Adsorption Properties of Cholesterol-Reduced Egg Yolk Low-Density Lipoprotein at Oil-in-Water Interfaces

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The adsorption properties of cholesterol-reduced egg yolk low-density lipoprotein (CR-LDL) at oil-in-water interfaces were studied. The CR-LDL was prepared by absorbing cholesterol to β-cyclodextrin (CD). The CR-LDL formed larger emulsion particles at low protein concentration. Concentration of protein at the interface was greater for emulsions made by CR-LDL when compared to the control LDL at pH 7.0 and 3.5, a result attributed to formation of lipoprotein aggregates by removing the cholesterol in LDL. The emulsion stability of CR-LDL at low protein concentration was lower at pH 7.0 than at pH 3.5, whereas the LDL emulsions showed considerable stability during aging for 1 month. Electrophoretic analysis of the adsorbed polypeptides revealed the preferential adsorption of LDL polypeptides at the interface. Increase in protein concentration resulted in higher phosphatidylethanolamine (PE) and lower phosphatidylcholine (PC) levels at the interface, whereas the opposite trend was observed at pH 3.5. Time-dependent polymerization of lipoproteins through hydrophobic interaction at the interface was detected by SDS−PAGE analysis. Removing the cholesterol from egg yolk LDL caused changes in phospholipid−protein interactions at the interface, which could be explained the instability of CR-LDL emulsion.

Keywords: *Egg yolk; low-density lipoprotein; cholesterol; β-cyclodextrin; emulsion; stability; phospholipids*

INTRODUCTION

Hen's egg yolk provides excellent functional properties to a variety of food products such as mayonnaise, ice cream, bakery items, and salad dressings. Egg yolk contains various emulsifying agents such as hydrophobic and hydrophilic proteins, phospholipids, and cholesterol (Kiosseoglou and Sherman, 1983; Carrillo and Kokini, 1988). Egg yolk consists of a soluble plasma (~78% of the total liquid yolk) that is composed of livetins and low-density lipoprotein (LDL) (McCully et al., 1962). LDL contains ~12.5% protein and ~80% lipids. The lipid in LDL consists of 70% neutral lipid, 26% phospholipids [71−76%, phosphatidylcholine (PC), 16−20% phosphatidylethanolamine (PE), and 8−9% sphingomyelin and lysophospholipids], and 4% free cholesterol (Martin et al., 1963). LDL has been considered the major factor governing the emulsifying properties of egg yolk. Protein−phospholipid complexes (lipoproteins) are the components of egg yolk responsible for stabilizing an emulsion (Vincent et al., 1966; Mizutani and Nakamura, 1985). In an emulsion prepared with egg yolk, the contribution of proteins to emulsifying activity is higher than that of phospholipids (Bringe et al., 1996). The egg yolk proteins exhibit a higher adsorbing capacity than globular proteins, because they have a more flexible structure and a greater surface hydrophobicity (Kiosseoglou and Sherman, 1983). The emulsifying properties and heat stability of protein emulsion were improved substantially through the formation of a complex between lysolecithin and free fatty acids (Mine et al., 1992, 1993, 1994). Emulsifying capacity and heat stability of egg yolk were also improved by fermentation with pancreatic phospholipase (Dutilh and Groger, 1981). These results indicate that the emulsifying properties of egg yolk lipoproteins might be closely related to the structure of phospholipid−protein complexes and their interactions at an oil-in-water interface. On the other hand, concerns regarding the relationship between cholesterol or oxidized cholesterol products and coronary heart disease have resulted in various technologies being examined to reduce the cholesterol content in egg yolk (Anonymous, 1988; Froning, 1994). These include solvent extraction (Warren et al., 1988), supercritical fluid extraction (Froning et al., 1990), microbial and enzymatic degradation (Dehal et al., 1991), and complexing with β-cyclodextrin (CD) (Smith et al., 1995). The removal of cholesterol by adsorption to CD is an alternative approach. A few papers have reported on the functional properties of low-cholesterol egg yolk (Froning et al., 1990; Paraskevopoulou and Kisseoglou, 1995; Bringe et al., 1996; Awad et al., 1997). However, no information is available on adsorption behavior and the effect of cholesterol reduction from egg yolk on its phospholipid−apoprotein interactions at an oil-in-water interface. The objective of this study is to investigate the adsorption properties of cholesterol-reduced LDL (CR-LDL) and phospholipid−protein interactions at an oil-in-water interface.

