a 0.1 M Tris–HCl buffer (pH 8.6) containing 0.5% SDS. Gel chromatography resolved apoproteins of LDL into three distinct peaks (Fig. 5). The first peak (A) contained apoproteins from 80 to 130 kDa (apoproteins A), the second (B) contained two apoproteins: 60 and 65 kDa (apoproteins B), and the third (C) contained the apoprotein of 15 kDa in a nearly pure state (apoprotein C), as demonstrated by SDS gel electrophoresis (Fig. 5).

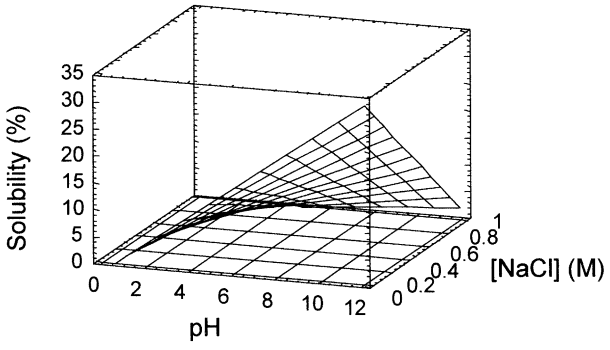

Fig. 4. Effect of pH and ionic strength on the solubility of LDL apoproteins. Protein concentration: 1 mg/ml.

3.6. Apoprotein C sequence

The N-terminal amino acid sequence of apoprotein C of yolk LDL was established from sequence determination of peptides derived from Edman degradation (Fig. 6). The sequence has been established as far as the 49th amino acid. After screening banks of protein sequences, we observed over 90% homologies with the known sequence of hen blood apo VLDL-II. Consequently, apo VLDL-II could be an efficient model for studying apoprotein C of yolk LDL.

According to the N-terminal amino acid sequence of apoprotein C of yolk LDL and apo VLDL II of hen blood, their hydropathy profiles (Fig. 7) have been deduced. The profiles exhibited an amphipolar structure with a succession of hydrophobic and hydrophilic domains, making these apoproteins potentially good surface active agents.

3.7. Secondary structure of apoproteins

Circular dichroïsm, showed (Fig. 8), whatever the apoprotein, a double minimum at 208 and 222 nm, and a maximum at 191–193 nm, synonymous with a considerable proportion of α-helix chain. Sodium dodecyl sulphate (SDS) was used to solubilize apoproteins and it is suggested that SDS could lead to a denaturation and

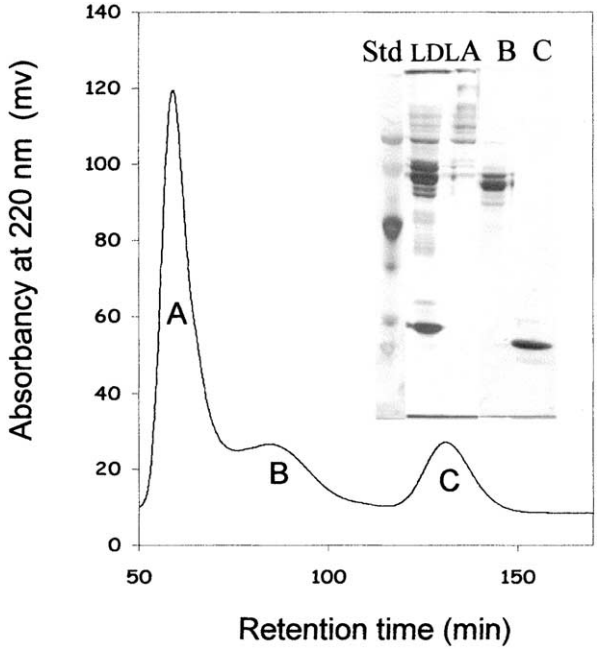

Fig. 5. Gel filtration chromatography of apoproteins extracted from LDL and SDS-polyacrylamide gel electrophoresis of LDL and LDL apoproteins. Chromatography: Ultrogel AcA 34 column (Sepracor/IBF), protein concentration: 2 mg/ml, in a 0.05 M Tris–HCl buffer (pH 8.2) containing 0.5% SDS. Electrophoresis: Low-molecular weight calibration Kit (Amersham Pharmacia Biotech) was used as protein standard (Std). Stacking and running gels: 3.5% and 10% acrylamide, respectively.

