Fusion of Low Density Lipoproteins with Cholesterol Ester-Phospholipid Microemulsions

PREVENTION OF PARTICLE FUSION BY APOLIPOPROTEIN A-I

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Little is known about the mechanism and control of lipoprotein particle fusion, although apoproteins are presumed to be important in maintenance of particle structure. This study characterizes the interaction of apo-B-containing low density lipoproteins (LDL) with cholesterol ester microemulsions (CEME) in the presence and absence of apo-A-I to determine if a role for these apoproteins in particle integrity could be ascertained. CEME are an apoprotein-free analog of LDL formed by sonication of radiolabeled phospholipid (surface) and cholesterol ester (core). Incubation of CEME with LDL followed by precipitation of LDL with MnCl₂ resulted in coprecipitation of CEME with LDL that was time-, temperature-, and concentration-, but not pH (pH 6–9), dependent and occurred over a wide range of CEME and LDL particle compositions. Particles from the incubation were larger than the unincubated particles and intermediate in density and electrrophoretic mobility between the starting LDL and CEME. Differential scanning calorimetry experiments suggested that CEME surface and core lipids had mixed with those of LDL. When particles from incubations were exposed to an anti-apo-B column, radiolabeled surface and core molecules originating from the CEME particles bound to the column. Particles eluted at low pH from the anti-apo-B column were irregularly shaped and had excess surface material as judged by electron microscopy. Incubation of CEME with LDL in the presence of apo-A-I (2:1 CEME cholesterol-to-apo-A-I mass ratio) greatly reduced the interaction of the LDL and CEME particles. We conclude that the incubation of CEME with isolated LDL resulted in particle fusion that was prevented by apo-A-I.

Little is known about the factors responsible for maintaining particle integrity of the circulating lipoproteins in blood plasma. The two major lipoprotein classes, LDL and HDL, coexist as separate, discrete families of particles with different chemical and physical properties. Indeed, particle integrity occurs even in the presence of numerous transfer mechanisms for both lipids and proteins, and although many of the lipid molecules of LDL and HDL can exchange, these lipoprotein classes remain distinct. The apoproteins of each lipoprotein family are presumed to function in the maintenance of particle heterogeneity and integrity. Loss of apo-A-I from HDL has been hypothesized to lead to fusion of HDL with other particles (1), but for the most part, HDL appear to remain intact and can be isolated from blood of almost all species.

Even when LDL and HDL are isolated and concentrated together during ultracentrifugation at a density of 1.2 g/ml (2), particle integrity is preserved. Therefore, we decided to test the hypothesis that interactions of apo-A-I and apo-B, the major apoproteins of LDL and HDL, respectively, prevent the fusion of these two lipoprotein classes. We have used stable, apoprotein-free model lipoprotein particles termed cholesterol ester microemulsions (CEME) that have a surface monolayer of phospholipid covering a core of some 2000 cholesterol ester molecules (3). The CEME were incubated with LDL alone and then with mixtures of LDL and apo-A-I. We observed interactions (fusion) between the LDL and microemulsions that were prevented by apo-A-I, suggesting the presence of apo-A-I, apo-B interactions that play an important role in the maintenance of LDL particle integrity.

EXPERIMENTAL PROCEDURES

Materials

Cholesterol esters were purchased from Nu Chek Prep (Elysian, MN). Dimyristoylphosphatidylcholine (DMPC), egg yolk lecithin, triolein, and bovine serum albumin (BSA) were obtained from Sigma. Cholesterol [1,4C]oleate, dipalmitoylphosphatidylcholine (2-[9,10-3H]palmitoyl and [1,4C]dipalmitoyl), and (1,2-3H)cholesterol were purchased from New England Nuclear. [3H]Cholesterol linoleate was made using linoleoyl chloride (Nu Chek Prep) and [1,2-3H]cholesterol as described previously (4). All lipids were checked for purity by thin layer chromatography before use and were estimated to be >95% pure. Radiolabeled cholesterol ester was purified routinely by high pressure liquid chromatography before use (5).

