With this model, $1/\beta_1^{app}$ (as a function of y) gives a straight line if $k_1' = k_2' = k_3' = k_4' = k'$ or if $k_2 = k_3 = k_4 = 0$. In the first case, $\beta_1^{app}/\beta_2^{app}$, $\beta_2^{app}/\beta_3^{app}$, and $\beta_3^{app}/\beta_4^{app}$ as a function of y also give straight lines, and the intercepts on the x axis of all the curves are equal to $1/k'$. In the other case, when $k_2 = k_3 = k_4 = 0$, $1/\beta_1^{app}$ as a function of y gives us the intrinsic constants k_1 and k_1'. Then we consider the function $\beta_1^{app}/\beta_2^{app}$. A straight line, is indicative of two possibilities: (1) $k_1' = k_2' = k_3' = k_4' = k'$ (this is the case examined before) or (2) $\delta_{1,1,0,0} = +\infty$, $\delta_{1,1,0,0}k_1k_2 = \beta_2$, and $\delta_{1,0,1,0} = \delta_{1,0,0,1} = \delta_{0,1,1,0} = \delta_{0,0,1,1} = \delta_{0,1,0,1} = 0$.

This last case can be interpreted as a sequential binding of ligand L^1 to the two first sites. The same reasoning can be applied to the functions $\beta_2^{app}/\beta_3^{app}$ and $\beta_3^{app}/\beta_4^{app}$. To summarize, if we consider a sequential binding of Ca^{2+} to calmodulin detected by the scheme

$$\mathrm{CaM} \underset{}{\overset{\beta_1}{\rightleftharpoons}} \mathrm{CaMCa} \overset{\beta_2/\beta_1}{\rightleftharpoons} \mathrm{CaMCa_2} \overset{\beta_3/\beta_2}{\rightleftharpoons} \mathrm{CaMCa_3} \overset{\beta_4/\beta_3}{\rightleftharpoons} \mathrm{CaMCa_4}$$

β^{app} must obey

$$\frac{1}{\beta_1^{app}} = \frac{(1 + k_1'y)}{\beta_1} \qquad \frac{\beta_2^{app}}{\beta_3^{app}} = \frac{\beta_2(1 + k_3'y)}{\beta_3}$$

$$\frac{\beta_1^{app}}{\beta_2^{app}} = \frac{\beta_1(1 + k_2'y)}{\beta_2} \qquad \frac{\beta_3^{app}}{\beta_4^{app}} = \frac{\beta_3(1 + k_4'y)}{\beta_4} \tag{26}$$

To satisfy the last model, binding of Ca^{2+} to calmodulin must exhibit the following properties. Calmodulin must have four specific Ca^{2+} binding sites. An increase in the concentration of Mg^{2+} of K^+ must decrease the affinity of Ca^{2+} for calmodulin in a nonsaturable manner. The representation of $1/\beta_1^{app}$, $\beta_1^{app}/\beta_2^{app}$, $\beta_2^{app}/\beta_3^{app}$, and $\beta_3^{app}/\beta_4^{app}$ as a function of y must give straight lines with different intercepts on the x axis.

General Conclusions. Finally, we can draw the following conclusions. The experimental data of ion binding to a macromolecule can be analyzed by using a stepwise equilibrium model. More generally, if we have a system described by a stepwise equilibrium model, the same analysis can be done, if we can associate to each complex a signal (for instance spectrophotometric signal, average number of ligand bound per molecule, reaction rate for an enzymatic reaction, etc.). The information given by the experimental data allows us to propose a kinetic scheme, the first step in the kinetic analysis of a given system. The use of effectors (other ions, pH, temperature, etc.) acting in a different way on each step of the kinetic scheme allows the determination of the molecular mechanism of ion binding. In the case where the signal is a spectrophotometric one, some information on the molecular mechanism of conformational changes can also be obtained.