MATERIALS AND METHODS

Preparation of CR-LDL from Egg Yolk. LDL was prepared from fresh egg yolk according to a modification of the method of Raju and Mahadevan (1974). LDL concentration was determined from protein concentration using a modified Lowry procedure (Markwell et al., 1978). Extraction

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Table 1. Lipid Composition of Egg Yolk LDL and CR-LDL[a]

	mg/g of solid				
sample	triglycerides	cholesterol	phosphatidylcholine	phosphatidylethanolamine	others
LDL (control)	497.6[a]	47.2[a]	213.6[a]	28.8[a]	13.7[a]
CR-LDL (1)	512.8[b]	24.3[b]	195.6[b]	23.6[b]	12.6[a]
CR-LDL (2)	516.1[b]	3.4[c]	193.8[b]	26.6[a]	4.7[b]

[a] Means within a column with the same letter are not significantly different at the 0.05 level ($n = 4$).

of cholesterol from LDL was carried out using CD. The LDL solution (6.0%, w/v) was heated to 50 °C in a water bath, and CD (Wacker Chemicals, Adrian, MI) was added at CD/cholesterol molar ratios of 2 and 4. The sample was mixed for 45 min at 50 °C and cooled at 4 °C for 1 h. The slurry was centrifuged for 30 min at 8000*g* at 10 °C. The supernatant containing the CR-LDL was decanted and used for the preparation of emulsions. The determination of egg yolk lipid composition and the reduction ratio of cholesterol in CR-LDL were measured using flame ionization detection (TLC-FID) on an Iatroscan system (Iatroscan MK-5, Iatron Laboratories, Inc., Tokyo, Japan); the procedure is described in detail below.

Purification of Triolein. Crude triolein (65% purity) was purchased from Sigma Chemical Co. (St. Louis, MO). It was purified by silica gel column chromatography (hexane/diethyl ether, 97:3, v/v), the solvent was evaporated in a rotary evaporator, and the triolein was saturated with nitrogen gas (Aluko and Mine, 1997). The purity of the triolein preparation was >99% after analysis by TLC-FID using Iatroscan system.

Determination of Emulsifying Properties. The LDL and CR-LDL preparations were diluted with various buffers (50 mM acetate and imidazole buffers containing 0.1 and 1.5 M NaCl, pH 3.5 and 7.0, respectively) to give a final LDL concentration of 0.44−4.0% (w/v) in the aqueous phase. Emulsions were prepared by homogenizing 2.0 mL of each LDL or CR-LDL solution with 0.5 mL of pure triolein (>99%) for 1 min at a speed of 22 000 rpm using a Polytron PT 2000 homogenizer (Brinkmann Instruments, Inc., Westbury, NY). Each emulsion was mixed with 2 volumes of 0.1% sodium dodecyl sulfate (SDS) solution to prevent flocculation, and the droplet size distribution ($d_{3.2}$) of emulsions was determined using Milli-Q water as a dispersant on a Mastersizer X (Malvern Instruments Ltd., Malvern, U.K.) with optical parameters defined by the manufacturer's presentation code 0303. The emulsions were centrifuged at 20 °C and 5000*g* for 30 min, and the cream was washed with 5 mL of appropriate buffer with each washing followed by centrifugation. The subnatants were pooled together and filtered through a 0.22 μm filter. The protein contents were determined according to the modified Lowry method (Markwell et al., 1978). Since the particle size distribution of the emulsions (from Mastersizer results) showed that <0.5% of the droplets were <0.3 μm, the filtrate should not contain emulsified particle to the extent that the protein content of the subnatant solutions will be overestimated. The surface concentration was estimated as the difference between protein concentration of the subnatant solution and the total protein used to make the emulsion. The washed cream was treated with a solution of 10% (w/v) SDS in 0.1 M Tris buffer, pH 8.0, containing 5% (v/v) 2-mercaptoethanol (ME). The protein composition of the supernatant was determined by SDS−polyacrylamide gel electrophoresis (SDS−PAGE) on 4−15% gradient gels using the Bio-Rad Mini Protean II electrophoresis cell at a constant voltage of 20 mA/gel. The gels were stained using a Coomassie blue procedure followed by destaining with a solution containing acetic acid/methanol/water (1:8:12, v/v/v).