Methods

Cholesterol Ester Microemulsion and LDL Preparation—CEME preparations were made as previously described with slight modifications (3). After sonication, the microemulsions were centrifuged at 1,000 g/ml for 1 h at 40,000 rpm in an SW 40 rotor (T = 20°C). The larger emulsion particles at the top of the tube were removed after the tube was sliced. The infranatant was removed without disturbing the Ti pellet and recentrifuged (d = 1.006 g/ml) for 18 h at 40,000 rpm (SW 40 rotor at 20°C). The contents of the tube (microemulsions) were removed except for the material in the bottom 1.5 cm of the tube. This 18-h centrifugation helped eliminate any
contaminating vesicles produced during sonication as monitored by electron microscopy. The microemulsions were then centrifuged by recentrifugation at 1.d. 1225 g/ml (3). The microemulsions were finally fractionated by size exclusion chromatography on Sepharose CL-4B (see below). To minimize size heterogeneity, the material from the side fractions were used for most investigations. For differential scanning calorimetry and immunoblotting studies, the entire included peak was used. Most preparations were made with cholesterol linolate and dimyristoylphosphatidylcholine containing [3H]+cholesterol linolate (10-15 μCi) and [3H]dipalmitoylphosphatidylcholine (2-5 μCi). For some studies, cholesterol oleate-dimyristoylphosphatidylcholine microemulsions containing [4C]cholesterol oleate and [4C]dipalmitoylphosphatidylcholine were used. Unlabeled cholesterol linolate-egg yolk lecithin microemulsions were also made. Trilinole microemulsions were made from trilinole and egg yolk lecithin as described (6). The LDL used in these studies was isolated from the plasma of nonhuman primates by ultracentrifugation and agarose column chromatography (7).

**Incubations**—Most incubations were performed with equivalent total cholesterol concentrations of LDL and CEME (20-30 μg each tube). Nonhuman primate LDL contains 75% of the total cholesterol as esterified cholesterol. CEME containing approximately 10²⁸ mg of cholesterol oleate and 10¹⁶ mg of phospholipid was added in the incubation tubes containing LDL. The volume of each incubation was adjusted to 0.38 ml with 0.1 m sodium phosphate buffer, 0.01% EDTA, and 0.01% NaN3, pH 7.4 (phosphate buffer). After incubation, the LDL was precipitated by addition of 0.22 ml of 12% BSA (in phosphate buffer) and 17 μl of 1 M MnCl2 (8). After 10 min at room temperature, the precipitate was pelleted by centrifugation at 1500 g for 15 min. An aliquot of the supernatant was added to BisOcount (Research Products International Corp., Mount Prospect, IL) for liquid scintillation counting. Control incubations consisted of the same concentration of microemulsions or LDL alone. A trace amount of [3H]-LDL was prepared (9) and added to the LDL tube to monitor the efficiency of the precipitation method.

In some cases, apo-A-I purified to homogeneity (10) or BSA was included in the incubations. Ten min before the addition of LDL, apo-A-I was added to the CEME at a 2:1 weight ratio of CEME total cholesterol to apo-A-I. In other experiments, the 0.22-mal aliquot of 12% BSA used for the precipitation procedure was added before the addition of LDL, giving a final concentration of 4% BSA during incubation.

To determine the effect of pH on the interaction of CEME and LDL, the pH of the microemulsion + buffer was adjusted to values between 6 and 9 with HCl or NaOH. The LDL was then added in a small volume (15-20 μl) at zero time without altering the pH of the solution.

**Column Chromatography**—Incubations for subsequent column chromatographic analysis were performed using 650-1300 μg of microemulsion cholesterol ester and 3100 pg of LDL cholesterol as esterified cholesterol. CEME containing approximately 650-1300 pg of microemulsion cholesterol ester and 3100 pg of LDL cholesterol were added to the LDL incubation tubes containing LDL. The volume of each incubation was adjusted to 3.8 ml with 0.1 m sodium phosphate buffer, 0.01% EDTA, and 0.01% NaN3, pH 7.4 (phosphate buffer). After incubation, the LDL was precipitated by addition of 0.22 ml of 12% BSA (in phosphate buffer) and 17 μl of 1 M MnCl2 (8). After 10 min at room temperature, the precipitate was pelleted by centrifugation at 1500 g for 15 min. An aliquot of the supernatant was added to BisOcount (Research Products International Corp., Mount Prospect, IL) for liquid scintillation counting. Control incubations consisted of the same concentration of microemulsions or LDL alone. A trace amount of [3H]-LDL was prepared (9) and added to the LDL tube to monitor the efficiency of the precipitation method.