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Interaction of Apolipoprotein B from Human Serum Low-Density Lipoprotein with Egg Yolk Phosphatidylcholine†

Robert M. Watt and Jacqueline A. Reynolds*

ABSTRACT: A binary complex of apolipoprotein B and egg yolk lecithin has been formed which contains 250–350 mol of lipid/500 000 g of protein. This particle retains many of the structural properties of native human low-density serum lipoprotein (LDL) as evidenced by the state of association of the protein, the circular dichroic spectrum, and immunological characteristics. Apolipoprotein B does not interact with lipid vesicles but rather binds a small number of phospholipid molecules in water-soluble form. This study represents the first partial reconstitution of native LDL from the delipidated apoprotein and is the initial step in a systematic investigation of the lipid binding properties of apolipoprotein B.

Apolipoprotein B (apo B)[1] from human serum low-density lipoprotein (LDL) has been delipidated by substituting a number of different detergents for the naturally occurring amphiphilic ligands (phospholipid, cholesterol, cholesteryl esters, and triglycerides) (Helenius & Simons, 1971; Ikai & Hasegawa, 1978; Steele & Reynolds, 1979a; Watt & Reynolds, 1980). In all detergents thus far investigated the protein maintains its native dimeric state (500 000 g/mol of complex). In nonionic detergents the secondary structure is also similar to that of the native protein as evidenced by the circular dichroic spectra and by immunological studies. The large alteration in apparent helical and β pleated sheet content which has been reported when sodium dodecyl sulfate ($NaDodSO_4$) is bound is readily reversed by exchanging the ionic detergent for $C_{12}E_8$ (Watt & Reynolds, 1980).

In the absence of bound amphiphiles apo B aggregates irreversibly, and a large increase in β pleated sheet structure is observed. Consequently, any study of the interaction of naturally occurring lipids with apo B must be carried out as a competition binding experiment, i.e., lipid must be substituted for the bound detergent. Exchange of bound amphiphiles

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[1] Abbreviations used: LDL, human low-density serum lipoproteins; HDL, human high-density serum lipoproteins; VLDL, human very low density serum lipoproteins; apo B, apolipoprotein B; PL, phospholipid; Apo B–PL, soluble complex of apolipoprotein B and phospholipid; $C_{12}E_8$, octaethylene glycol *n*-dodecyl monoether; $NaDodSO_4$, sodium dodecyl sulfate; cmc, critical micelle concentration.

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having relatively high aqueous solubilities in the monomeric form (high cmc's) is experimentally straightforward since both ligands can equilibrate rapidly among the detergent–protein complexes. However, phospholipids have extremely low cmc's ($<10^{-10}$ M) and hence equilibrate very slowly among their self-associated form (liposomes), lipid–detergent mixed micelles and detergent–protein mixed micelles. The experimental problem then in obtaining an apo B–phospholipid complex from the apoB–detergent particle is to produce a ternary complex of detergent–phospholipid–apo B before lowering the detergent concentration. The protocol used in this study is derived from principles identical with those used in this laboratory for the reconstitution of membrane proteins into phospholipid vesicles (Mimms et al., 1981) and circumvents the inherent kinetic problems in the system.

In the present work we have substituted egg yolk lecithin for $NaDodSO_4$ as the bound ligand on apo B to produce a phospholipid–protein particle devoid of detergent. Characterization of these particles reveals that the native quaternary structure of the protein is maintained (500 000 g/mol of complex) and the secondary structure is closely similar to that of apo B in its native milieu.

The ultimate goal of our studies is to reconstitute an apo B–lipid complex which is similar to that found in vivo, and this requires progressing from the two-component system, apo B–phospholipid, to more complex systems which include cholesterol, cholesteryl esters, and triglycerides. The effect of each bound component on the protein moiety and on the relative amounts of the other naturally occurring bound lipids can only be investigated in a systematic manner, and the present work represents the first step in this process.