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                  1                  11                  21
Apo C             KSIIDRERRD   LLVIPDAAAA   YIYEAVNKVS
ApoVLDL II        KSIIDRERRD   TLVIPDAAAA   YIYEAVNKVS

                31                  41                  51
Apo C             PR-AGQFLLD   -VSQTTVV
ApoVLDL II        PRRAGEFLLD   TVSQT-VVSGI  NFLINTAER

                61                  71                  81
ApoVLDL II        LTKLAEQLME   KIKDLCYTKV   LG
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Fig. 6. N-terminal amino acid sequence of apoprotein C of yolk LDL and apoprotein VLDL II of hen blood.

a loss of biological activity of proteins, and indeed an induction of α-helix structures (Montserret, McLeish, Bockmann, Geourjon, & Penin, 2000). But, in the case of membrane proteins or amphiphilic proteins, such as LDL apoproteins, SDS is known to mimic the hydrophobic environment existing in biological membranes without changing protein conformation (Montserret et al., 2000).

4. Discussion

4.1. Structure and composition of yolk LDL

The bulk composition, the proportions of proteins, total lipids and phospholipids are in good agreement with those noticed previously (Martin et al., 1964). The fatty acid composition of total lipids was also in accordance with the literature (Kuksis, 1992). However, it is known that the nature of dietary fatty acids significantly influences the fatty acid composition of total lipids of yolk LDL (Kuksis, 1992).

There were five major apoproteins for LDL, in accordance with previous studies (Itoh, Abe & Adachi, 1983; Yamauchi, Kurisaki & Sasago, 1976). Yamauchi et al. (1976) indicated that 130 and 65 kDa apoproteins were glycoproteins and that the apoprotein of 15 kDa (apoprotein C) was unique in containing SH groups. Furthermore, in the present study, after sequencing the N-terminal chain of apoprotein C, there was a very important homology with apo VLDLII, a blood apoprotein of laying hen. Apo VLDL II contains two identical polypeptide chains of 82 amino acid residues which are linked by a single disulfide bond at residue 76 (Jackson, Lin, Chan, & Means, 1977). Consequently, we consider that apo VLDL II could be a good model for improving understanding of the structure-function relationships of yolk LDL apoprotein C.

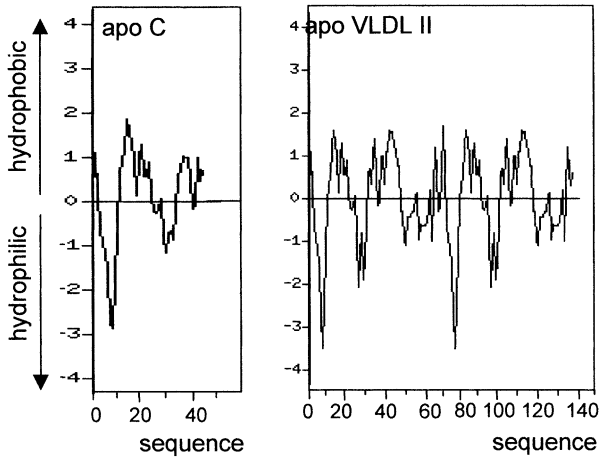

Fig. 7. Hydropathy profile of apoprotein C of yolk LDL and apoprotein VLDL II of hen blood, with hydrophobicity index based on Kyte and Doolittle (1982). Regions above the dotted line indicate hydrophobic domains while regions below indicate hydrophilic domains. The profile was computed using an interval of nine amino acids.

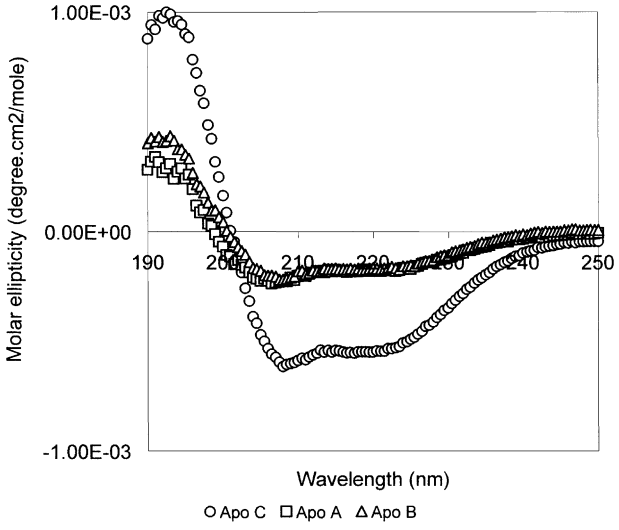

Fig. 8. Far UV CD spectra (190–250 nm) of apoproteins A, B and C of yolk LDL. Protein concentration: 1.2 mg/ml, in a 0.05 M Tris–HCl buffer (pH 8.2) containing 0.5% SDS.