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**Column Chromatography**—Incubations for subsequent column chromatographic analysis were performed using 650-1300 μg of microemulsion cholesterol ester and 3100 pg of LDL cholesterol (LDL/CEME ratio = 2.4 to 4.8). The volumes of the incubations were adjusted to 3 ml with a solution of 0.9% NaCl, 0.01% EDTA, 0.01% NaN3, pH 8 (hereafter referred to as saline). Control incubations consisted of microemulsion or LDL alone at the same concentration. After incubation (2-8 h), the samples were applied to a 1.6 × 85-cm Sepharose CL-4B column (Pharmacia, Piscataway, NJ) that was equilibrated and LDL was eluted with phosphate buffer (15). The column was equilibrated and LDL was eluted with buffers described by McVicar et al. (16).

**RESULTS**

Fig. 1 shows the results of incubations of equal concentrations of radiolabeled microemulsion (cholesterol linolate-DMPC) with LDL for various periods of time. As incubation time increased from 1 min to 2 h, there was a rapid decline in the amount of the microemulsion radiolabel (both [3H]cholesterol ester and [3H]-phospholipid) remaining in the supernatant. The decline was more rapid at 37 °C than at 0 °C, but after 24 h, the amount of radiolabel remaining in the supernatant was similar at both temperatures. There was no difference in the amount of cholesterol ester versus phospholipid label in the supernatant at any time point. When CEME were incubated without LDL present (CEME only, Fig. 1, top panel), most of the radioactivity in the microemulsions remained in the supernatant; thus, the MnCl2 did not precipitate microemulsions at all temperatures. Greater than 90% of the [3H]-LDL included peak was precipitated by the MnCl2 (Fig. 1, top panel). In several incubations, the mass of cholesterol remaining in the supernatant after LDL precipitation was measured and found to agree with the results derived from radiolabeled tracers.

The effect of concentration on the interaction of CEME with LDL is shown in Table I. The ratio of LDL-to-microemulsion cholesterol concentration was varied from 6 to 9. As the amount of LDL relative to microemulsion increased, more of the microemulsion radiolabel precipitated with the LDL. The [3H]-cholesterol ester label in the supernatant was slightly lower than the [3H]cholesterol ester label at all LDL/microemulsion ratios. At all concentration ratios, the LDL was efficiently precipitated as indicated by the low amount of [3H]-LDL remaining in the supernatant.

The effect of pH on CEME-LDL interaction was also studied. Microemulsions + LDL (1:1 cholesterol ratio) were incubated for 6 h at 37 °C at pH values of 6 to 9. After precipitation with MnCl2, the microemulsion radiolabel remaining in the supernatant was as follows (mean ± S.E., n = 3): 18.8 ± 3.7% CE, 22.6 ± 4.8% PL, pH 6; 24.1 ± 13.6% CE, 24.4 ± 4.8% PL, pH 7; 24.4 ± 14.4% CE, 24.8 ± 9.2% PL, pH 8, and 22.8 ± 15.2% CE, 25.2 ± 9.3% PL, pH 9. In control
incubations over the same pH range, only 5–14% of the $^{125}$I-LDL radioactivity remained in the supernatant after precipitation. When microemulsions were incubated alone at pH 6–9, >75% of the phospholipid and >83% of the cholesterol ester label remained in the supernatant after the precipitation procedure.

Agarose electrophoresis was performed on the LDL, CEME (cholesterol linoleate-DMPC), and incubation mixtures of LDL + CEME. The results of a typical experiment are shown in Fig. 2. LDL alone had typical β mobility (Lane A). Microemulsions alone had a slight migration out of the well toward the cathode. Incubation of microemulsions with LDL (1 h at 37 °C) resulted in a diffuse band which was intermediate in mobility between LDL and microemulsions. As the amount of LDL relative to microemulsion increased, the resulting band became more diffuse and moved closer to the β position of LDL.