Experimental Procedures

Materials

All materials used in this study, unless otherwise indicated, were standard reagent grade. Sodium dodecyl sulfate was British Drug House Chemical Corp. "specially pure" grade purchased from Gallard-Schlesinger. Sodium dodecyl [^{35}S]-sulfate was obtained from Amersham. Sepharose CL-4B was a product of Pharmacia Fine Chemicals, and 0.7 × 50 cm glass chromatographic columns were from Bio-Rad Laboratories. Egg yolk lecithin used in this study was from Lipid Products; thin-layer chromatography on silica gel G (Supelco) in chloroform–methanol–water–acetic acid (65:25:4:1), followed by charring with 50% H_2SO_4, revealed only one spot. High-pressure liquid chromatography on Ultrasphere Si in hexane–2-propanol–water (6:8:1.4) showed <0.5% contamination by fatty acid or lysolecithin. Regenerated cellulose membranes (RC-54), which have an average pore size of 200–350 Å, were from Schleicher & Schuell.

Methods

Preparation of Holo-LDL$_2$. Holo-LDL$_2$ (d = 1.02–1.05 g/cm^3) was isolated from fasting, normal human volunteers by centrifugal flotation employing the procedures described in detail by Steele & Reynolds (1979a). After isolation, the two free sulfhydryls per 250 000 g of protein were alkylated with iodoacetamide and the reaction was terminated by dialysis against a buffer solution containing 20 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Tes), adjusted to an ionic strength at 0.3 with NaCl, pH 7.4 (hereafter referred to as standard Tes buffer). Holo-LDL$_2$ was then passed through sterile 0.22-μm Millipore filters into sterile test tubes for storage at 4 °C.

Analytical Procedures. $NaDodSO_4$–polyacrylamide gel electrophoresis on 5% acrylamide gels was used routinely to monitor the purity of all apo B preparations. The buffer system of Weber & Osborn (1969) was employed, except that the running buffer contained 0.1% $NaDodSO_4$ and 50 mM sodium phosphate, pH 7.2. Apo B migrated in this gel system as a single band. The method of Lowry et al. (1951), with a final concentration of 2% $NaDodSO_4$ in both experimental samples and bovine serum albumin standards, was used to determine protein concentration. Lipid phosphorus was assayed according to the micromethod of Bartlett (1959). Radioactive ligands were measured in a Beckman LS-100C liquid scintillation counter, using ACS (Amersham) scintillation fluid. Sodium dodecyl sulfate ($NaDodSO_4$) concentrations were determined with the methylene blue assay as described by Reynolds et al. 1967.

Preparation of Delipidated Apo B. Delipidated apo B was obtained from holo-LDL$_2$ by using sodium dodecyl sulfate as described previously by Steele & Reynolds (1979a). Briefly, holo-LDL$_2$ was incubated with $NaDodSO_4$ (30 mg of $NaDodSO_4$/mg of apo B) for 3 h at room temperature, followed by gel filtration chromatography on a Sepharose CL-4B column equilibrated with standard Tes buffer containing 2.5 mM $NaDodSO_4$, pH 7.4. Appropriate fractions were pooled and concentrated on an Amicon XM-100A Diaflo membrane. Repeated analysis of the detergent–protein complex has demonstrated that it contains <1 mol of phospholipid, cholesteryl ester, and triglyceride per 250 000 g of protein. Three to four moles of free cholesterol per 250 000 g of protein, however, are still bound under these conditions (Steele & Reynolds, 1979a).

Circular Dichroism. Circular dichroic spectra of holo-LDL$_2$ and the reconstituted apo B–PL particle were recorded on a Jobin-Yvon Dichrographe III calibrated with *d*-10-camphorsulfonic acid. One millimeter cells were used and a mean residue weight of 112.5 was utilized to calculate values of molar ellipticity.

Sedimentation Equilibrium. A Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner was used in sedimentation equilibrium studies of apo B–PL particles. A sample of apo B–PL particles, obtained immediately after elution from a gel filtration column (see Figure 1), was introduced into the centrifuge cell, sealed under argon, and centrifuged at speeds ranging from 5200 to 6000 rpm. The mass of apo B per particle was determined from the slope of plots of ln OD vs. r^2 which yield $M_p(1 - \phi'\rho)$ directly. This term can be expanded to $M_p[(1 - \bar{v}_p\rho) + \delta_{PL}(1 - \bar{v}_{PL}\rho)]$, where M_p is the molecular weight of the protein, $\bar{v}_p$ and $\bar{v}_{PL}$ are the partial specific volumes of apo B and phospholipid, ρ is the solution density, and δ_{PL} is the grams of phospholipid bound per gram of protein (Reynolds & Tanford, 1976). $\bar{v}_p$ and $\bar{v}_{PL}$ values of 0.725 cm^3/g and 0.98 cm^3/g, respectively, were employed, while δ_{PL} was measured directly. Initial protein concentrations in the fractions studied were 0.12 mg/mL or less. All experiments were performed at 14 °C.