Lipid Analysis of LDL, CR-LDL, and Oil-in-Water Interfaces. The creams were extracted with ≈5 volumes of chloroform/methanol (2:1, v/v), and the solvent layer was evaporated using a rotary evaporator; the residue was dissolved in 5 mL of 5% (v/v) ethyl acetate in hexane and loaded onto a prepacked Sep-Pak silica cartridge (Waters Co., Milford, MA). The column had been previously dehydrated in succession with 5 mL of ethyl acetate, 10 mL of 50% (v/v) ethyl acetate in acetone, 5 mL of ethyl acetate, and 20 mL of hexane. The unadsorbed lipids (triolein) were washed off the column with 20 mL of 5% (v/v) ethyl acetate in hexane, and a portion of the eluate was collected and analyzed as described below for phospholipids and cholesterol. The phospholipids and cholesterol were then eluted from the column with 20 mL of methanol/water (98:2, v/v), and all of the eluate was collected and transferred into evaporation flasks. The contents of the flasks were evaporated to dryness on a rotary evaporator, and the residue was dissolved in 0.1 mL of chloroform/methanol (2:1, v/v) solvent. For lipid composition analysis of LDL and CR-LDLs, lipids were extracted with 5 volumes of chloroform/methanol (2:1, v/v) and the solvent layer was evaporated as described above. The triglyceride, phospholipid, and cholesterol contents were then analyzed by TLC-FID using the Iatroscan system. The samples were developed for TLC-FID as follows: 1 μL of sample was applied onto the Chromarods (Chromarod-S3), dried, and developed first in chloroform/methanol/water (70:30:3, v/v/v) for 10 min. The Chromarods were dried and developed in a second solvent system containing petroleum ether/diethyl ether/acetic acid (80:30:0.2, v/v/v) for 30 min. After drying, the Chromarods were loaded onto the Iatroscan system, and the area under each peak was determined and used to calculate the concentration of PC, PE, and cholesterol. Egg PC (QP Corp., Tokyo, Japan), cholesterol (Sigma), and bovine liver PE (Avanti Polar Lipids Inc., Alabaster, AL) were used as standards.

Statistical Analysis. Lipids composition and cholesterol reduction of LDL and CR-LDL were performed using a Statistical Analysis System (SAS, 1988) program. Student's *t* test was used to determine significant differences between treatments. The level of statistical siginificance was 0.05.

RESULTS AND DISCUSSION

Cholesterol Reduction from Egg Yolk LDL. The control LDL contained 14.5% protein and 79.5% lipid, whereas 48.5% and 92.7% CR-LDLs contained 77.1 and 74.5% lipid, respectively. The decreased lipid content of CR-LDL was attributed to removal of cholesterol and other lipid components by CD. The significant decrease of protein was not observed by removing cholesterol from LDL. Table 1 shows lipid composition of LDL and CR-LDL. Cholesterol content of LDL comprised 5.9% (w/w) of the total lipid by TLC-FID analysis. Triglycerides are the major lipid in LDL. The slight increase in triglyceride concentration in CR-LDL might be due to losses of other lipids during processing. The major egg yolk phospholipids are PC and PE. The concentration of PC and PE was slightly lower ($P < 0.05$) in CR-LDL than in control LDL, probably due to their partial absorption to CD during the cholesterol reduction process. Table 2 shows the effect of the CD/cholesterol molar ratio on the extraction of cholesterol from egg yolk LDL. The reductions of cholesterol in LDL were 48.5 and 92.7% for molar ratios of 2 and 4 of CD/cholesterol, respectively. The removal of cholesterol from liquid egg yolk by adsorption to CD has been described by several researchers (Haggin, 1992; Smith et al., 1995). The most important factors influencing cholesterol reduction were dilution of egg yolk to a defined water/solid ratio (2.9) and CD concentration at a CD/cholesterol molar ratio of 4.0 (Smith et al., 1995). CD remaining in the

Table 2. Effect of CD/Cholesterol Molar Ratio on the Reduction of Cholesterol from Egg Yolk LDL[a]

sample	ratio of CD/cholesterol (molar ratio)	cholesterol reduction (%)	residual CD (%)
LDL (control)	0	0	0
CR-LDL (1)	2	48.5 ± 0.7	0.025 ± 0.004
CR-LDL (2)	4	92.7 ± 0.9	0.074 ± 0.008

[a] Data are the average of triplicate measurements ± standard deviation.