Size changes in the incubation mixture were investigated by size exclusion chromatography (Fig. 3). Incubation of CEME (cholesterol linoleate-DMPC) for 8 h at 37 °C in the absence of LDL (Fig. 3, top left) did not alter the elution position ($V_0 = 104$ ml) compared to unincubated microemulsions ($V_0 = 105$ ml) (data not shown). The microemulsion cholesterol ester and phospholipid radiolabel eluted in a homogeneous peak. A vertical line has been drawn through the center of the peak for reference. LDL incubated alone (Fig. 3, center left) for 8 h at 37 °C also eluted in the same position ($V_0 = 99$ ml) as unincubated LDL (data not shown). When LDL + microemulsions were incubated for 2 h at 37 °C, a shoulder on the main peak emerged at $V_0 = 91$ ml for the cholesterol ester and phospholipid radioactivity and the optical density profile (Fig. 3, bottom left). Although most of the optical density was the result of LDL protein absorption, microemulsions do scatter light and contributed to the optical density profile. Additional incubations carried out for 5 and 8 h showed a larger change in the elution position ($V_0 = 86–87$ ml) for the radioactivity peaks and the optical density profile (Fig. 3, right).

Fig. 4 shows the results of density gradient centrifugation of CEME (cholesterol olate-DMPC), LDL, and microemulsion + LDL. The top panel shows that the microemulsions floated to the top of the density gradient. Eighty % of the $^{14}$C-cholesterol ester and $^3$H-phospholipid was recovered in

![Fig. 1. Incubation of CEME (30 µg of cholesterol) at 37 °C, LDL (30 µg of cholesterol) at 37 °C, or CEME + LDL (30 µg of cholesterol each) at 37 °C (top panel) and 0 °C (bottom panel). CEME were composed of cholesterol linoleate (CL) and DMPC mass with $[^3]$H-cholesterol linoleate and $[^14]$C[DPPC radiolabel. The volume of each incubation tube was adjusted to 0.38 ml with 0.1 M phosphate buffer, pH 7.4. The LDL-only incubation had a trace amount of $^{125}$I-LDL. At the indicated times, LDL was rapidly precipitated as described under “Methods.” Each point is the mean of four separate experiments using two LDL and two CEME (cholesterol linoleate-DMPC) preparations.](image)

![Fig. 2. Agarose electrophoresis of CEME, LDL, and CEME + LDL. CEME were composed of cholesterol linoleate and DMPC. Samples were incubated for 1 h at 37 °C and subjected to agarose electrophoresis. The anode is at the top of the gel. Lanes (micrograms of cholesterol): Lane A, LDL (5 µg); Lane B, CEME (5 µg); Lane C, CEME (5 µg) + LDL (5 µg); Lane D, CEME (5 µg) + LDL (10 µg); Lane E, CEME (5 µg) + LDL (15 µg).](image)
Fig. 3. Column chromatography of CEME, LDL, and incubation mixtures of CEME + LDL on Sepharose CL-4B (1.6 × 85-cm column). CEME were composed of cholesterol linoleate (CL) and DMPC mass with [3H]cholesterol linoleate and [14C]DPPC. Incubations of 650–1300 μg of CEME cholesterol and 3100 μg of LDL cholesterol in a total volume of 3 ml of 0.01 M Tris, 0.15 M NaCl, 0.01% azide, 0.01% EDTA, pH 8, were conducted for the indicated times. The ratio of LDL-to-CEME cholesterol is given for each incubation. The column had a flow rate of 30 ml/h, and 10 min fractions (~4.8 ml) were collected for analysis. A vertical line has been drawn at 105 ml to mark the center of the CEME elution peak in subsequent column runs.

the d < 1.020 g/ml fraction. The LDL (middle panel) was distributed in the bottom half of the gradient tube with a mean peak density of 1.036 g/ml. When the same microemulsion and LDL preparations (1:1 cholesterol ratio) were incubated together for 17 h at 37 °C and subjected to density gradient centrifugation, several changes were observed. The optical density peak in the position of LDL was no longer clearly visible, and the optical density was increased in the middle part of the gradient (tubes 8–30) compared to the starting microemulsion (bottom panel). The peaks of the [3H] cholesterol ester, 3H-phospholipid, and absorbance profiles shifted to tubes 4–5 near the top of the gradient. The percentage of the total radiolabel in the d < 1.020 g/ml fraction decreased from 80% (microemulsion only) to 66% when microemulsion + LDL were incubated. The percentage of radiolabel in the d > 1.030 g/ml fraction increased from 14% (microemulsion only) to 27% (microemulsion + LDL). The percentage of radiolabel in the d > 1.030 g/ml fraction was unchanged.