Electron Microscopy. Apo B–PL particles were negatively stained with 2% uranyl acetate by using the flotation techniques of Valentine et al. (1968) as described by Fowler & Erickson (1979). A Joelco 100-C electron microscope was used to examine the specimens.

Results

Preparation of Apo B–Phospholipid Particles. The apo B–$NaDodSO_4$ complex obtained as described under Methods was concentrated to 1–2 mg/mL in standard Tes buffer, pH 7.4. Trace amounts of NaDod[^{35}S]SO_4 were added to a 1-mL aliquot in order to obtain a specific activity of 1500–2000 cpm/μg of $NaDodSO_4$. The concentration of free or unbound

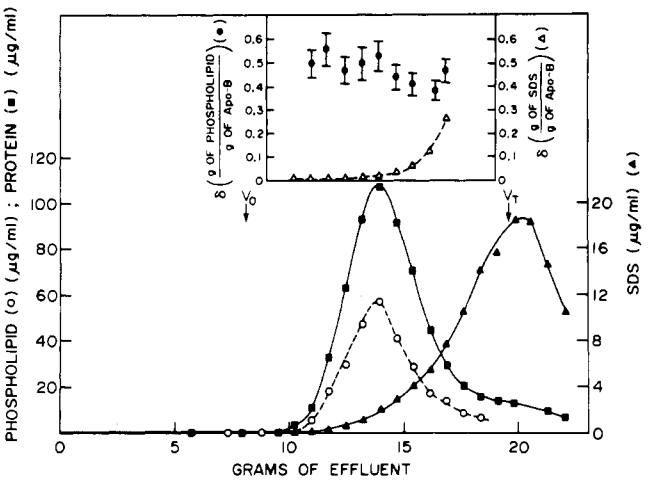

FIGURE 1: Gel filtration chromatography of apo B–PL particles. After flotation of contaminating phospholipid vesicles at $d = 1.10$ g/cm^3, gel filtration chromatography was employed to free the preparation of both sucrose and residual detergent. 420 μg of apo B–egg yolk phosphatidylcholine particles in 530 μL of solution was applied to a 50 × 0.7 cm Sepharose CL-4B column. The eluent was 20 mM sodium carbonate–bicarbonate, pH 10.0, adjusted to an ionic strength of 0.3 with NaCl. Arrows mark the void (V_0) and total (V_T) volumes of the column.

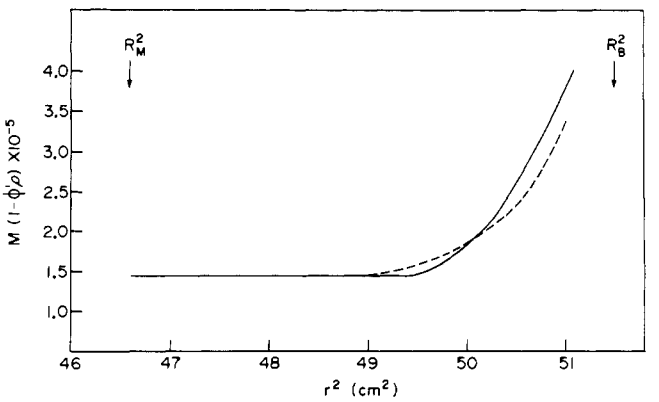

FIGURE 2: $M(1 - \phi'\rho)$ vs. r^2 obtained from sedimentation equilibrium studies at 5200 (---) and 5600 (—) rpm. Centrifugation was performed at 14 °C. The meniscus (R_M^2) and cell bottom (R_B^2) were 46.6 and 51.5 cm^2, respectively.