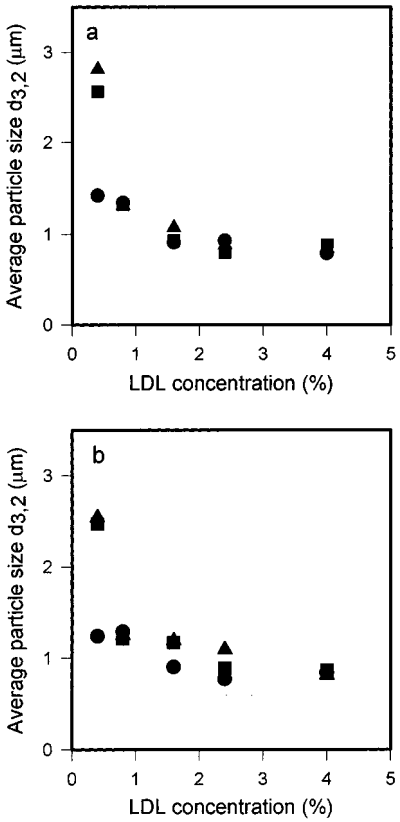

Figure 1. Mean droplet size of emulsions (20% oil, v/v) stabilized by egg yolk LDL (●), 48.5% CR-LDL (■), and 92.7% CR-LDL (▲) as a function of LDL concentration at pH 7.0 (a) and 3.5 (b).

sample after centrifugation was determined using a freshly prepared phenolphthalein solution (Smith et al., 1995). The value for each sample was negligible.

Emulsifying Properties of CR-LDL. The change in particle size of the emulsions as a function of LDL concentration and pH is shown in Figure 1. The mean particle size of emulsions decreased with increase of LDL concentration used to make the emulsion. Generally, there was a decrease in particle size with increasing amount of emulsifier. The latter observation is supported by the fact that an increase in the concentration of surface active agents generally leads to a reduction in interfacial surface tension of the droplets, facilitating their breakup into smaller droplets (Parker, 1987). At low surfactant concentrations, the system produces larger emulsion droplets, since there is insufficient surfactant to cover all of the freshly created oil surface so that the droplet size increases. At high surfactant concentration, the system produces maximal interfacial area under the preceding thermodynamic conditions. At concentrations of LDL > 2.4%, the droplet sizes of emulsions plateaued at 0.8−0.9 μm. There was no noticeable change in particle size for pH

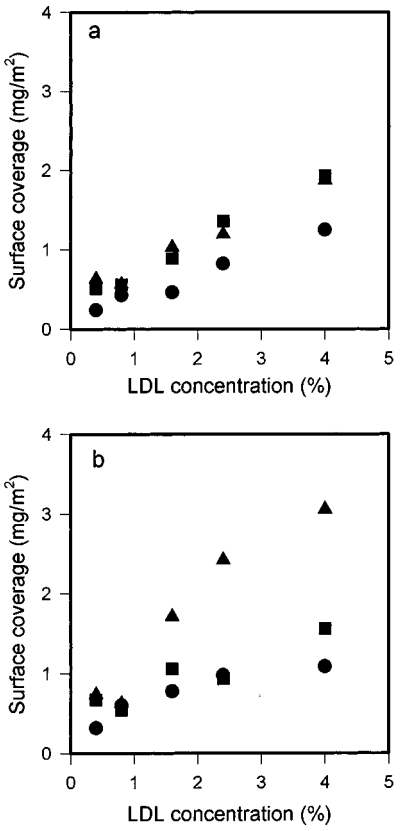

Figure 2. Surface protein coverage of emulsions (20% oil, v/v) stabilized by egg yolk LDL (●), 48.5% CR-LDL (■), and 92.7% CR-LDL (▲) as a function of LDL concentration at pH 7.0 (a) and 3.5 (b).