DSC experiments were performed to determine if admixing of microemulsion and LDL lipids occurred during incubation. Fig. 5 shows the results from an incubation of a cholesterol linoleate-DMPC microemulsion with LDL. The LDL had a peak melting temperature of 39 °C and an enthalpy typical of cholesterol ester liquid crystalline-to-liquid transition (6.8–1.6 cal/g). The microemulsion had a peak melting temperature of 25 °C (Fig. 5, middle panel). This transition was the result of the DMPC gel-to-liquid crystalline transition; the cholest-
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FIG. 4. Density gradient centrifugation of CEME, LDL, and CEME + LDL after a 17-h incubation at 37 °C. CEME were composed of cholesterol oleate and DMPC mass with cholesterol [14C] oleate ("C-CO) and [3H]DPPC. Each incubation tube had a volume of 7.5 ml of saline containing either 1.1 mg of CEME cholesterol, 1.3 mg of LDL cholesterol, or 1.1 mg of CEME + 1.3 mg of LDL cholesterol. Density gradient centrifugation was performed as detailed under “Methods.” The LDL profile (middle panel) has a 3-fold vertical expansion relative to the other two profiles.

terol linoleate transition, which normally occurs at 30–40 °C, had a lower enthalpy and was obscured by the DMPC transition. The bottom panel of Fig. 5 shows a DSC scan of LDL and CEME incubated for 6 h at 37 °C (half the amount of each shown in the top and middle panels). The phospholipid transition was greatly decreased, while the enthalpy of the higher temperature endotherm decreased by 40% compared to that of LDL and the peak temperature increased from 39 (LDL only) to 42 °C (microemulsion + LDL).

Additional DSC studies were conducted using triolein microemulsions composed of triolein and egg yolk lecithin. These data are shown in Fig. 6. The DSC analysis for LDL alone is shown at the top of Fig. 6. The liquid crystalline-to-liquid transition for the cholesterol esters ranged from −35 to 60 °C. The triolein microemulsion preparation had no transitions between 0 and 60 °C (Fig. 6, middle). When the same amounts of triolein microemulsion and LDL were incubated together for 1.5 h at 37 °C and examined by DSC (Fig. 6, bottom), the LDL cholesterol ester transition was greatly reduced.

The morphology of the incubation product was examined by electron microscopy. LDL alone or LDL + CEME were incubated overnight at 37 °C (~1:1 ratio of LDL-to-microemulsion cholesterol) and applied to an anti-apo-B column to selectively retain the apo-B-containing particles. After thorough washing of the column, the samples were eluted with 1
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of these agents. Fig. 8 shows the time course of incubations for control (no addition), apo-A-I, and BSA additions; similar results are not available for the high salt additions as the KBr interfered with LDL precipitation. The control data were similar to previous incubations of microemulsions + LDL. The addition of BSA (4% final concentration) did not prevent the interaction (coprecipitation) of the microemulsions with LDL. In fact, the BSA promoted the coprecipitation of microemulsion lipids with the LDL, affecting microemulsion phospholipid to a greater extent than cholesterol ester. Addition of apo-A-I (2:1 microemulsion cholesterol-to-apo-A-I ratio) to the CEME preparation decreased the interaction of the microemulsions with the LDL for up to 24 h.

Column chromatography was performed with these incubation mixtures, and the results are shown in Fig. 9. The elution profile of the CEME + LDL + apo-A-I incubation was similar to that of the unreacted microemulsion preparation (Fig. 9, top panel versus Fig. 3, upper left). However, the ascending edge of the column peak in Fig. 9 did appear asymmetric. The 3H and 14C radioactivity peaks and absorbance peak eluted at ~102-104 ml. The absorbance peak at ~141 ml was presumably unbound apo-A-I. In the BSA incubation (Fig. 9, middle panel), there was a clear shift in the microemulsion radiolabel peaks to lower elution volumes ([3H]cholesterol ester = 95 ml; [14C]phosphatidylcholine = 100 ml). Small amounts of microemulsion radioactivity were also seen at Vr and at the major peak for BSA (Vr = 163 ml). Incubation of CEME + LDL in the presence of high salt for 5 h at 37 °C did not prevent the interaction of the reactants. The count profile for the microemulsions was broad and centered at Vr ~80 ml. The absorbance peak was heterogeneous with at least three peaks at 60 (Vt), 80, and 100 ml; the peak at Vt was the result of the KBr refractive index change.