detergent (initially 2.5 mM) was lowered to between 0.25 and 0.5 mM by using a continuous-flow dialysis cell. (Typically 7–8 h were required to reduce the free detergent concentration to this level.) This $NaDodSO_4$ concentration is still sufficiently high to maintain apo B in a soluble and disaggregated state but reduces the number of free detergent micelles present in the solution (Steele & Reynolds, 1979a). The apo B–$NaDodSO_4$ solution was transfered to a conical test tube which contained a thin film of dried egg yolk phosphatidylcholine on the walls and was allowed to stand for 6–8 h at room temperature. The resultant solution was then stirred for an additional 3–4 h. Phosphatidylcholine was present in the film at a calculated molar excess of 2000:1, lipid/apo B. The lipid–protein–detergent solution was dialyzed against 2 L of 20 mM carbonate buffer, pH 10, for 24 h to remove a substantial portion of the detergent. Solid sucrose was added to a final density of 1.1 g/cm^3 and the solution spun at top speed in a Beckman airfuge for 1 h in cellulose nitrate tubes that had been presaturated with phospholipid. Free phospholipid vesicles containing no protein floated to the top, and the infranate contained >95% of the apo B complexed to lipid. (For example, 1 mg of total protein in the sample centrifuged in the airfuge produced 0.95 ± 0.04 mg of protein in the infranate and <0.03 mg of protein with the floating liposomes.) The infranate was collected from the bottom of the centrifuge tube by using a Hamilton syringe and was applied to a Sepharose CL-4B gel filtration column which had been presaturated with phospholipid. In order to avoid transferring free lipid vesicles from the centrifuge tubes, we withdrew only the lower 60% of the infranate for application to the column.

Gel Filtration Chromatography. The elution profile of apo B–PL particles chromatographed on a Sepharose CL-4B column, equilibrated with a 20 mM carbonate buffer adjusted to an ionic strength of 0.3 with NaCl, pH 10, is presented in Figure 1. All of the protein applied to the column eluted as a single, broad fraction with a peak at 14 g of effluent and a K_{av} of 0.51. As illustrated in the figure, phospholipid coeluted from the column with protein. Occasionally, a small amount of phospholipid was detected in the void volume of the column, due to incomplete removal of free phospholipid vesicles during the centrifugation step of the preparative procedure. In no experiment was protein found with the void volume liposomes. The small amount of residual $NaDodSO_4$ was also removed during gel chromatography and eluted at the total volume in a peak overlapping the protein–phospholipid complex. The total amount of residual $NaDodSO_4$ at this step was somewhat variable depending upon total dialysis times and volumes.

Immediately after all the protein eluted from the column, the concentrations of protein, phospholipid, and $NaDodSO_4$ were assayed. The binding of phospholipid to apo B (plotted in the insert to Figure 1 in terms of grams of phospholipid bound per gram of apo B) is constant throughout the leading two-thirds of the peak at 0.5 g/g corresponding to 320 mol of lipid/500 000 g of protein. Slightly lower binding levels are observed on the descending side of the peak together with coelution of the residual $NaDodSO_4$. Phospholipid binding to apo B in repetitive experiments has varied from 230 to 370 mol/500 000 g of protein. Protein recovery in all cases was close to 100% providing the centrifuge tubes and the gel filtration column were presaturated with phospholipid.

Characterization of Apo B–PL Particles. Apo B–PL particles were characterized by analytical ultracentrifugation, circular dichroism, and electron microscopy. Sedimentation equilibrium studies were performed to determine both the state of association of apo B in these complexes and the homogeneity of the particle preparation. Samples were taken immediately after elution from a gel filtration column, introduced into a double-sector centrifuge cell, purged with argon, and centrifuged to equilibrium (see Experimental Procedures).