7.0 and 3.5. The CR-LDL formed larger emulsion droplets than LDL at 0.4% at pH 7.0 and 3.5. The results would indicate that LDL is a better emulsifier at low concentrations than CR-LDL at neutral and acid pH values. Figure 2 shows the amount of protein present on the surface of the emulsion as a function of LDL concentration. At pH 7.0, the surface protein concentration of LDL emulsion increased from 0.24 to 1.25 mg/m^2 with increasing LDL concentration. The concentration of protein at the interface was greater for emulsions stabilized with CR-LDL at pH 7.0. However, there was no noticeable difference between 48.5 and 92.7% CR-LDL. On the other hand, the surface protein concentration was markedly different at pH 3.5. The LDL surface protein ranged from 0.32 to 1.08 mg/m^2. However, the 48.5 and 92.7% CR-LDL formed much thicker films, ranging from 0.67 to 1.56 and from 0.74 to 3.01 mg/m^2, respectively. These data suggest that the adsorption behavior of CR-LDL at an oil-in-water interface is different with LDL at pH 3.5. The higher surface protein concentration of 92.7% CR-LDL at pH 3.5 when compared to the control LDL can be explained on the basis of increased coagulation of lipoproteins at low pH values. More recently, we reported that egg yolk LDL micelles break down when the micelles come into contact with the interface and that rearrangement of lipoproteins, cholesterol, and phospholipids occurs following adsorption at an oil-in-water interface (Mine, 1998). ^{31}P NMR and enzymatic hydrolysis studies showed that the membrane fluidity of egg yolk LDL is high and the interactions of protein−phospholipid may not be so strong (Mine, 1997) as proposed by Burley (1975). There is no information regarding the role of cholesterol on structural change of egg yolk LDL. In

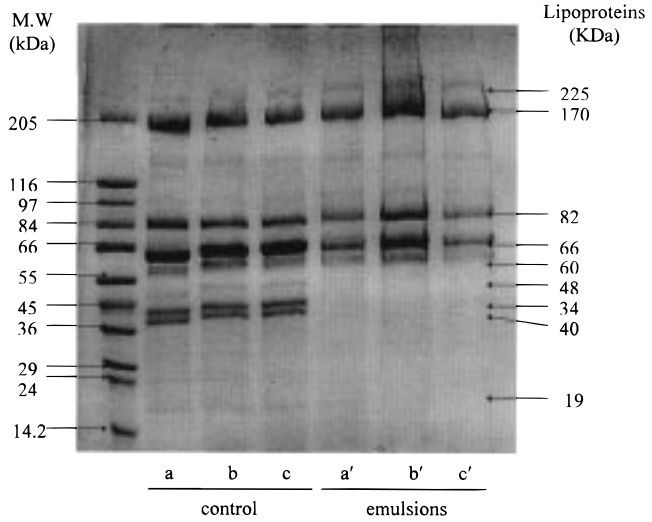

Figure 3. SDS−PAGE profiles of egg yolk lipoproteins: (lanes a a′) LDL; (lanes b, b′) 48.5% CR-LDL; (lanes c, c′) 92.7% CR-LDL; (lanes a−c) control; (lanes a′−c′) emulsions stabilized by 2.4% LDL or CR-LDLs. Left lane is molecular markers: 205 kDa (myosin), 116 kDa (*β*-galactosidase), 97 kDa (phosphorylase *b*), 84 kDa (fructose-6-phosphate kinase), 66 kDa (bovine serum albumin), 55 kDa (glutamic dehydrogenase), 45 kDa (ovalbumin), 36 kDa (glyceraldehyde-3-phosphate dehydrogenase), 29 kDa (carbonic anhydrase), 24 kDa (trypsinogen), 14.5 kDa (trypsin inhibitor).

general, it is believed that cholesterol is an important component in stabilizing biological cell membranes. Removing the cholesterol from egg yolk LDL may cause the structural change of phospholipid−protein interaction in LDL micelles. These changes may be the major factor for forming thicker films when LDL micelles come into the interface.