DISCUSSION

Lipoproteins are spherical emulsion particles composed of a core of nonpolar lipid surrounded by a surface monolayer of polar constituents (19). Emulsion particles similar in lipid composition to lipoproteins form a continuum of particle sizes which are determined by the ratio of surface-to-core material (20). However, lipoproteins form distinct populations and subpopulations of particles which can be separated by size and density. During postprandial metabolic events, the size and density of certain lipoproteins are altered by particle fusion and exchange reactions (21, 22). Redundant surface material generated by the lipolysis of chyomicron triglyceride forms discoidal or vesicular particles which can fuse with HDL (1, 22-24) and result in HDL subfractions of different chemical and physical properties (21, 22). Alternatively, some HDL apo-A-I may exchange onto the chylomicron surface material leading to unstable HDL particles which can fuse with other lipoprotein classes (1). In vitro incubation of vesicles with HDL or LDL results in particle fusion or aggregation, respectively (25-28). In the face of plasma exchange and fusion reactions which continuously scramble the surface and core components of the lipoproteins, it is unclear what factors are responsible for maintaining distinct classes of lipoprotein particles.

Apo-proteins are assumed to be one factor important in maintaining lipoprotein class integrity although there is little direct evidence to support such a role. In model systems, protein is necessary to promote vesicle fusion (29), yet the apoprotein moeity of lipoprotein particles is thought to stabilize the particle. An explanation for such paradoxical findings is that certain apoproteins function to promote particle fusion while others prevent it. Since LDL and HDL are the two major plasma lipoprotein classes which contain the two
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major apoproteins in plasma (apo-B and apo-A-I), we decided to test the hypothesis that apo-A-I/apo-B interactions are important in maintaining the two separate lipoprotein classes found in fasting plasma. We took advantage of a well-defined apoprotein-free lipoprotein model, CEME, and characterized the interaction of CEME with apo-B-containing LDL. We then characterized the interaction after the addition of apo-A-I to the system.

Incubation of LDL with CEME for brief periods of time (<1 h) resulted in the coprecipitation of microemulsion lipid with LDL. These results suggest an interaction between LDL and microemulsion particles which is strong enough to allow microemulsion particles to precipitate with LDL particles. The interaction resulted in some marked changes in physical properties of microemulsion particles. The hydrodynamic size of microemulsion particles incubated in the presence of LDL was much larger than microemulsion particles or LDL incubated alone (Fig. 3). By gel filtration, the elution volume of the incubation product decreased by 10–15 ml compared to unmodified microemulsion or LDL. Using the elution position of an iodinated LDL, tracer of known molecular weight, it was estimated that the mean molecular weight of the incubation product was \( >8.0 \times 10^6 \) and the molecular weight of the starting LDL and microemulsions was 4.2 and 3.0 \( \times 10^6 \), respectively. Incubation of LDL with microemulsions also altered the charge-to-mass ratio so that the incubation product migrated intermediate between LDL and microemulsions on 1% agarose gels (Fig. 2). This could have been due to an increased size of the incubation product or to a masking of the surface charge of the LDL particle. In addition, the density of the microemulsion particles increased upon incubation with LDL (Fig. 4). The increase in density suggested that microemulsion lipids had become associated with LDL particles.

Fig. 7. Negative stain electron micrographs of LDL, CEME, and LDL + CEME. CEME was composed of cholesterol oleate and DMPC. Particles shown in Panels C and D were adsorbed to an anti-apo-B column after incubation and eluted with 1 M acetic acid as described under “Methods.” Panel A, LDL incubated for 19 h at 37°C; Panel B, CEME; Panel C, LDL (700 µg of cholesterol) incubated 19 h at 37°C and subjected to immunoaffinity chromatography; Panel D, CEME (500 µg of cholesterol) + LDL (700 µg of cholesterol) incubated for 19 h at 37°C and subjected to immunoaffinity chromatography. Arrowheads in Panel C indicate particles with angular shapes. Arrowheads in Panel D indicate particles showing excess surface material in the form of a flap. Bar marker indicates 1000 Å.