A sample of apo B–egg yolk phosphatidylcholine obtained from a gel filtration experiment similar to that in Figure 1 and containing 0.36 g of lipid/g of protein was centrifuged to equilibrium at 5200 and 5600 rpm. Plots of ln OD vs. r^2 were nonlinear and revealed the presence of at least two species of complex. Figure 2 shows $M_p(1 - \phi'\rho)$ as a function of r^2 for both centrifuge speeds. Sixty-five percent of the complexes observed have a $M_p(1 - \phi'\rho)$ of 1.434×10^5 corresponding to a protein molecular weight of 535 000. Since this homogeneous fraction of apo B–phospholipid complexes is stable for several days and does not aggregate as the concentration is increased during analytical ultracentrifugation, we conclude that the higher molecular weight material comprising 30–35% of the total sample was formed during the process of detergent–lipid exchange.

Circular Dichroic Spectra of Apo B–PL Particles. The circular dichroic spectra of apo B–PL particles were recorded and compared to that of holo-LDL in order to assess the secondary structure of the reconstituted particles. These spectra are presented in Figure 3. The curves labeled 1 and

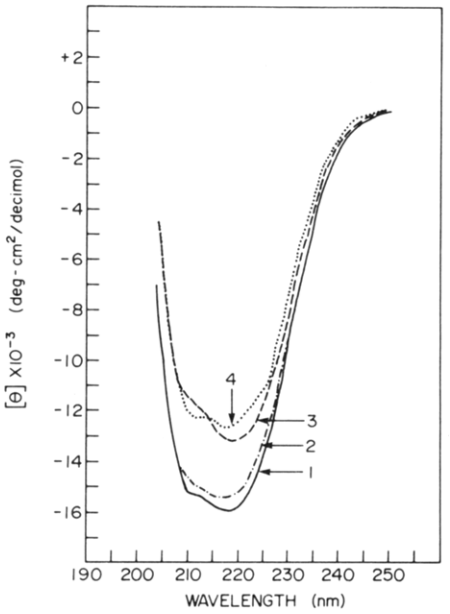

FIGURE 3: Circular dichroic spectra of apo B–PL particles and LDL_2. Spectra of fractions taken from the gel filtration column shown in Figure 1 were recorded. Samples eluting at 12.5 (curve 1), 14.0 (curve 2), and 16.4 (curve 4) g of effluent were compared to holo-LDL (curve 3). Spectra were taken at room temperature in 1-mm rectangular cells at protein concentrations of 0.05–0.1 mg/mL.

2 were obtained from fractions at the leading edge and peak positions of elution from the gel filtration column. Curves 3 and 4 are holo-LDL and a trailing edge fraction, respectively. All four spectra show a pronounced minimum near 217 nm indicative of significant β pleated sheet structure. The absolute magnitudes appear to depend upon the amount and type of bound ligand present. Thus, slightly larger ellipticities are observed in the two fractions containing 0.5 g of lipid/g of protein as compared to that containing 0.35 g of lipid/g of protein.

The circular dichroic spectra of apo B–amphiphile complexes in general appear to be little affected by the type of ligand bound (Steele & Reynolds, 1979a; Watt & Reynolds, 1980), indicating that the secondary structure is not greatly altered so long as the appropriate hydrophobic milieu is maintained. It is particularly significant that no shape changes are observed in the spectra when apo B is liganded with sodium dodecyl sulfate below the critical micelle concentration, $C_{12}E_8$, or egg yolk lecithin. The absolute magnitude of the negative ellipticity is altered by varying the level of phospholipid binding, but no significant shift in the wavelength of the minimum is observed (Figure 3).

The apo B–phospholipid complex cross-reacts with rabbit antibodies elicited to holo-LDL_2 as well as with the complexes formed between apo B and $C_{12}E_8$ or sodium dodecyl sulfate below the critical micelle concentration. Precipitin lines in all three cases appeared to fuse with those formed between the antibody and holo-LDL_2 suggesting that the major portion of the antigenic determinants was intact. An exhaustive study of the antigenic properties of the complex was not undertaken since even a rigorous demonstration of *total* identity between the reconstituted particle and holo-LDL_2 would not exclude the possibility of some minor structural changes. (For example, some portion of the antibody population may recognize amino acid sequences rather than three-dimensional structure and thus would be insensitive to changes in the latter.)