Protein Composition at the Oil-in-Water Interface. It has been reported that LDL consists of about six major polypeptides that range in molecular mass from about 10 to 180 kDa and several unidentified minor polypeptides (Raju and Mahadevan, 1974; Burley and Sleigh, 1980). SDS−PAGE analysis indicated LDL was composed of nine major polypeptides of 19−225 kDa and some minor polypeptides. From the migration patterns of polypeptides in SDS−PAGE gels (Figure 3), the preferential adsorption was observed among polypeptides in LDL emulsions. Almost all major polypeptides with high molecular mass (>60 kDa) in LDL components were adsorbed on the oil surface, but four major polypeptides with molecular mass <48 kDa remained in serum. Such results were obtained with CR-LDL emulsions at LDL concentration ranging from 0.4 to 4.0%. Even at the lower concentration (0.8%) of LDL, these four polypeptides did not adsorb at an oil-in-water interface. The molecular sizes of these unadsorbed polypeptides were estimated at about 48, 43, 40, and 19 kDa, respectively.

Phospholipids and Cholesterol Compositions at the Interface. The compositions of cholesterol and phospholipids at the oil-in-water interfaces are shown in Figure 4. The emulsifying properties of egg yolk LDL have been attributed in part to the phospholipid−protein complex, which can interact with the oil phase through hydrophobic groups and also with the aqueous phase through the charged phospholipid molecules. The percentage of phospholipids and cholesterol in the LDL was PC, 66.6%, PE, 19.0%, and cholesterol, 16.4%. These results were similar to previously reported values

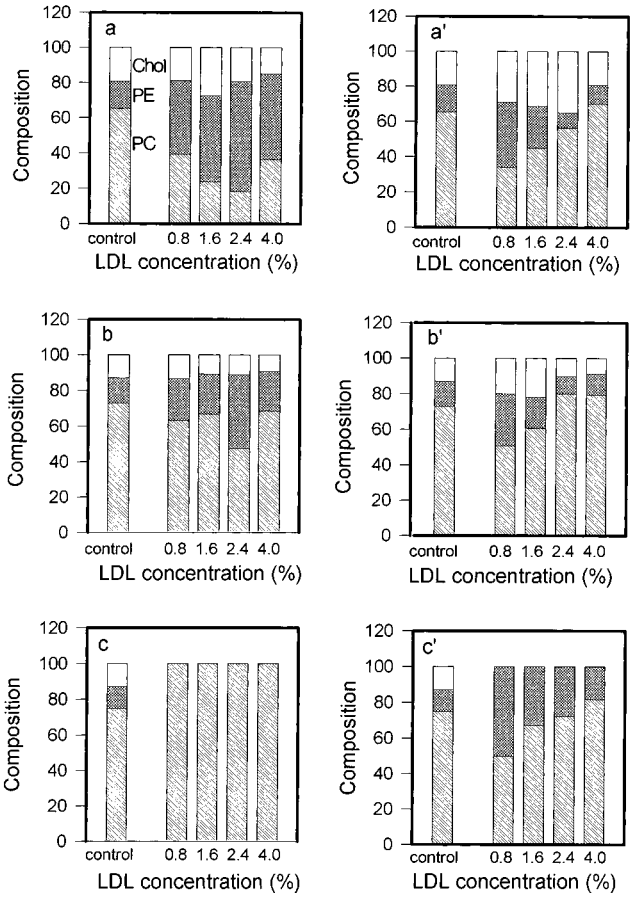

Figure 4. Composition of cholesterol and phospholipids at oil-in-water interfaces stabilized by egg yolk LDL (a, a′), 48.5% CR-LDL (b, b′), and 92.7% CR-LDL (c, c′) as a function of LDL concentration at pH 7.0 (a−c) and 3.5 (a′−c′).

(Martin et al., 1963). At pH 7.0, PC level at the interface decreased while that of PE increased with increasing LDL concentration, whereas the opposite trend was observed at pH 3.5. These data indicate that PC is preferentially bound to the interface at pH 7.0 and low protein concentrations when compared to pH 3.5. The results can be explained on the basis of differences in affinities of the PC and PE molecules with apoproteins at different pH values. It is known that the quaternary headgroup of PC is a stronger base than the primary headgroup of PE. Therefore, at pH 7.0, PC has more charges on the headgroup and the electrostatic interactions between the PC molecules and apoprotein are weaker than the PE−apoprotein interaction. For the low LDL concentration, the insufficient apoprotein in LDL cannot cover all of the freshly created oil surface, so that the PC can adsorb tightly. The proportion of PE increased with increasing protein concentration. However, the negative charges on PC at pH 3.5 were reduced, and the electrostatic interaction between PC molecules and apoproteins becomes greater as compared to results seen at pH 7.0. Such increased interactions would enable the PC molecule to bind more to the interface with increasing protein concentration at pH 3.5, when compared to the weakly primary base headgroup of PE (Scholfield, 1989). Interestingly, the ratio of PC increased and that of PE decreased at 4.0% LDL concentration at pH 7.0, unlike the trend observed at pH 3.5. As described above, the plateau was reached for particle size at 2.4% LDL. The decreased cholesterol level at higher protein concentration may be as a result