4.2. Relationship between structure/composition and emulsifying properties of yolk LDL

It seems that LDL are soluble whatever the pH and ionic strength conditions, and that heat treatment (75 °C) or static high-pressure (5000 bars) alter the integrity of yolk LDL, while homogenisation (rotor/stator or dynamic high-pressure) has very limited effects. These results mean that LDL can be used to prepare emulsions under a wide range of conditions, due to their excellent solubility and that, after homogenisation, LDL micelles are in their native state.

Further evidence shows that LDL are disrupted at the oil–water interface (Kiosseoglou and Sharman, 1983; Shenton, 1979), and these findings have recently been confirmed using a Langmuir balance (Martinet, Saulnier, Beaumal, Courthaudon, & Anton, 2002). The mechanism whereby LDL micelles are disrupted at the oil–water interface is not known but it is likely that LDL protein–protein bonds are weakened by surface forces (Kiosseoglou and Sherman, 1983). Then, liberated triglycerides merge with the oil phase whereas phospholipids and apoproteins compete to adsorb at the oil-water interface. The liberated apoproteins are supposed to be totally insoluble and the adsorption process almost irreversible. This is corroborated in the present study, as it is demonstrated that level and range of LDL apoprotein solubility are very limited (30% at basic pH and low ionic strength). Under turbulent conditions occurring during homogenisation, adsorption of non-soluble proteins is generally observed, due to convective movements (Walstra, 1983). Under these conditions, the poor solubility observed for LDL apoproteins would not be a drawback for emulsion formation.

Previous studies (Martinet et al., 2001), have demonstrated the preferential adsorption at the oil–water interface of the 15 kDa yolk LDL apoprotein (apoprotein C). The strong amphipathic nature of apoprotein C, results in a high adsorbing activity at the oil–water interface.

The yolk apoproteins bound to the phospholipid layer of LDL particles are very different from aqueous proteins. In this phospholipid layer, the majority of apoprotein hydrophobic groups, are in exposed sites over the outer surface, whilst hydrophilic groups tend to face inwards, protected from the lipidic medium. This inverted configuration would account for the great affinity of the apoproteins for the lipid phase, once liberated.

Generally, α-helices are amphipathic, containing two clearly defined faces, one of which is hydrophilic (due to distribution of charged amino acid residues) and the other hydrophobic (Segrest, Garber, Brouillette, Harver, & Anantharamaian, 1994). It is likely that α-helices are spread on the hydrophobic side at the oil–water interface, covering a majority of the interfacial area and therefore controlling the decrease of interfacial tension and the interactions between oil droplets. These findings are supported by results of Camejo, Colacocco, and Rapport (1968), showing that apoproteins of lipoproteins have an exceptionally great penetrating power at the oil–water interface as compared to globular albumin. Furthermore, competition studies have shown that capacities of LDL apoproteins to displace other proteins from the oil–water interface were characteristic of very flexible proteins (Mine & Keeratiurai, 2000). It is likely that disordered structures, existing between α-helix domains, improve the flexibility of apoprotein C. Therefore, it appears that apoprotein C may be able to rearrange rapidly at the interface and cover the majority of the interfacial area and therefore control the interactions between oil droplets.

Consequently, it seems that apoproteins of yolk LDL, and particularly the apoprotein of 15 kDa, have a great capacity to adsorb at the oil–water interface in emulsions, due to their structure and composition, which combines amphipathic character and flexibility. This could explain the excellent emulsifying properties of egg yolk. We have now to confirm these findings by directly studying the adsorption capacity of the apoprotein C at the oil–water interface and also defining the role of phospholipids in this adsorption.

Acknowledgements

We thanks Pays de la Loire Province and INRA TPA Department for providing V. Martinet a Ph.D. grant to carry out this study.

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