Fig. 8. Incubation of LDL (30 µg of cholesterol) + CEME (30 µg of cholesterol) in the presence of apo-A-I (15 µg), saline (control), or BSA (4.4% final concentration) at 37°C. The CEME preparation was composed of cholesterol linoleate and DMPC mass with \(^{3}H\)cholesterol linoleate (open symbols) and \(^{14}C\)DPPC (closed symbols). The total volume of the incubation tube was adjusted to 0.36 ml with 0.1 M phosphate buffer, pH 7.4. The LDL was rapidly precipitated at the indicated time points as described under “Methods.” Results are the mean of six (apo-A-I), three (control), and two (BSA) experiments using three CEME and four LDL preparations.
We examined a number of parameters which might influence the interaction of LDL with CEME. The interaction was time-, temperature-, and concentration-dependent but was independent of pH over the range of 6-9. The chemical composition of LDL and microemulsions had little effect on the interaction of the particles. LDL were obtained from animals consuming either saturated or polyunsaturated fat diets. These LDL had cholesterol ester cores which had order-to-disorder transitions ranging from diets. These LDL had cholesterol ester order-to-disorder transition at triglyceride:cholesterol ratios >0.2 (30). Therefore, incubation of LDL with triglyceride microemulsions was studied. If particle integrity was maintained upon incubation of triolein microemulsions with LDL (i.e., aggregation, the cholesterol ester order-to-disorder transition of the LDL would be preserved. However, the LDL cholesterol ester transition was greatly decreased when triolein microemulsions were incubated with LDL (Fig. 6), which suggests that the particle cores became mixed and triolein had solubilized the ordered cholesterol ester phase. Similar data were found for the surface transition. LDL had no surface phospholipid transition above 0 °C, while CEME with DMPC as the surface phospholipid had a transition of 25 °C (Fig. 5). Upon incubation of the LDL with microemulsions, the surface DMPC transition was greatly reduced, suggesting that the LDL surface had mixed with the microemulsion surface which abolished the DMPC liquid crystalline-to-liquid transition.

The results of electron microscopy also suggested that CEME and LDL underwent fusion. When incubation mixtures of microemulsions and LDL were viewed by electron microscopy after separation of unreacted microemulsions by immunoabsorption chromatography, there was evidence of excess surface material (Fig. 7, Panel D). Although the stoichiometry of LDL and microemulsion in the fusion product is not known, if one microemulsion fused with one LDL (assuming particle diameters of 220 Å), the fusion product would require 22% less surface. This calculation involves the assumptions that the fusion product was spherical and the surface area/PL molecule did not change. However, a number of the resulting particles were not spherical when viewed by negative stain electron microscopy. The presence of abnormally shaped particles and excess surface material precludes the calculation of per particle compositions.

These results suggest that apo-B may act to promote particle fusion. Apoprotein-free CEME particles incubated for up to 8 h showed no evidence of fusion (Fig. 2). In another study, Hunter et al (27) found that tryptic digestion of LDL prevented its aggregation with vesicles, suggesting that apo-B was important in particle aggregation. Addition of apo-A-I to the LDL-microemulsion incubation specifically inhibited the fusion between the particles, but 3 m KBr or 4% BSA did not (Figs. 8 and 9). These results suggest that a specific interaction between apo-A-I and apo-B may help maintain the structural integrity of LDL and HDL particles. However, we cannot exclude the possibility that some other constituent of LDL (cholesterol, sphingomyelin, etc.) may be responsible for fusion with microemulsions and that there may be an apo-A-I-lipid interaction which prevents particle fusion.

Fusion may be a biologically important mechanism for the generation of lipoprotein subfraction heterogeneity. Since apo-A-I is equally stable in solution or on the HDL surface, loss of apo-A-I from HDL may lead to fusion of HDL with other lipoprotein particles (31-33). This may provide a mechanism for the constant remodeling of HDL subfractions via fusion with lipid transfer reactions acting to fine tune the composition of the subfraction population. Recent data suggest that the in vitro conversion of small spherical HDL derived from lecithin:cholesterol acyltransferase-deficient patients to larger particles in the presence of purified lecithin:cholesterol acyltransferase may involve fusion (34).
observation suggests that fusion mediated by lecithin:cholesterol acyltransferase and apo-A-I may play a key role in the maturation of nascent HDL to mature plasma HDL. Thus, particle fusion could be an important mechanism for the generation of lipoprotein class heterogeneity. The data of the present paper suggest that fusion may be controlled by specific apoproteins.

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