Electron Microscopy. Figure 4 shows a negatively stained preparation of apo B–phospholipid complexes compared to holo-LDL. The average diameter of single particles of the

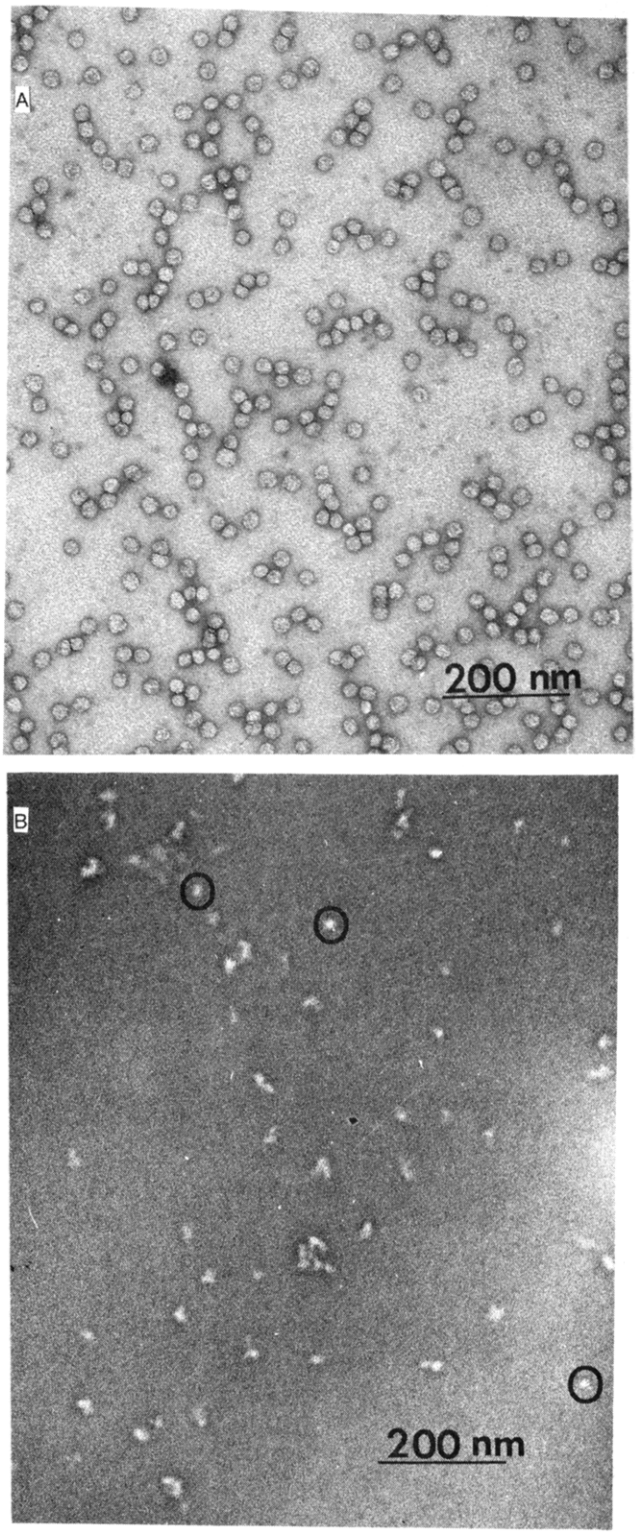

FIGURE 4: Electron micrograph of negatively stained holo-LDL (A) and apo B–egg yolk phosphatidylcholine (B). Single, reconstituted apo B–phospholipid particles are circled in (B). Magnification, 85 000×.

reconstituted material is ~14 nm, which is in reasonable agreement with the diameter of an unhydrated sphere containing 500 000 g of apo B and 250 000 g of egg yolk lecithin (12.8 nm). Analytical ultracentrifugation of this material indicated between 30 and 35% was of higher molecular weight, and this is also observed in the electron micrograph shown in Figure 4B. Several areas of aggregated species are seen which may in part have resulted from the staining procedure as well as being representative of the higher molecular weight material

present in ultracentrifugation experiments. The electron micrograph of negatively stained holo-LDL shows much better contrast than that of the reconstituted particle even though the experimental procedure for sample preparation was identical. The reconstituted particle is smaller than holo-LDL, and this in itself will lead to reduced contrast in negative-stain electron microscopy. Alternatively, the apo B–phospholipid complex may not be perfectly spherical but rather a flattened ellipsoid.