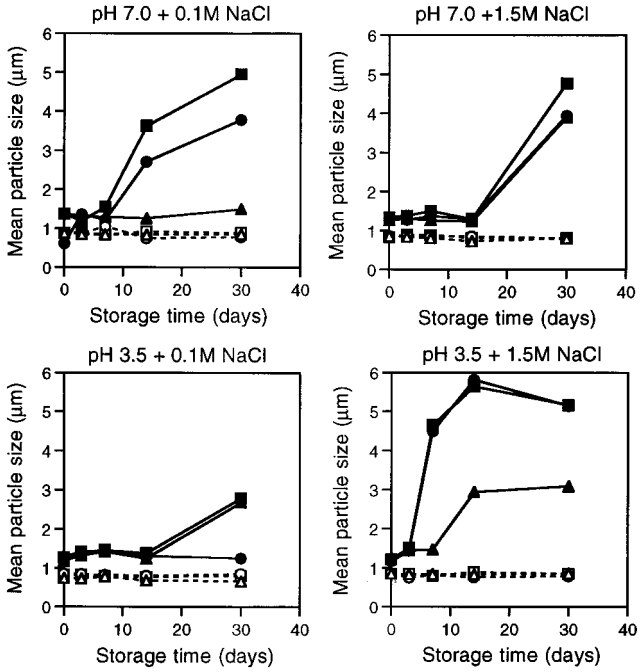

Figure 5. Changes of mean particle size of emulsions (20%, v/v) stabilized by egg yolk LDL (△, ▲), 48.8% CR-LDL (○, ●), and 92.7% CR-LDL (□, ■) as a function of storage time at pH 7.0 and 3.5 containing 0.1 and 1.5 M NaCl: (△, ○, □) 4.0% LDL or CR-LDL concentration; (▲, ●, ■) 0.8% LDL or CR-LDL concentration

of competitive adsorption by the apoproteins and phospholipids. Cholesterol showed less affinity to the interface at pH 7.0 and 3.5, while it decreased at 4.0% LDL. Cholesterol is a hydrophobic lipid. At low pH, the interaction between cholesterol and phospholipids or apoprotein could increase. For 92.7% CR-LDL emulsions, very different phospholipid compositions were observed at different pH values. The PE molecule could not adsorb to the interface at pH 7.0, while it was observed at the interface at pH 3.5. The level of PE decreased and that of PC increased with increasing CR-LDL concentration, similar to LDL emulsions. The lipid composition from 48.8% CR-LDL emulsion showed intermediate patterns at both pH values between LDL and 92.7% CR-LDL sample. The interactions of cholesterol with apoprotein or phospholipids are not well understood. The results indicate that cholesterol in LDL would play an important role as a "bridge" to facilitate the interaction of PE at the interface. However, PE can penetrate at the interface at low pH values because of reduced electrostatic interaction with PC and apoproteins. The differences of lipid composition at the interface can be related to the characterization of LDL and CR-LDL emulsions such as stability.

Stability of LDL and CR-LDL Emulsions. The stability of the emulsions containing 0.8 and 4.0% LDL or CR-LDLs at different conditions is shown in Figure 5. Regardless of pH and NaCl concentration, the emulsions stabilized with high LDL concentration were stable for up to 1 month. At pH 7.0 and 0.1 M NaCl, the CR-LDLs were unstable after 2 weeks and the mean particle size of the emulsion increases with increasing aging time. However, the CR-LDL emulsions were more stable at high NaCl concentration at pH 7.0 for up to 2 weeks. Interestingly, CR-LDL emulsions were stable at pH 3.5 and 0.1 M NaCl with low surface concentrations, while coalescence/flocculation of the emulsions were increased at 1.5 M NaCl level, resulting in an

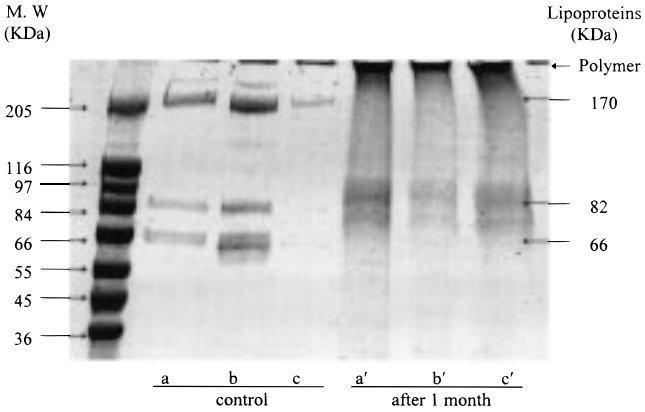

Figure 6. Analysis of adsorbed lipoproteins by SDS−PAGE. Emulsions (20% oil, v/v) were made with LDL (a, a′), 48.5% CR-LDL (b, b′), and 92.7% CR-LDL (c, c′) containing 2.4% lipoproteins: (a, b, c) adsorbed lipoproteins after 24 h; (a′, b′, c′) adsorbed lipoproteins after 1 month.

increase in emulsion particle size. It has been stated that emulsions stabilized with protein should be more unstable at low pH values; however, the present results do not support this. The lipid compositions was also analyzed for emulsions that had undergone 3 weeks of aging. The PC was more dissociated from the interface of CR-LDL emulsions when compared to the control LDL emulsions. The PC molecules were retained on the emulsions at pH 7.0 and 1.5 M NaCl, whereas they were dissociated from the interface at pH 3.5 and 1.5 M NaCl (data are not shown). Among egg yolk proteins, the apoproteins have a more flexible structure and a greater surface hydrophobicity and exhibit a higher adsorbing capacity than other globular proteins such as phosvitin and livetin (Kisseoglou and Sherman, 1983; Aluko and Mine, 1997, 1998). The time-dependent polymerization of lipoprotein through hydrophobic interaction at the oil-in-water interface in emulsions was observed by SDS−PAGE analysis (Figure 6). There was no polymerization of adsorbed apoproteins in emulsions immediately after emulsion formation with LDL and CR-LDLs. While only a little polymerization of apoprotein was detected in the emulsion after 24 h (Figure 6 a−c), the amount of polymerized apoprotein in emulsions increased with time on storing the emulsions for 1 month at 4 °C (Figure 6a′−c′). There was no remarkable difference between LDL and CR-LDL emulsions. In this respect, the emulsion stability of LDL is closely related to phospholipid−apoprotein interaction at the interface. The results indicate that the motional freedom of PC molecules is affected by the interaction of PE, cholesterol, and apoprotein. The pH and salt concentration are also important factors affecting the phospholipid−protein interaction at the interface. At pH 7.0 and low NaCl concentration, the motional freedom of PC molecules of CR-LDL emulsions is relatively high and may be easily dissociated from the interface during aging. On the other hand, the charged density of the PC is neutralized in the presence of high NaCl concentration or lower pH values, resulting in a decrease in the dissociation of PC from the interface. The PE molecules at the interface may play an important role by acting as a bridge between the PC and oil phase or apoproteins. At pH 3.5 and 0.1 M NaCl, the results were similar to those at pH 7.0 and 1.5 M NaCl, which showed that lowering the pH reduces the charge of PC and apoproteins, whereas the presence of high salts at low pH values may decrease the binding affinity of the phos-

pholipids at the interface, resulting in the breakdown of emulsion droplets.

Conclusion. The effect of cholesterol reduction of egg yolk LDL on its adsorption properties was investigated. Our results demonstrate that the cholesterol is an important component in the stabilization of LDL emulsions. Removing cholesterol from LDL caused the formation of larger particle sizes at the low protein concentration and the formation of much thicker films at the interface as a result of apoprotein aggregates. It was found that removing the cholesterol from LDL changed the phospholipid−apoprotein interactions at the interface, and these changes can be responsible for the instability of CR-LDL emulsions. The mechanism of interfacial adsorption of lipoproteins including the cholesterol at the interface is not fully clear at present. Further studies on phospholipid−protein interactions related to structure−function relationships would be useful for better understanding of egg yolk lipoprotein functionalities.

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