Complexes of apo B with the nonionic detergent $C_{12}E_8$ contain ~930 mol of detergent/500 000 g of protein (Watt & Reynolds, 1980). Electron microscopic studies of these particles demonstrated that they were asymmetric rods ~80 by 4.5–5.5 nm (Zampighi et al., 1980). The apo B–phospholipid complex shown in Figure 4B is clearly a more symmetric structure indicating that interaction of this protein with an amphiphile containing two hydrophobic tails rather than one leads to a major alteration in the tertiary structure of the apo B–amphiphile complex.

Experiments at Neutral pH. Preliminary studies conducted at pH 7.4 rather than 10 resulted in a population of apo B–egg yolk phosphatidylcholine particles that were highly aggregated. Similar behavior was noted when nonionic detergents were bound to apo B at neutral pH (Watt & Reynolds, 1980; Zampighi et al., 1980), and it was suggested that this aggregation occurred through interactions between the water-soluble portions of the protein since the presence of significant charge on the complex by either increasing the pH or adding ionic detergents dissociated the aggregates to a state containing 500 000 g of protein/mol of complex.

Discussion

Apo B has been recombined with egg yolk phosphatidylcholine to produce a water-soluble complex in which substantial features of the native structure of the protein are intact, i.e., the protein is dimeric, the circular dichroic spectrum is similar to that of holo-LDL with a negative ellipticity minimum at 218 nm, and the complex contains the majority of the antigenic determinants found in the native particle. Undoubtedly, there are ligand-induced differences in the structure of apo B which depend upon the nature of the bound amphiphile, and we would anticipate some structural alterations when cholesteryl esters, for example, are added to the apo B–phospholipid complex. It is clear, however, from the data presented on apo B–phospholipid complexes that such differences will be difficult to detect since major changes in secondary structure (circular dichroism in the wavelength range 200–250 nm) or in the major antigenic determinants recognized by antisera raised against holo-LDL do not occur. In this context it will be particularly important to investigate the effects of bound ligands on the interaction of reconstituted apo B complexes with specific receptors. Additional information on the state of the protein in the reconstituted particles may be obtained by using antibodies directed to *specific* determinants on the protein [e.g., Schonfeld et al. (1979)]. Other investigators have reported "reconstitution" of LDL-like particles, but in all cases native lipid was not totally removed from the protein. Lipase-treated VLDL (Deckelbaum et al., 1979) or heptane-extracted and lyophilized LDL (Krieger et al., 1978; Steinberg et al., 1978) were used as the starting material for reconstitution.

The maximum amount of phospholipid binding to apo B which we have observed is 320 ± 50 mol/500 000 g of protein. This is considerably less than the 600 mol/500 000 g of protein found in holo-LDL. Whether our maximum binding is kinetically limited due to the low monomer solubility of egg yolk phosphatidycholine or represents a true difference in saturation level in the absence of the other naturally occurring lipids found in the native complex cannot be determined unequivocally. This binary system, however, offers the opportunity for systematic investigation of the interaction of apo B–phospholipid complexes with the other lipid components found in holo-LDL, i.e., cholesteryl ester, cholesterol, and triglycerides.

Two important observations on the properties of apo B have emerged from this and previous studies. The protein maintains its dimeric structure in the presence of all bound amphiphiles thus far investigated, suggesting strong protein–protein interactions. Secondly, apo B is not found associated with the free phospholipid vesicles generated during detergent removal in our exchange experiments. Instead, the protein packages a small number of phospholipid molecules in water-soluble form—a property similar to the interaction of lipids with the apoproteins from HDL but unlike the association of membrane-bound proteins with phospholipid vesicles.

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